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Società Italiana di Parassitologia

XXVII Congresso Nazionale

Lettura Magistrale
Relazioni ai Simposi e Tavola Rotonda
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Alghero 26-29 giugno 2012

Editors
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Nota Editoriale

A distanza di 32 anni il Congresso della Società Italiana di Parassitologia ritorna ad Alghero dove si era tenuto nel 1980. Ad ospitarlo è il Quarté Sayàl, un ex convento edificato nel 1722 e stabilimento vitivinicolo dell'Azienda Sella & Mosca dal 1902 al 1961.
Il programma scientifico si articola in quattro Simposi tematici, una Tavola Rotonda e dodici Sessioni di comunicazioni orali e poster.
Il Congresso sarà aperto dalla Lettura Magistrale "Americani, comunisti e zanzare" tenuta dalla Professoressa Eugenia Tognotti.
I quattro Simposi, cui partecipano esperti italiani e stranieri con 33 relazioni, trattano di problematiche parassitologiche di rilevante interesse per la sanità pubblica e per le produzioni zootecniche:

- **Epidemiologia della trichinellosi nell'uomo e negli animali in Italia e in Europa**
- **Aggiornamenti sulla leishmaniosi e novità per il suo controllo**
- **La dicroceliosi negli animali da reddito: diagnosi, terapia e strategie di controllo**
- **Protozoi di interesse medico**

La Tavola Rotonda "L'Echinococcosi Cistica in Sardegna: quali possibilità di controllo", con l'intervento dei massimi esperti nazionali ed internazionali, istituzioni pubbliche, veterinari e medici delle AASSLL, si propone di fare il punto sulla situazione della parassitosi nella nostra isola ed individuare strategie per i suo controllo.
Le sessioni scientifiche contemplano 225 contributi scientifici sia sotto forma di comunicazioni orali sia come poster su argomenti inerenti: Terapia e farmacoresistenza, Entomologia medica e veterinaria, Parassiti e fauna selvatica, Sistematica molecolare ed evoluzione, Malattie trasmesse da artropodi, Epidemiologia delle malattie parassitarie (degli animali da reddito, degli animali d'affezione e dell'uomo), Leishmaniosi e filariosi, Biologia molecolare, Parassiti e fauna acquatica, Diagnostica delle malattie parassitarie.
Un particolare ringraziamento va al Prof. Giuseppe Cringoli, Series Editor della Collana Monografica Mappe Parassitologiche, che con la sua consueta disponibilità ha consentito la stampa di questa Special Issue, che raccoglie la Lettura Magistrale, le Relazioni dei Simposi, della Tavola Rotonda e gli abstract comunicazioni e dei poster presentati al Congresso.
Mi è gradito ringraziare, anche a nome del Comitato Organizzatore, il Direttivo della Società, i Referee, i Soci e tutti coloro che, in varia misura hanno contribuito all'organizzazione dell'evento. Rivolgo un caloroso benvenuto a tutti i congressisti augurando loro un proficuo lavoro ed un piacevole soggiorno ad Alghero.

Il Presidente del Comitato Organizzatore
Prof. Giovanni Garippa

LETTURA MAGISTRALE

Americani, comunisti e zanzare

Eugenia Tognotti
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Abstract: Between 1946 and 1950 the Rockefeller Foundation (RF) carried out in Sardinia an experiment in malaria control by species eradication in using a new weapon, DDT. The powerful chemical promised a technological solution of the ancient problem of malaria, opening a new dazzling era for malariology. 257 metric tons of the insecticide was applied over the island and the sum of 12 millions of dollars was spent in four years. But the tensions of the Cold War did condition the antimalarial Campaign. The RF was viewed as working hand-in-hand with the U.S. government in the Mediterranean. Between 1947 and 1948, Communist press attacked several times ERLAAS, the Regional Agency for the Anti-Anopheles Struggle in Sardinia founded by the RF. The communist press claimed that the ERLAAS vehicles were secretly armed and equipped to "take over" Sardinia and that the antimalarial campaign was paving the way for the transformation of the island into an enormous US air base. This situation prompted the RF to speed the operations and transform the original scientific objective. Ultimately, the project became a public health campaign against malaria. The disease was eliminated, but the main objective of the complete eradication of the indigenous vector was not achieved: breeding places of A. Labranchiae mosquitoes are still present in Sardinia, in some isolated areas

Il progetto di eradicazione della malaria in Sardegna maturò negli anni della seconda guerra mondiale per il concorrere di due condizioni favorevoli: la comparsa sulla scena del DDT (dicloro-difenil-tricloroetano), la cui azione insetticida fu scoperta nel 1939; e la disponibilità dei fondi dell'UNRRA (United Nation Relief and Rehabilitation Administration), istituita nel 1943 con lo scopo di pianificare gli aiuti per la ricostruzione delle zone devastate dalla guerra. (Snowden F. 2005, The Conquest of Malaria. Italy, 1900-1962, Yale University Press). Dopo i primi test effettuati negli Stati Uniti, nel 1944 il DDT – che sviluppava anche un'azione residua – venne sperimentato per la prima volta in Italia a Castel Volturno (a nord di Napoli), dall'Unità Dimostrativa per il Controllo della Malaria della RF, guidata dal malariologo Patrick Russel e dall'entomologo Fred Soper. Spruzzato sulle pareti delle abitazioni e delle stalle, si rivelò immediatamente efficace nel ridurre la densità dei vettori. Nel luglio dello stesso anno vennero trattate numerose abitazioni a Ostia, e nel giugno del 1945 la sperimentazione fu estesa al Delta del Tevere (Tognotti E., 2009, Emerg Infect Dis 15(9):1460-6). La straordinaria efficacia dimostrata dalla potente arma chimica, fece maturare, tra gli esperti americani della Rockefeller Foundation, che collaboravano con l'igienista e malariologo italiano Alberto Missiroli, l'idea che fosse ora possibile conseguire l'eradicazione della malaria dall'Italia, e che tale risultato potesse essere ottenuto (in alcune situazioni) addirittura attraverso l'eliminazione definitiva dei vettori. Sotto la spinta dei malariologi alleati furono quindi varati, nel 1946, due piani, con obiettivi e metodi d'intervento diversi: un piano (concepito da Missiroli) volto all'eradicazione della malaria mediante lo spruzzamento intradomiciliare di DDT; e un progetto per l'eradicazione dei vettori malarici dalla Sardegna. Area di malaria endemica, l'isola aveva, dall'ultimo quarto del XIX secolo, il ben poco invidiabile primato di regione italiana con i più alti tassi di mortalità per quella malattia. La malaria sarda rientrava, secondo la classificazione del malariologo Angelo Celli, nel tipo «epidemico sud-Italia», a cui appartenevano anche la Maremma toscana e romana, Italia meridionale e insulare. Essa presentava un certo numero di caratteri particolari:

a) il carattere pandemico, cioè la generale diffusione della malattia tra la popolazione.
b) la gravità delle forme morbose, per cui «although malaria was present all over Italy, the Sardinian malaria situation had to be considered more threatening because of the high prevalence of P. falciparum and its associated mortality rates» (Brown J, 1983, Studies in Sardinian Archaeology I: 209-235)
c) la durata eccezionalmente lunga del periodo epidemico.

Rispetto alle altre aree del Mediterraneo, la Sardegna aveva un tasso assai più elevato di mortalità per le estivo-autunnali durante il picco epidemico di agosto e settembre.
Le trasmissioni e le ricadute non conoscevano praticamente sosta nel corso dell'anno, e ciò a causa della compresenza di due specie di parassita malarico: *P. vivax*, agente della terzana semplice, attivo per l'intero anno, e il maligno *P. falciparum*, che predominava sull'altro ed era responsabile del picco epidemico estivo: la varietà geografica sarda, inoltre, si distingueva per la speciale virulenza clinica dell'infezione che richiedeva una dose curativa di chinino più elevata, stando alle testimonianze dei medici impegnati nelle grandi campagne antimalariche (Lustig A., Sclavo A., Alivia M., 1911).
Ai malariologi della RF – che avevano conseguito successi significativi in Egitto e in Brasile – si proponeva quindi la possibilità di con-

durre un grandioso esperimento naturale su larga scala col DDT per la totale eliminazione del vettore indigeno *A. labranchiae*, in un'area endemica del Mediterraneo. (Logan J, 1953, The Sardinian project. An experiment in the eradication of an indigenous vector. Baltimora). La messa a punto del piano, il «Sardinian project», richiese quasi un anno di contatti a vari livelli – l'UNRRA, il governo italiano, l'Istituto Superiore di Sanità, l'Alto Commissariato per l'igiene e la sanità pubblica. A dicembre del 1945 prese corpo e il 12 aprile 1946 arrivò il decreto istitutivo di un ente apposito l'ERLAAS (Ente Regionale per la Lotta Anti-Anofelica in Sardegna), la cui prima riunione si tenne a Cagliari il 14 maggio, presente una piccola pattuglia di malariologi, parassitologi, igienisti, entomologi, esperti di tecniche di eradicazione, medici, autorità politiche e sanitarie, tecnici idraulici, agrari e forestali. Presiedeva l'Alto Commissario per la Sardegna, il generale Pietro Pinna – «most interested and cooperative». Nel suo indirizzo di saluto, definì quella che stava per iniziare «la più santa delle guerre, molto più santa di quelle che si sono finora combattute» (RF Archives, First meeting of Committee, 1946).

Per le prime operazioni il governo italiano aveva stanziato trecento milioni, con una garanzia di 500.000 dollari da parte dell'UNRRA per trasporti e materiali: spruzzatori a getto continuo e intermittenti a mano, stivaloni di gomma, tute da lavoro, caschi coloniali, brandine da campo e, naturalmente, tonnellate di DDT.

In clima di "guerra fredda" e di crisi degli equilibri internazionali, l'esigenza di procedere con le operazioni portò ad una sottovalutazione delle difficoltà organizzative e logistiche, mentre mancava un approfondito studio entomologico ed epidemiologico preventivo.

Tra dubbi e incertezze la campagna di eradicazione partì nel novembre del 1946, dopo un'indagine preliminare del servizio entomologico che aveva ampliato le già cospicue conoscenze sulla biologia e sull'ecologia della specie indigena *labranchiae*, appurando anche la presenza di altre specie selvatiche e con pochissimi rapporti con l'uomo: l'*algeriensis* e la *claviger*, e altre meno diffuse e circoscritte ad alcune zone quali *A. plumbeus, A. marteri*, *A. hispaniola* (Garrett Jones C., RF Archives, 1948).

Questa acquisizione poneva un grosso problema non solo scientifico, ma anche economico e organizzativo: il progetto originario consisteva nel tentativo di eradicazione di *A. labranchia*e, ritenuta l'unica presente nell'isola, ciò che spiega la genericità della parola «lotta anti-anofelica» che compariva nella denominazione stessa dell'ERLAAS. Il programma doveva ora essere adeguato alla nuova necessità di eradicare tutte le specie, data anche l'impossibilità di tracciare una netta distinzione tra i siti di riproduzione. La prima parte del programma consistette nell'irrorazione con l'insetticida delle case di Cagliari e dintorni per poi proseguire nel resto dell'isola. Nel giugno del 1947 il lavoro era compiuto nell'85% di tutti i distretti, con un incoraggiante decremento del numero di insetti adulti, di cui davano conto le esplorazioni condotte a marzo nel Cagliaritano (basso e alto Campidano, Iglesiente, Sulcis, Trexenta, Salto di Quirra, Sarrabus) e nel Nuorese (Baronia, altopiano di nord-est, altopiano di Nuoro, bacino del Tirso, Barbagia di Ollolai, Gennargentu, Baronia). (Tognotti E., 2009, Emerg Infect Dis 15(9):1460-6). Ma sul più grandioso esperimento naturale mai condotto dal momento della scoperta del ciclo di trasmissione della malaria, cominciarono a pesare le tensioni della Guerra Fredda. Nel maggio 1947 i il governo democristiano di Alcide De Gasperi, aveva escluso i partiti comunista e socialista del suo gabinetto, come risultato della crescente pressione anticomunista della politica estera americana. Questo improvviso cambiamento, dopo l'elezione del Presidente Truman, aveva portato ad una sostituzione del Commissario italiano per la salute e l'igiene. I comunisti italiani cominciarono a criticare l'ERLAAS, sospettato di essere la *longa manus* del potere americano in Italia. (Stapleton D.H., 2000, Parassitologia, 42: 21).

Nei mesi successivi, dopo cambiamenti repentini nelle alleanze internazionali in conseguenza della fondazione del Cominform (Settembre), i comunisti intensificarono le loro polemiche contro il Piano Marshall. Questa situazione indusse i responsabili del "Sardinian project" a procedere più rapidamente. "Gli occhi del mondo" si erano concentrati sull'esperimento ed era di primaria importanza andare avanti a tutti i costi prima dell'arrivo di una crisi. Ciò comportò una trasformazione graduale dell'obiettivo originario della eradicazione di un vettore indigeno. L'esperienza portata avanti fino allora dimostrava in modo conclusivo che, a causa della topografia della Sardegna, l'eliminazione di *A. labranchiae* era tecnicamente molto più difficile di quanto non fosse l'eradicazione di *A. gambiae* dal Brasile o Egitto: e che l'amministrazione di un servizio di eradicazione in Italia era più complessa. Nell'imminenza delle Elezioni politiche del 1948 (18 aprile), - considerato come uno spartiacque potenziale non solo per l'Italia - le tensioni aumentarono. Il governo americano sosteneva la DC. L'amministrazione Truman dichiarò che nessun ulteriore aiuto economico sarebbe arrivato in Italia se il partito comunista avesse vinto le elezioni. Gli attacchi della stampa comunista alla RF aumentarono: la campagna di eradicazione e la guerra alle zanzare vettrici, era vista come un pretesto per occupare l'isola. Alcuni giornali scrissero che i veicoli ERLAAS erano stati segretamente armati ed equipaggiati allo scopo. In questa situazione la RF decise di continuare nell'esperimento, modificando senza clamore l'obiettivo originario – l'eradicazione del vettore malarico – in quello di «quasi eradicazione sperimentale del genere». Nel marzo del 1948 il lavoro anti-alate era terminato: ogni pezzo di muro o di soffitto dei ricoveri costruiti dall'uomo nell'isola – compresi i nuraghi, i ponti, le grotte, le caverne e i pozzi di miniera – era stato trattato. (Tognotti E., 2008,

Per una storia della malaria in Italia. Il caso deal Sardegna, Milano).
Nell'estate del 1948 fu realizzata la parte più impegnativa del programma, la campagna intensiva anti-larva che interessò 1.200.000 potenziali focolai: praticamente ogni luogo d'acqua dell'isola, che doveva essere trattato ogni settimana per tutta la stagione e ispezionato alla ricerca di larve una volta al mese.
Alla fine dell'estate del 1948, i direttori delle operazioni potevano annunciare che "la negatività era stata raggiunta, anche se non era ancora garantita".

Focolai positivi delle varie specie anofeliche nel mese di settembre prima e dopo le campagne anti-anofeliche (stime)

Specie	prima del 1947	1948	perc. dim.
A. labranchiae	70.000	45	99,36
A. claviger	210.000	482	99,77
A. algeriensis	70.000	148	99,79

Contro i focolai residui si svilupparono, ancora nel corso dell'autunno, le operazioni di disinfestazione antilarva. Ma, per quanto circoscritte e in numero limitato, aree positive esistevano ancora al 1949, anno di transizione tra la campagna anti-larva e i test di eradicazione. Ma se l'obiettivo dell'eradicazione delle resistentissime «indigenous mosquitos», non era stato raggiunto, la malaria era stata eradicata dall'isola di Sardegna. (Brown P.J., 1998, Parassitologia , 40:117–30).
Nel 1950, per la prima volta nella storia dell'isola, nessun caso autoctono di malaria fu registrato dalle autorità sanitarie: (ISTAT, 1958, Cause di morte).

Casi di malaria dal 1947 al 1952

Anni	Numeri
1947	39.303
1948	15.121
1949	1.314
1950	40*
1951	9*
1952	0

* Tutti casi di ricaduta. ** 8 ricadute.

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SIMPOSIO 1

EPIDEMIOLOGIA DELLA TRICHINELLOSI NELL'UOMO E NEGLI ANIMALI IN ITALIA E IN EUROPA

We can still learn something from trichinellosis: from a public health problem to a model for studying immune mediated diseases

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Trichinellosis is a zoonosis caused by the parasitic nematode *Trichinella* which is nowadays distinguishable in eight different species and several other genotypes. Two main clades can be considered: one of encapsulating species (*T. spiralis*, *T. britovi*, *T. murrelli*, *T. nativa*, *T. nelsoni*) and another which include non-encapsulating ones (*T. pseudospiralis*, *T. papuae*, *T. zimbabwensis*); these can infect not only mammals but also birds (*T. pseudospiralis*.) and reptiles (*T. papuae* and *T. zimbabwensis*). This zoonosis has an ubiquitous distribution, however in these last years it represent a particularly relevant public health problem in Eastern Europe countries, China and Argentina (Murrell KD, Pozio E, 2011, Emerg Infect Dis, 17: 2194-2202). Since it is a food borne diseases the transmission occurs by the consumption of raw or undercooked meat from different animal species (mammals, birds, and reptiles). Trichinellosis, like other helminth infections, is characterised by a host immune response which shifts rapidly from a T helper (Th) 1 activation to a stable Th2 polarization, responsible for blood eosinophilia and increased levels of total IgE (Bruschi F, Chiumiento L, 2012, Endocr Metab Immune Disord-Drug Targets, 12: 4-15), for this reason, infection with *Trichinella* represents a very useful experimental model to study immune mediated diseases such as allergy, for example or molecules modulating them (Del Prete G et al, 2008, J Allergy Clin Immunol, 122: 908-913). The protective role of Th2 polarization is far to be fully elucidated and recently even a beneficial effect for the parasite of eosinophil activation has been highlighted (Fabre et al, 2009, J Immunol, 182: 1577-1583). Eosinophils in trichinellosis would act as regulatory the Th1 response, rather than effector, cells (Gebreselassie et al, 2012, J Immunol, 188: 417-425).

Can be Trichinella-Induced Th2 polarization beneficial in immune mediated diseases?

This nematode is unique, being characterized by intracellular parasitism. It interferes with host immune response, establishing a chronic infection for the entire life of the host. This long-term host-parasite relationship is due to *Trichinella* immune evasion strategies. In fact, the parasite can modulate and even suppress host immune response to persist and ensure his survival in host organism.

It has been shown that *T. spiralis* muscle larvae excretory/secretory products (TspES) suppress *in vitro* dendritic cell (DC) maturation induced by both S- (from *E. coli*) and R-form (from *N. meningitidis*) lipopolysaccharide (LPS), in a TLR4 restricted way, furthermore TspES were also able to interfere with the expression of several genes related to the TLR-mediated signal transduction pathways. The incubation of spleen cells from mice transgenic for ovalbumin (OVA)-TCR with OVA and TspES-pulsed DC resulted in the expansion of CD4+ CD25+Foxp3+ T cells which are T regulatory cells with suppressive activity, producing TGF-β (Aranzamendi et al, 2012, Parasite Immunol, 34: 210-223).

Many experimental studies have shown an ameliorating effect of *Trichinella* infection in some immune-mediated diseases and now the mechanisms underlying this phenomenon are much more understandable. During the infection, parasite stimulates host Treg and Th2 response that mutually inhibits the Th1 polarization resulting in an alternative strategy to down-regulate excessive Th1 polarization in chronic autoimmune diseases. Research regarding the potential immunomodulatory activity of *Trichinella* infection was applied to different Th1 diseases, like experimental colitis, where pathology is associated with production of IFN-γ and other pro-inflammatory cytokines. Infection with the parasite ameliorates in fact experimental colitis induced by DNBS (dinitrobenzene sulfonic acid) in mice, by down regulating myeloperoxidase activity in colonic tissue. This effect is due to an emerging Th2-type immune response characterized by high IL-4 and IL-13 production by spleen cells in *T. spiralis* infected mice (Khan WI et al, 2002, Infect Immun, 70: 5931-5937). Another Th1 disease is represented by the experimental autoimmune encephalomyelitis (EAE), an animal model of brain inflammation accompanied by demyelination of the central nervous system (CNS) mediated by myelin specific Th1 and Th17cells (Domingues HS et al, 2010, PLoS One, 5: e15531). *T. spiralis* infection in rats affected by EAE reduces the severity of the disease, from the clinical and histological point of view (Gruden-Movsesijan A et al, 2008, Exp Parasitol, 118: 641-647). These effects seem to be related to high IL-10 production by T reg cells (Gruden-Movsesijan A et al, 2010, Parasite Immunol, 32: 450-459). The nonencapsulating species

T. pseudospiralis can suppress EAE by reducing the inflammatory infiltration and demyelination in spinal cord and brain, by down regulating Th17 and Th1 responses as shown by reduced expression of pro-inflammatory cytokines, IL-17, IL-6, IL-1 , IFN-γ, and TNF-α in the spinal cords of infected EAE, compared to uninfected animals (Wu Z et al, 2010, Parasitol Res, 107: 1173-1188).
NOD (Non-obese diabetic) mice represent a very useful model of type 1 diabetes and autoimmune diseases. NOD mice infected with *T. spiralis* remained diabetes free with a prevalence of only 10% at week 36 to 37, whereas 80% of uninfected NOD mice are diabetic by 22 to 23 weeks of age.
This is not due to a lower ability to produce auto-antibodies, in fact titres of total immunoglobulin insulin-specific antibodies increased significantly in both uninfected and *T. spiralis*-infected NOD mice. As expected, there was an increase in proliferation of spleen cells to *T. spiralis* antigen, following infection of BALB/c mice with this parasite; but this didn't occur when infected NOD mice were evaluated.
The presence of helminth infection is not necessarily accompanied by a reduction in the production of Th1-associated IFN-γ, but spleen cells produce significantly more IL-4 in *T. spiralis* NOD mice than in uninfected animals, in response to *Trichinella* antigens. The percentages of infiltrating pancreatic CD4+ T cells producing IFN-γ, IL-10, and IL-4, after a period of unmodified situation, at 22 to 23 weeks of age, had increased in NOD mice protected from diabetes by *T. spiralis* infection (Saunders KA et al, 2007, Infect Immun, 75: 397-407).
T. spiralis infection reduced the levels of TNF-α in bronchoalveolar lavage fluid and the cellular recruitment into the airways of mice co-infected with influenza A virus. Infiltration of inflammatory cells such as neutrophils and CD4+ and CD8+ lymphocytes was reduced, resulting in a faster recovery of experimental animals. The generalized increase in vascular permeability in pulmonary tissues, typical of influenza was suppressed in co-infected mice, but this blunting effect was observed only during the early phase of trichinellosis. Moreover, the number of IL-10 producing cells, and the levels of this cytokine at local level, were reduced, suggesting that the effects of helminth infection is not due to a reduction of IL-10 production (Furze RC et al, 2006, Infect. Immun, 74: 1924-1932).
It was recently shown that *T. spiralis* infection reduces also airway allergy inflammation in mice (Park HK et al, 2011, Exp Parasitol, 127: 539-544). The effects of *T. spiralis* infection on animal models of immunological diseases or co-infection are summarised in the Table 1.

Table 1. Effects of Trichinella spp. infection on animal models of immunological diseases or co-infection.

Immunological disease or co-infection	Infection with	Effect	Mechanism
Experimental colitis	*T. spiralis*	Amelioration	Decreased levels of MPO induced by Th2 polarization
Experimental autoimmune encephalomyelitis	*T. spiralis*	Amelioration	Increased IL-10 levels
Experimental autoimmune encephalomyelitis	*T. pseudospiralis*	Amelioration	Reduced IL-17and IFN-γ production
Type I diabetes	*T. spiralis*	Amelioration	Th2 polarization
Influenza A virus	*T. spiralis*	Amelioration	Decreased TNF-α levels
Allergy	*T. spiralis*	Amelioration	?

CONCLUSIONS: The "hygiene hypothesis" (Strachan D P, 1989, Br Med J, 299: 1259-1260) postulates that the increased incidence of autoimmune diseases such as diabetes or multiple sclerosis as well as of atopic conditions in the industrialised world derives from a decreased exposure to infectious agents. *Trichinella* infection in animal models can ameliorate the outcome of such diseases, but a lot of research is still needed to understand the fine mechanisms of such effects (Bruschi F, 2012, Endocr Metab Immune Disord-Drug Targets, 12: 1-2).

Epidemiology of *Trichinella* infections in humans and animals of Italy and Europe

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AIM: Parasites of the genus *Trichinella* are zoonotic nematodes circulating in wild animals and sometimes also in domestic pigs of all the continents but Antartica. The aim of the present work was to review the epidemiological patterns of *Trichinella* infections in humans and animals of Italy and Europe in the last years based on the increased information availability and to show the lights and shadows of control measure underway in the European Union (EU) since 2006.

MATERIALS AND METHODS: Published and unpublished information originated from the scientific and gray literature and reports of National Reference Laboratories for *Trichinella*, the database of the International *Trichinella* Reference Center and the annual EFSA reports.

RESULTS: Out of the four *Trichinella* species circulating in Europe, *T. britovi* is the most widespread in all the MS but Cyprus, Denmark, Ireland, Malta and UK. The main hosts are carnivores (e.g. foxes, raccoon dogs, wolves, mustelids) but also wild boars are infected mainly in central and northern European countries. Domestic pigs are rarely infected. *Trichinella spiralis* shows foci in Spain, the Ireland isle, Germany, Estonia, Latvia, Lithuania, Poland, Czech Republic, Hungary, Slovakia, Slovenia, Romania, and Bulgaria. The main hosts are domestic and wild swine even if in the Ireland isle, the cycle is maintained by the fox. *Trichinella nativa* is restricted to wild carnivores of Finland, Estonia, Latvia, Lithuania and Sweden, but two and one infected foxes have been also detected in Germany and Poland, respectively. *Trichinella pseudospiralis* foci have been described in Bulgaria, Czech Rep, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Netherlands, Slovakia and Sweden. The most important reservoir is the wild boar, whereas carnivores are rarely infected. In the domestic habitat, *Trichinella* parasites circulate among free-ranging and backyard pigs of some countries, whereas no infection has been documented in pigs farmed under high containment level conditions. Out of about 260 million pigs slaughter every year in the EU, an average of 800 pigs per year have been detected with *Trichinella* infection in the last five years. From 1986 to 2009, 45,117 *Trichinella* infections have been documented in humans of EU. Of them, 63% of the infections occurred in Romania, 9.1% in Bulgaria, 8.8% in Lithuania, 6.8% in Poland, 2.8% in Spain, 2.7% in France, 2.6% in Italy, and 4.2% in other 10 countries. No deaths were documented in this period in EU countries, but 24 deaths occurred in non-EU countries of Europe. The main source of infection was pork from domestic pigs and wild boars, but horse meat was the main source of infections in France and Italy and dog meat in Slovakia. In 2011, a person died for trichinellosis due to the consumption of pork from a hunted wild boar in Spain. In the last 10 years in Italy, 52 cases of infections in humans has been documented for the consumption of free-ranging and backyard pigs (26 cases in the Sardinia island), hunted wild boars (8 cases in Abruzzi and Piedmont regions), imported pork from Romania (11 cases), and imported horse meat from Eastern Europe (7 cases).
Epidemiological data clearly show that *Trichinella* infections cannot be eradicated in the EU due to the presence of a sylvatic cycle which plays the most important role in the maintenance of these parasites in nature. The greatest percentage of the pig population is farmed under high containment level conditions which easily prevent the *Trichinella* transmission. However, most of economic resources and efforts to control this infection are focused on these animals, whereas free-ranging and backyard pigs which are at high risk for these parasites, frequently escape from any control resulting in the transmission of these pathogens to other pigs and humans. In addition, most of hunted wild boars are not tested for *Trichinella* since they are not for the market, resulting in the transmission of the infection to humans.

CONCLUSIONS: *Trichinella* testing of fattening pigs from controlled housing conditions can be stopped, whereas, pigs from non-controlled housing should be tested to reach the double result of the consumer protection and collection of epidemiological information (sentinel animals). A risk-based surveillance system should be put in practice by a strict control for *Trichinella* of home slaughtered pigs, making compulsory the control for *Trichinella* to all backyard and free-ranging pigs and wildlife used for human consumption independently if they are for the market or for the own consumption; moving funds and efforts from the control of *Trichinella* in fattening pigs from controlled housing to the control of *Trichinella* in pigs from non-controlled housing (e.g. backyard and free-ranging).

Epidemiological situation of trichinellosis in Spain

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As points out the title of the Simposium designed for the 2012 National Congress of the SoIPa, where this paper is enclosed, "The trichinellosis: is an always current zoonoses", and this is the case for more than 175 years. Since that Paget observed small cysts of this nematode in the muscles of a man in 1835, and Zenker, in 1860, identified the first case of trichinosis attributed it to *Trichinella*, really research and in the fight against this parasitosis has not ceased to this day. It is clear that the autoheteroxenus character of its life cycle, the fact that a same animal can act as a definitive host hosting adult parasites in his gut and also as intermediate host to house the larval stages in his digestive system and finally in the muscles, without having to exit the environment to complete their biology, in addition to having a great spectrum of possible hosts, it has enabled a diffusion in the very broad nature throughout its history. In Spain, and specifically in the region in which work, Extremadura, in the Southwest, border with Portugal, this zoonosis continues to present certain prevalence in the pig and wild boar, occur relatively often human disease outbreaks. This is due to the large number of extensive pig farms in the South of Spain. In addition, still continues to be deeply rooted the regime of domestic or house-to-house sacrifices. We must not forget that the pig is one of the largest livestock sectors of the Spanish economy, especially in the south areas of Spain, documenting in Extremadura a total of 12.218 farms with 1.637.254 animals, of which 924.981 animals are pigs reared in organic farms, so it must provide health care that this host deserves, and focus our efforts on getting the placing on the market of a product of great quality and with greater health guarantees. On the other hand, should add hunting, which enjoys a great importance in Extremadura, with a high number of killed wild boars per year, one of the main hosts of Trichinella. In fact, in the 2009-2010 season bring, only in Extremadura region, 38.297 pieces of hunting, of which 13.031 correspond to wild boars. In addition, it's considered that each year bring approximately 33,000 foxes in this region, another important wild reservoirs of *Trichinella.* Therefore, must take into account that the rise of hunting, especially wild boar requires greater attention in all its aspects, both economic and management, as obviously the health due to his relationship with the man. The zoonotic character of the parasitación makes that every year diagnose several human outbreaks in Spain (Rodríguez-Osorio et al., 1999;) Gómez-García et al., 2003; Rodriguez de las Parras et al., 2004; Gallardo et al., 2007). One of the most serious recent outbreaks which occurred in Spain happens at the beginning of 2011 in Huesca, close to the Pyrenees, where a person dies by positive consumption of wild boar. So, Spain is no stranger, much less, to the 167 million pigs annually investigated in the European Union in search of *Trichinella* (Alban et al., 2011). While the most important source of human infection worldwide is the pig, the majority of outbreaks that occurred in Europe in recent decades are linked to horse and wild boar meat. Indeed, Prof. Pozio indicates, in 1998, that the increase in wild boar in Europe has led to an increase of human trichinosis. Although historically, the method of diagnosis used for the detection of muscle larvae of *Trichinella* has been trichinelloscopia, currently to publish the regulation 20752005 of the European Commission, which establishes "specific rules for official controls on the presence of trichinae" in meat" and the Spanish national contingency Plan against *Trichinella* (may, 2010), method applicable only in exceptional circumstances in which meat is not marketed and is distributed only in a field family, all inspections are handled by the digestion of collective samples with use of magnetic stirrer, being this technique the official method of diagnosis of the trichinellosis. Since this implementation, the detection of *Trichinella* in Spain increased clearly affirming that in nature the majority of infections present a low or very low intensity of infection. According to Pozio et al. (2009) and in agreement with the ITRC of Rome, in Spain has detected the presence of *Trichinella* in pig, wild boar, fox, wild cat, wolf, marten and wild dog. The presence of this nematode in rats has also been demonstrated in Extremadura and in Granada, south of Spain. In addition, serologically have been demonstrated antibodies against the parasite in horses and hunting dogs. Obviously, he has also been diagnosed in humans.

In the year 2009, Blanco et al. indicate a prevalence of *Trichinella* in wild boar in Extremadura of 0.15 %, placing the prevalence in the domestic pig in 0,021%, reducing significantly the more intensive pig farms. In a very recent study, from the end of 2011, Gamito-Santos, comparing the ELISA technique with official diagnostic techniques, denounces the 0.18 % in wild boar and a 0,0047 % in home slaughter pigs, which are the main cause of human outbreaks. It also detects the presence of *Trichinella* in foxes in a 2.86 % of investigated animal, and this is the main wild reservoir of the parasite. These data confirm that the parasite is fairly widespread mainly in the wild areas, so it requires great efforts for their prevention and control.

Trichinellosis is still the most important zoonotic disease in Southeastern and Eastern Europe

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Human trichinellosis may be acquired through the consumption of undercooked meat (e.g., pork, horse and game meat) containing infective larvae of Trichinella spp. The global prevalence is difficult to evaluate, but as many as 11 million people may be infected worldwide. No region has experienced a more marked threat of trichinellosis than that of Southeastern and Eastern Europe due to political and economic changes of the1990s. The disastrous impact of war was clearly evident in countries of the former Republic of Yugoslavia, especially Croatia. Changes in pig production practices appear to account for much of the increase in human and pig infections in Russia and Lithuania. It is clear that trichinella infection in animals is a continuing threat to food safety especially in resource-poor areas. The following prevalence rates from Croatia clearly demonstrate the high risk for humans with traditional food habits of eating cured pork products. In the period between 1997 and 1999, 600 240 slaughtered pigs were tested for Trichinella and 0,16% were found to be positive. A decade later (2009) the prevalence showed a significant decrease whereby only 0,01% of 950 000 pigs tested positive. This decrease was a result of 10 years of government funded intensive monitoring and control activities. The greatest success within the eradication program was achieved through continuous rodent control at all sites where infected pigs were detected, prompt disposal of infected swine carcasses and compensation to the owners for condemned pigs.

Updating on trichinellosis in Sardinia

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AIM: up to 2004 Sardinia was considered free from Trichinella, in fact there has never been any report about trichinellosis in humans or animals, besides, previous epidemiological surveys confirmed the absence of the parasite. A study carried out during 1959-1962 (Arru E. et al, 1962) on 802 muscle samples from pigs, foxes, stray dogs, cats and wildcats (*Felis silvestris lybica*) has given negative results in all tested animals.

One more survey was carried out from 1994 to 1996 by the Istituto Zooprofilattico della Sardegna (Pintore A. et al 1996), serum samples from 4427pigs, 668 wild boars and 8 foxes were tested by ELISA and they were all negative for *Trichinella* antibodies. In the same study muscle samples from 2036 pigs (among those detected serologically) and from 32 foxes, were tested by artificial digestion leading to negative results.

The first evidence of *Trichinella* in Sardinia occurred in 2005 when two separate outbreaks (April and December) resulted in the hospitalization of 19 persons showing clinical patterns due to a massive *Trichinella* infection (Pozio, E. et al 2006). In both cases the established source of infection was the consumption of fresh homemade pork sausages made from free ranging pigs that were bred and slaughtered out of any veterinary control in the Orgosolo municipality. A following epidemiological survey on 681 free ranging and backyard pigs from Orgosolo municipality showed 4 muscle samples positive for *Trichinella* sp (Cossu P. et al, 2006).

One more person was hospitalized in 2007 after the consumption of fresh homemade product obtained from a free ranging sow from the Orgosolo municipality. (Pozio E. et al, 2009).

In 2008 the Istituto Zooprofilattico della Sardegna found *Trichinella* larvae (*T. britovi* and *T. spiralis* mixed infection) in muscle samples from a Polish regularly slaughtered horse whose meat was promptly confiscated and destroyed avoiding any kind of consequences to human population (Liciardi M. et al, 2009).

This was the last report untill January 2011, when a new **human outbreak** caused the hospitalization of 6 more persons showing gastroenteric symptoms, fever and muscular pain. In order to stop the spreading of the disease the mayor of Orgosolo demanded, in agreement with the Regional Health Department, the veterinary control (*Trichinella* larvae test) of muscle samples from illegally bred free ranging pigs slaughetered for private domestic consumption.

The aim of the present work was to investigate the spread of *T. britovi* in pig populations and hunted wild animals in the entire province of Nuoro and in the adjoining province of Ogliastra during the 2010-2011 and 2011-2012 winters (fig.1). Furthermore in this particular Sardinian area extensive pig farming is often practiced therefore the interaction between pigs and wild animals is very common.

MATERIALS AND METHODS: from October 2010 to February 2012 at the Istituto Zooprofilattico Sperimentale of Sardinia, muscle samples (5 g of diaphragm pillars from pigs and wild boars; 10 g of foreleg muscle from foxes and martens) from 5541 animals were tested to detect *Trichinella* sp. larvae by artificial digestion according to Regulation (EC) No. 2075/2005. In particular the survey was performed on 351 samples from free ranging pigs illegally raised in the Orgosolo municipality, 470 samples of legally breed backyards pigs slaughtered in the farms, 208 specimens from wild boars and 28 red foxes hunted in the

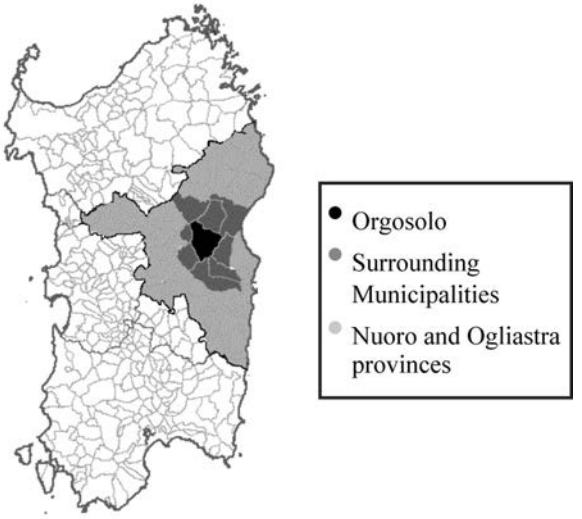

Fig. 1. Investigated area

Table 1. Results

	BACKYARDS PIGS SLAUGHTERED IN FARM		FREE RANGING PIGS		WILD BOARS		FOXES		MARTENS	
AREAS	Total	Positive samples	Total	Positive samples	Total	Positive samples	Total	Positive samples	Total	Positive samples
Orgosolo municipality	470	0	351	9	208	0	28	10	1	0
Municipalities surrounding Orgosolo	308	0	0	0	866	0	18	0	1	0
Nuoro and Ogliastra provinces	863	0	0	0	2390	0	32	0	5	0
Total	1641	0	351	9	3464	0	78	10	7	0

Orgosolo municipality and 1 marten (*Martes martes*) found dead in the same area. The survey involved the control of bordering territories through the detection of 308 samples from farm slaughtered pigs, 866 wild boars, 18 red foxes and 1 marten. Besides the neighbouring provinces of Nuoro and Ogliastra were investigated with 863 samples of farm slaughtered pigs, 2390 wild boars, 32 red foxes and 5 martens.
Trichinella sp. larvae were collected from each positive sample, stored in 90% ethyl alcohol and sent to the International Trichinella Reference Center of Rome for the identification at species level by multiplex PCR.

RESULTS: *Trichinella* larvae, were detected only in animals from the Orgosolo municipality. Specifically, larvae were collected from 9 (2.6%) free-ranging pigs, between the ages of 1 to 10 with an average age of 5.3 years and from 10 (35.7%) foxes. Infected pigs and foxes had an average larval burden of 127 larvae/g (range 0.4-543) and 79 larvae/g (range 3.4 - 565), respectively. All the other domestic (1983) and wild (3539) animals tested negative regardless their geographical origin (Table 1). Multiplex PCR identified all the larvae as *Trichinella britovi***.**

CONCLUSIONS: This is the first report of *T. britovi* in wild animals of Sardinia. The presence of this *Trichinella* species in the red fox is not surprising since this carnivore species plays the major role of reservoir of *T. britovi* in Europe (Pozio et al, 2009) and consequently, it can be considered as a sentinel animal to assess the spreading of this zoonotic parasite in nature. At present, *T. britovi* infection is confined to the Orgosolo municipality even if its distribution area seems to be wider than the one previously reported in free-ranging pigs (Cossu P. et al, 2006) and closer to neighbouring municipalities where it could rapidly spread. The presence of *T. britovi* in uncontrolled and illegal free-ranging pigs and the existence of this pig breeding in many areas of Sardinia, could result in the spreading of this zoonotic pathogen in other areas of the island. We can speculate that the cannibalism may occur among the illegal free-ranging pigs as a consequence of the endemic presence of the African Swine Fever in this area, and that this may favour the spreading of *T. britovi.* The discovery of *T. britovi* in foxes suggests that all the attempts to eradicate this infection from pigs of the Sardinia island could be frustrated by the presence of the sylvatic cycle.
The habit of breeding free ranging pigs and of slaughtering them out of veterinary control represents the main public health risk factor in Sardinia, as confirmed by all tests performed. Moreover typical homemade products from those pigs represent the ideal source of contamination because they are ready to eat while swine meat is generally consumed after a proper heat.

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Regulation (EC) No. 2075/2005

Animal *Trichinellosis*: Italian application of Reg. EC 2075/2005 Assessment and perspectives

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The aim of this work is demonstrating an upward trend in the number of Italian swine holdings officially recognised as *Trichinella*-free achieved through the application of animal health measures provided by Reg. EC No 2075/2005 (Annex IV, Chapters I and II).

The Ministry of health prepared an electronic form for the Regional Veterinary Services *Trichinella* control activities reporting whose data are used to draft the Annual Report to the European Commission.

The application of swine farm biosafety rules described by the regulation led to an increase of holdings officially recognised as *Trichinella*-free which made possible a sharp reduction of number of animals to be tested at the slaughterhouse.

The integrated strategy adopted by Italy for the surveillance and control of *Trichinellosis* has allowed to highlight some critical points in the officially recognition of *Trichinella*-free holdings and regions procedure provided by the regulation. These critical points notified before to the European Commission and then to the Codex Alimentarius (FAO/WHO), are now under evaluation by the OIE who is going to synthesized the opinions received, included the Italian one, in the Chapter 8.13.3 "*Trichinellosis free herd*" of the Terrestrial Animal Health Code.

SIMPOSIO 2

AGGIORNAMENTI SULLA LEISHMANIOSI E NOVITÀ PER IL SUO CONTROLLO

Update on epidemiology and treatment of canine leishmaniosis

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Dogs are considered the most important peridomestic reservoir of *L. infantum* infection to humans and sand flies are the only arthropods that are adapted to biologic transmission of *Leishmania*.
However, recently different authors have been evaluated other arthropods as potential vectors of *Leishmania*, like ticks and fleas but no experimental infection evidence has been shown that they have a role in natural transmission of the protozoan.
Other non vectorial routes of transmission are nowadays discussing: direct dog- to-dog transmission has been implicated as being responsible for transmission of infection in non-endemic areas in the absence of apparent vectors (e.g. USA); however, this has not been confirmed yet by experimental evidence. Also vertical transmission of infection in dogs appears to be rare but possible and recently, venereal transmission has been reported in dogs. Finally transmission of infection by infected canine blood products has been documented and is of special concern in areas where blood donors could be carriers of infection. Nevertheless, non-sandfly modes of transmission probably play only a marginal role in the natural history and epidemiology of canine leishmaniosis.
Added to these alternative routes of transmission the movement of dogs from non-endemic areas to endemic areas and vice versa, have changed dramatically the epidemiology of this important disease all over Europe.
Very recently, new secondary reservoirs have been proven like hares, in an important outbreak of human leishmaniosis occurred in Madrid (Spain) proven by xenodiagnosis.
On the other hand, the medical treatment of canine leishmaniosis (CanL) recent progress has been shown. The main drugs used for therapy of the disease are able to improve clinical signs and/or clinicopathological abnormalities temporarily or cure dogs clinically, but none of these treatments reliably eliminates the infection. Studies on treatments with these drugs, alone or in combination, have shown that most treated animals cure clinically but remain carriers of the parasite and might relapse back to a clinical disease.
Actually, the drugs licensed in Europe specifically against CanL are meglumine antimoniate, miltefosine and domperidone (only in Spain and Italy). The combination of meglumine antimoniate (N-methylglucamine antimoniate, 75-100 mg/kg for 4 – 6 weeks, S.C.) with allopurinol (10 mg/kg twice a day for at least 6-12 months P.O.) or the use of miltefosine (2 mg/kg daily P.O, 28 days) in combination with allopurinol (the same dosage as in combination with antimonials) are considered the most effective therapy and constitutes the first line protocol against the disease.
Recently the use of domperidone (1 mg/kg, 1 month, P.O) for the treatment of dogs with subclinical disease has demonstrated good clinical results.
Other drugs, such as marbofloxacin (2 mg/kg once a day for 28 days P.O.), metronidazole (25 mg/kg) combined with spiramycin (150000 U) for 3 months P.O., have been evaluated for CanL therapy, but these have mostly been suggested as additions to be administered in combination with the core drugs or as a second-line therapy for dogs that do not respond well to other medicine.
Overall, the expected clinical response to treatment of sick dogs can vary from poor to good depending on their initial clinicopathological status and individual response to therapy. Dogs with renal insufficiency are expected to have a lower recovery rate in comparison to those without kidney compromise. The majority of dogs experience clinical improvement within the first month of therapy, although in others, a longer period of therapy is required before any apparent improvement. Serum antibody titres and serum protein alterations are expected to require a longer period of time before normalization.
The clinicopathological parameters to be monitored during treatment would depend on the individual abnormalities. However, in general, it is recommended to perform complete CBC, biochemical profile and urinalysis including urine protein/creatinine ratio in proteinuric dogs. The frequency of monitoring clinicopathological parameters would vary in each patient but, clinicopathological parameters should, in most cases, be monitored more frequently initially, i.e. after the first month of treatment and then every 3-4 months. Later on, if the dog is fully recovered clinically with treatment then a recheck would be recommended every 6 months or once a year.

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Miltefosine: a new and more modern treatment for Canine Leishmaniasis

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Canine Leishmaniasis was described for the first time in Europe in the early 1900s; despite this, the first veterinary drug to treat this important protozoan disease in canine species, Aminopharma® (Amminosidine), was not registered until the 1990s in Italy. Despite its excellent efficacy in vitro, the treatment did not find favour among veterinarians in clinical practice, mainly because of its high nephro- and ototoxicity.
The second drug registered, by order of time, for the treatment of this disease, was Glucantime® (N-methylglucamine antimoniate), the veterinary equivalent of the almost identically-named Glucantim®, used in human medicine and later in the veterinary field for more than 40 years.
In autumn 2007, Virbac, registered in some European countries the first veterinary drug based on Miltefosine (Milteforan®) for the treatment of Canine Leishmaniasis.
The development of this specific formula for use with dogs required more than ten years clinical and laboratory research and the use of several hundred dogs affected with Leishmaniasis, to carry out studies for dose determination, clinical evaluation and safety.
From the moment of its commercial release to the time of writing, 300,000 dogs in Europe have benefitted from the use of this drug which, above all because of its ease of administration and its excellent tolerability profile, immediately found favour with Veterinarians and dog owners.

Miltefosine:

The history of Miltefosine

Miltefosine was discovered at the Max Planck Institute in Gottingen by Professor Hans Eibl and Professor Clemens Unger. Initially selected for its antitumor activity it was then developed for use against human Leishmaniasis by Zentaris in cooperation with the WHO. Anti-*Leishmania* activity was described *in vitro* and *in vivo* for the first time in 1987, with activity against visceral Leishmaniasis with oral administration in murine models demonstrated in 1992. The first descriptions of clinical trials for the treatment of visceral Leishmaniasis in India date back to 1998, and Miltefosine (Impavido®) was registered for the treatment of human visceral Leishmaniasis in India in March 2002, and 2004 in Germany.
Miltefosine is the first effective anti-*Leishmania* agent registered for oral administration and Virbac, in partnership with Zentaris, has developed a specific formulation for veterinary use in Europe: MILTEFORAN® in liquid formulation for oral administration containing 2% of Miltefosine (20 mg/ml)

Mode of action

Miltefosine (chemical name: hexadecylphosphocoline) is phospholipid analogue, composed of esters with different long-chain saturated and unsaturated alkyl groups. It has been shown that Miltefosine is structurally similar to compounds metabolised by protozoa parasites of the *Leishmania* family.
Several studies show that Miltefosine exercises its anti-*Leishmania* effect by attacking the metabolism pathways of the phospholipids in the parasite. Miltefosine is able to penetrate the cellular membrane and cause rapid and intense metabolism of the phospholipid ethers in the *Leishmania* species.
Miltefosine also interferes with cell communication pathways and the synthesis of parasite cell membranes in the following ways:

- it inhibits the biosynthesis of GPI (glycosylphosphatidylinositol) receptors: a key molecule for the intracellular survival of *Leishmania* amastigotes
- it disrupts the transduction signal by acting on *Leishmania*-specific phospholipase C and proteinkinase C

Because of these effects on the mitogenic pathways of the parasite, Miltefosine leads to death by apoptosis of the protozoan cell.
Specific studies have shown that *L. donovani* (*L. infantum* , the etiological agent of Canine Leishmaniasis, forms part of *L.donovani* complex) is the most sensitive species, while *L.major* is the least sensitive species to the activity of Miltefosine, even though - as shown below, it is still sensitive to relatively low doses of Miltefosine.
Miltefosine, in contrast to antimonials, not only acts by directly killing Leishmania parasites, it is also able to activate some immune functions of the host (Croft & Engel, 2006). In fact it stimulates activation of macrophages or of T cells and the production

of metabolites of oxygen and nitric oxide (Baneth & Shaw, 2002). Also recently (Wadhone et al., 2009) it was shown that Miltefosine performs its anti-*Leishmania* functions by activating macrophages. In fact it has been observed that Miltefosine induces the production of IFN-γ by the macrophages. This production is greatly reduced in macrophages infected with Leishmania, but is significantly restored in the presence of Miltefosine. Miltefosine also promotes the action of important anti-*Leishmania* enzymes (p38MAP kinases) and the interleukin-12 dependent Th1 response functions. *Leishmania* infection in macrophages induces a Th2 type response but treatment with Miltefosine is able to modify such a response towards the Th1 type.

Pharmacokinetics

In laboratory animals and in the canine species extensive pharmacokinetic studies have been carried out with Miltefosine, which has been assayed in plasma, urine and faeces through the use of HPLC-MSMS analyses (high performance liquid chromatography coupled with tandem mass spectrometry).
These studies have shown:
Rapid and complete absorption after oral administration.
In rats and dogs Miltefosine showed an absolute bioavailability of 82% and 94%, respectively, with maximum concentration achieved in a time (Tmax) ranging from 4 to 48 hours.
Low plasma clearance
In dogs, after repeated oral administration of Miltefosine in food for 28 days, plasma clearance was 3.40 ± 0.447 ml / kg / h, corresponding to a body elimination rate of approximately 0.06% in a dog of 10 kg. This suggests that in dogs the metabolic efficacy of transformation of Miltefosine in different metabolites is scarce and there is no observed first-pass hepatic metabolism.
Half-life (t 1/2)
Half-life in dogs was approximately 153 hours (153 ± 13.7 h) equivalent to 6.3 days. This long half-life may be explained by the low plasma clearance of Miltefosine. Considering this prolonged half-life in dogs, a "dynamic equilibrium" can be expected to be achieved after about 3 - 4 weeks of daily doses of Miltefosine. In dogs, repeated administration of 2mg/kg/day of Miltefosine for 28 days in fact leads to an increased plasma concentration of the active principle within the first two weeks of treatment, with achievement of a dynamic equilibrium to the end of treatment (28 days). At the end of treatment there is a slow and linear decrease in Miltefosine plasma with complete elimination in a further 4 weeks.
Wide distribution in target tissues
Miltefosine is widely distributed in the body. The tissue distribution of Miltefosine was found in high doses in the kidneys, liver, spleen and skin, key organs in which the *Leishmania* amastigotes are located.
Metabolism
Miltefosine undergoes a slow metabolism in the liver to choline (a natural compound) and choline-containing metabolites.
Elimination pathways
Miltefosine is only partially excreted in the faeces. Only about 10% of the dose administered parenterally is eliminated in the faeces while the remaining 90% is, as stated, eliminated after extensive but slow hepatic metabolism. Urinary concentrations of Miltefosine are rather low and below the limits of quantification (20 mg / ml Miltefosine) showing that urine is in dogs a minor elimination pathway after oral administration of Miltefosine.
For this reason, Milteforan® can be selected for dogs with kidney failure. It is not necessary to adjust the dose with patients with renal failure and there should be no danger of overdose of Miltefosine in the case of dogs with renal failure treated with the registered dosage regimen.

Therapeutic protocols

Milteforan® should be used at a dose of 2 mg/kg sid for at least 28 days. It is important to remember that due to the long half-life of Miltefosine (approximately 6.3 days), the active principle administered for 4 weeks will remain active in the body of the patient for a further 4 weeks.
Based also on recent works (Miro et al., 2009), on the publication of the latest treatment protocols, on treatment guidelines and the WHO treatment recommendations, it is evident that the combined use of Milteforan® and allopurinol for the 28 days of treatment and then continuation with allopurinol alone for a further period of not less than 6 months (Ginel et al 1998). gives the best therapeutic results.

Clinical efficacy

The numerous clinical studies carried out for registration of the veterinary drug showed excellent efficacy of Milteforan® administered for 28 days, at a does of 2 mg/kg sid. The same studies show an efficacy equivalent to Glucantime® administered at a dose (recommended by the recent therapeutic guidelines) of 50 mg/kg bid (or 100 mg/kg sid) for 4 weeks. Use of the Real Time Quantitative PCR (RT-QPCR) showed that by the 28th day of treatment (end of the treatment period), both drugs were successful in reducing parasitic load by 95-98%. Although, thanks to the special kinetics

of Miltefosine, Milteforan allows further Leishmanicidal action for the 4 weeks following the end of the administration.

Tolerability

Gastrointestinal tolerability
Milteforan is usually well tolerated The only side effects verified during administration are of a gastrointestinal type and are dose correlated (they increase with increases in the dose / kg administered). Among these, the most frequent are vomiting and diarrhoea. All clinical trials performed with the use of Milteforan® have shown that gastrointestinal side effects are mild, transient and usually self-limiting.
These undesired side effects can occur at any time during treatment but usually occur between 5 and 10 days after the start of treatment with Milteforan®, and they last on average for 1 or 2 days. Administration of the product in the pet's food makes these side effects sporadic and they are only detected in less than 5% of treated animals (Miro et al., 2009)

Renal tolerability
The kidneys are potentially affected in all dogs with Leishmaniasis, and renal disease may be the only detectable pathology (Baneth et al., 2002, Costa et al., 2003) Chronic renal failure is a serious clinical manifestation of the development of the disease and is the leading cause of death in dogs with canine Leishmaniasis. Despite the high prevalence of kidney damage, increased levels of serum creatinine and urea, as a result of primary renal failure, becomes detectable only when a high percentage of nephrons are affected (>75%). Treatment with Glucantime® has sometimes been indicated as responsible for the deterioration in renal failure detectable in dogs during Leishmaniasis. Despite this, the available information on aspects of toxicology, pharmacokinetics and pharmacodynamics related to the use of this drug in dogs, is scarce and there are no specific guidelines for adjustment of the dosage of this drug in the course of renal failure. A study specifically carried out (Bianciardi et al., 2009 *Toxicol Pathol*), with Glucantime® and Milteforan® on healthy dogs (at standard recommended doses) showed that the impact on renal function and on the clinical condition of dogs subjected to Miltefosine treatment is extremely limited. Histological examination in these dogs in fact showed normal glomeruli and no damage under ultra-structural and immunofluorescence examination. Conversely, although without any clinical evidence of renal involvement, all dogs treated with N-methylglucamine antimoniate showed morphological changes consistent with severe tubular damage. Therefore, the pharmacological approach to therapy in dogs affected by Leishmaniasis, should be carefully evaluated especially in those subjects where there is already demonstrable renal damage.

Reproductive Tolerability
Milteforan® should not be used in pregnant bitches because toxicity studies have shown that Miltefosine has embryotoxic, and teratogenic foetotoxic effects. It is, in any case, a questionable choice to breed a dog suffering from Leishmaniasis. This disease is highly conditioned by the host immune status, and since pregnancy is an "immunosuppressant" physiological state it may cause or exacerbate symptomatic manifestations of the disease. Miscarriage is common in bitches affected with symptomatic Leishmaniasis.
However, Milteforan® can be used in breeding bitches between one litter and another.

Clinical follow-up

In spite of the different modes of action of Miltefosine, Vets can maintain their usual protocol for monitoring and follow-up. However, it is suggested that a first therapy check-up visit be planned for the end of the 28 day period to verify that the administration has been performed completely and correctly. Taking the pharmacokinetics of Milteforan® into account (8 weeks of action with 4 of administration) it is reasonable to conduct the second check-up around the 60th day.
Since some dogs can take several months to achieve a complete recovery or a noticeable improvement it is recommended that monthly visits are planned until complete resolution, or the best result for the particular case, is achieved.
Once cured, the dog should be rechecked every 6 months in endemic areas and at least every 12 months in non-endemic areas.
Response to treatment is obviously individual and subjective and should be evaluated individually based on the clinical evolution of the individual animal.
The use of 'Serum Protein Electrophoresis and of the A / G ratio, or of the serum antibody titre (IFAT), for the assessment of efficacy of therapy and prognosis, have been proposed over the past 20 years (Ceci et all. 1985; Bizzetti et all. 1989).
The publication, however, of more modern treatment protocols regarding Glucantime® and the introduction to the market of the new therapy based on Miltefosine, requires a review and reassessment of the parameters that were (and often still are) used to monitor the therapeutic efficacy of treatment and follow-up of dogs with Leishmaniasis The review of data from all studies conducted for comparison of efficacy of Milteforan and Glucantime indicates

how the A / G ratio tends, on average, over time, to normality in equal measure in both treatment groups. Based on the data obtained, the A / G ratio does not appear to be directly correlated with remission of clinical signs and, therefore, is neither a parameter indicative of the effectiveness of the therapeutic treatment nor a valid tool for determining the interruption of therapy.
Similarly, with regard to serum titres, several authors (Ginel et al.,1998; Solano-Gallego et al., 2001; Pennisi et al., 2000) have shown that there is no direct correlation between the serum titres at the moment of diagnosis and the severity of the clinical signs (Amusategui et al., 2003). Nor is there a correlation between the behaviour of serum titres and remission of clinical signs during treatment and follow-up (Mancianti et al., 1988). The humoral immune response during Leishmaniasis does not play a useful role in controlling the infection, and therefore a reasonable consequence is that the serum titres do not have a prognostic value
It therefore seems clear that both the A / G ratio and the anti-*Leishmania* antibody titre cannot be used to monitor progress achieved in dogs with Leishmaniasis or to determine their recovery.
Response to treatment should be evaluated on the basis of clinical examination (improvement or cure of clinical symptoms), haematobiochemical examinations (improvement or return to the normal range of parameters such as hematocrit, etc..) and improvement (assessed as tendency in time) of electrophoresis and the drastic reduction or disappearance of the parasite under microscopic examination of lymph node and/or bone marrow smears.
The future use of sophisticated techniques such as Real Time Quantitative PCR (currently restricted to experimental situations) may be of considerable importance in assessing the effect of treatment choice on the numerical value of Leishmania present in animals (bone marrow) before and after treatment.

Advantages

Milteforan®. offers several advantages over the current treatment option for canine Leishmaniasis (Glucantime®.):

- It is the first oral treatment administered in a single daily dose, which therefore allows greater ease of use than the two daily injections, which can be histolesive and / or difficult to perform, with a possible reduction of compliance by owners and consequent therapeutic failure.
- It has a precise treatment protocol which has been validated by many clinical evaluations, biochemistry, parasitology and comparative studies (Bianciardi P, et al., 2009 4th WorldLeish Congress Lucknow, India) unlike Glucantime® which still presents protocols and dosages that are not well-defined and, with particular reference to the treatment cycle, not scientifically proven.
- Miltefosine does not only act by killing the *Leishmania* parasite. It also stimulates the activation of macrophages and Th cells and the production of metabolites of oxygen and nitric oxide (Baneth & Shaw, 2002). Also recently (Wadhone et al., 2009) it was shown that Miltefosine performs its anti-*Leishmania* functions by activating macrophages and is able to modify the immune response from the Th2 type to the Th1 type.
- Clinical trials performed for registration showed a greater renal and hepatic tolerance with more stable parameters (creatinine, GGT). Milteforan® is not therefore contraindicated in animals with renal failure.
- A recent study (Bianciardi et al., 2009, *Toxicol Pathol*), with Glucantime and Miltefosine in healthy dogs (at standard recommended doses) showed that the impact on renal function and on the clinical condition of dogs subjected to Miltefosine treatment is extremely limited. Conversely, although without any clinical evidence of renal involvement, all dogs treated with N-methylglucamine antimoniate showed morphological changes consistent with severe tubular damage.
- Milteforan® has a different mode of action than antimonials and it therefore ideal for animals that react badly to treatment with those compounds, or dogs which have undergone previous treatments that were ineffective or dogs for whom a future rotational therapy is planned, and even in those dogs where treatment with Glucantime® has previously been effective.

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New developments in prevention – what do I need to know about the new vaccine?

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Canine leishmaniosis (CanL), resulting, in the Mediterranean area, from infection with *Leishmania infantum* was described over 100 years ago. Since then much work has been done to try to understand the extent of the problem, and it is now estimated that 2.5 million dogs are infected in South-Western Europe alone, with the area affected spreading North.

With an understanding of the severity of this problem for dogs, and more especially since the discovery of the role the dog plays as a reservoir for human infection, significant effort has been devoted to obtaining a better understanding of the disease with a view to establishing the best means of prevention.

It has become clear in recent years that, although this disease is caused by infection with a parasite, it is in fact primarily an immunological disease. The key clinical signs are caused by an inappropriate, excessive humoral response, resulting in immune complexes which lead to many of the classic signs such as skin ulcers, uveitis, arthritis and of course finally to renal failure. In addition, the progressive rise in the parasite load experienced during progressive active infection is due to the predominance of Th2 cytokines acting on macrophages and therefore stimulating the alternative metabolism of L-Arginine to polyamides (the key nutrient source for the parasite during its intracellular stage) instead of to the leishmanicidal nitric oxide that is promoted by Th1 cytokines. It is therefore clear that the key underlying factor that, firstly, permits an initial infection to become progressive and uncontrolled, and secondly also causes a lot of the pathology of the disease itself is the inappropriate shift of the immune response away from a protective Th1 profile and towards a Th2 profile. It is for this reason that in recent years several authors have proposed that an effective vaccine will be the most effective control strategy for this disease.

The recent availability in the market of CaniLeish® (Virbac) provides this possibility to the veterinary profession. CaniLeish® is based on the excreted secreted proteins of *L. infantum* (LiESP), which are produced using the patented invention of the Institut de Recherche pour le Développement relating to a cell-free serum-free culture system, and the adjuvant QA-21.

During this session we will share some data obtained during the development of CaniLeish® vaccine showing the way in which it induces a specific Th1-dominated memory response against *L. infantum*, referring to both *in vitro* studies and also to an *in vivo* challenge model.

The effects of vaccination with CaniLeish® on the immune responses of dogs were first studied *in vitro*. Briefly, it was shown that the T lymphocytes of dogs vaccinated with CaniLeish® proliferate on exposure to *L. infantum* antigens, demonstrating a specific memory response. It was also shown that these proliferating lymphocytes are capable of generating IFN-γ, a key marker of a Th1 profile. Finally, it was shown that these lymphocytes are also capable of stimulating *L. infantum*-infected autologous macrophages to reduce the parasite load *in vitro*, and that this leishmanicidal activity correlated with induction of inducible nitric oxide (NO) synthase, and the production of NO derivatives. This confirms that the mode of action is consistent with the currently understood mechanisms by which macrophages kill *Leishmania* during protective immune responses.

The good in vitro results confirming the correct immune profile after vaccination provided the basis for proceeding to an experimental challenge model. In this study, naïve dogs were either vaccinated (using a vaccine containing 10% less antigen than a normal commercial dose as is common for studies for regulatory purposes) or kept as unvaccinated controls (n=10 for each group). One year after the vaccination, no annual booster vaccination was given but instead the dogs were challenged by an intravenous dose of infectious *L. infantum* promastigotes ($10^{8.5}$ parasites per dog). At the end of the 47 week follow-up period, there was a significant decrease in the number of PCR positive dogs in the vaccinated group (30%) compared to the controls (80%) using a kinetoplast qPCR technique on bone marrow samples (p=0.0246). Two of the vaccinated dogs which became both PCR and culture positive after the massive intravenous challenge returned to a PCR negative state by the end of the 47 week period. This was never seen in the control group.

These results then permitted progress to a natural challenge study which will be discussed in another session in this symposium.

Until now, the only methods available to reduce the risk of a dog developing this disease have been measures to reduce its level of exposure to the parasite. Nevertheless, taking into account the key role played by an inappropriate (Th2 dominated) immune response

in both permitting active progressive infection and also in the production of symptoms during the later stages of active infection, it is clear that finding a means to alter this to a favourable (Th1 dominated) specific response must be the basis of any ideal prevention programme. With a disease such as this where there are no options which can provide 100% protection, the optimal strategy for the future almost certainly lies in an integrated approach. The recent availability of a proven vaccine on the European market provides a rational basis for significantly reducing the risk of developing this disease for an individual dog, and will be an essential foundation in an optimal prevention plan when discussed with owners. As with all vaccines, this does not eliminate the need to take reasonable precautions to limit the level of challenge received by the immune system. In the case of CanL, this means that the continued use of repellent products during the sandfly transmission season will be complementary to the fundamental benefit provided by vaccination.

The availability of this new tool also provides us with a means to deal with some previously unanswerable problems as part of a global prevention strategy. For example, modification of the immune response is also the only means available to provide continued protective benefit outside the sandfly transmission season, when a high proportion of dogs in endemic areas will still be carrying at least a low parasite burden.

Evidence for protection against active infection and disease progression in naïve dogs vaccinated with LiESP/QA-21 (CaniLeish®) exposed to two consecutive *Leishmania infantum* transmission seasons

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AIM: Canine leishmaniosis is a sandfly-borne protozoan infection widespread in the Mediterranean basin, where *Leishmania infantum* is endemic. A chronic progression of the infection results in fatal disease in those dogs susceptible to the parasite, approximately 40% of infected animals. In the development of anti-*Leishmania* canine vaccines, efficacy evaluation requires that two outcomes are taken into account, anti-infective capacity (= protection from initial infection) and the ability to control acquired infections (= protection from progressive disease). Considering the chronic nature of leishmaniosis, frequent monitoring of challenged dogs is thus required during an appropriate period of time. Because an experimental *Leishmania* challenge cannot completely replicate the effects of natural acts of transmission by infected sandflies, a natural-challenge model was developed to test the efficacy of LiESP/QA-21 vaccine (CaniLeish®; Virbac, France) in endemic sites in Italy and Spain with known elevated intensity of parasite transmission.

MATERIALS AND METHODS: Vaccinated and unvaccinated (control) naïve dogs were exposed to natural transmission in open-air kennels over 2 consecutive transmission seasons. A large set of laboratory and clinical parameters was evaluated every 3 months, from month 9 post vaccination through month 24. Any infection detected was ascribed to one of 3 stages as established by previous longitudinal studies - subpatent infection (only transient or steady positive bone-marrow PCR), asymptomatic active infection (no clinicopathological signs, positive bone-marrow/lymph-node PCR and culture, and increasing antibody titres) and symptomatic active infection (parasitological findings as above, in association with clinicopathological signs).

RESULTS AND CONCLUSIONS: By the end of the study (month 24) a *Leishmania* PCR positive status was recorded in 26/39 (66.7%) control dogs and in 20/41 (48.8%) vaccinated dogs, with no significant difference between groups (p=0.16). These high infection rates on one hand confirm the elevated intensity of *Leishmania* transmission in the study sites and on the other hand show that LiESP/QA-21 vaccine did not protect dogs from initial infections. However, the infection stages detected differed significantly between the 2 groups. Active infections were recorded in 13 control (33.3%) versus 5 vaccinated dogs (12.2%) (p=0.025); furthermore, overt clinical disease associated with active infection developed in 9 control (23.1%) versus 3 vaccinated dogs (7.3%) (p=0.046). These findings indicate that the vaccine decreased the risk of developing progressive infections, potentially leading to fatal disease, by about four fold in natural conditions of high intensity of transmission.

SIMPOSIO 3

LA DICROCELIOSI NEGLI ANIMALI DA REDDITO: DIAGNOSI, TERAPIA E STRATEGIE DI CONTROLLO

Dicrocoelium dendriticum: epidemiological characteristics of a parasite of ruminants

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INTRODUCTION: Dicrocoelioses are parasites infections, caused by the species of the genus *Dicrocoelium* (Trematoda, *Dicrocoeliidae*), with a widespread presence among grazing ruminants. The most important species of this genus which infect ruminants are *D. dendriticum* (Rudolphi, 1819), *D. hospes* (Looss, 1907), *D. chinensis* (Tang *et al.*, 1983), *D. suppereri* (Hinaidy, 1983), but only *D. dendriticum* is frequently present in several countries such as Europe, Asia, North Africa and North America.

Dicrocoeliosis, commonly named "small liver fluke" disease, is poorly known and often underestimated by researchers and practitioners due to the multiple parasitic infections, fasciolosis in primis, which affect grazing ruminants and mask its pathology and to the difficulties in diagnosing it with coprological techniques (Otranto and Traversa, 2002).

Instead the economic and health significance of dicrocoeliosis isn't unimportant and is partly due to the direct losses occasioned by the confiscation of altered liver and to the indirect ones caused by the digestive disorders derived from the hepatobiliary alterations such as decreased animal weight, growth delay, reduced milk production. Moreover, the additional costs incurred by the application of anthelminthic treatments have to be considered (Manga-Gonzalez *et al*., 2001).

D. dendriticum can be also occasionally zoonotic; the human infection, without exhibiting symptoms, takes place through accidental ingestion of infected ants but it is common the presence of spurious infection after eating raw or undercooked liver of parasitized herbivores (Cabeza Barrera *et al.*, 2011).

The life cycle of *Dicrocoelium* spp. is extremely complex with land molluscs and ants as first and second intermediate hosts and ruminants as definitive hosts; consequently, the epidemiology of this parasitosis is influenced by presence, biology and ethology of the molluscs and ants populations, meteorological factors, soil types, vegetation, existence of definitive hosts (domestic or wild) receptive to the parasites, farming model, animal handling.

In this paper, in light of the latest information to understand the several factors influencing the diffusion of the parasitosis, the most important aspects of the epidemiology of dicrocoeliosis, produced by *D. dendriticum* are reviewed.

LIFE CYCLE: The life cycle of *D. dendriticum* takes about 6 months. The adults of the parasite live in the liver and bile ducts of definitive hosts where lay their embryonated eggs (containing a miracidium). Egg hatching and miracidium liberation occur in the intestine of numerous species of land molluscs. In snails, larval stages evolve in about 3-4 months by asexual multiplication, developing from miracidia to first and second generation sporocysts.

Numerous cercariae are formed from sporocysts and when they are well developed they migrate to the respiratory chambers of the mollusc where are covered in slime.

The cercariae are extruded from the snails in clusters of at least 5000 enveloped in "slime balls" which are ingested by different species of ants. The cercariae cross the craw of the ants, lose their tail and one of them, called the "brainworm", settles in the suboesophageal ganglion of the ant. The cercariae become metacercariae in the abdomen in 1-2 months. When the temperature falls, the brainworm alters the behaviour of the ant by causing tetania of its mandibular muscles and blocking the ant on herbage and grass. This promotes ingestion by the definitive host.

In the grazing ruminants the metacercariae excyst from the ants, due to the action of duodenal enzymes, and the young flukes migrate through the choledocous and the gall bladder to the large bile ducts and then to the small bile ducts where they become adult worms. When they are mature, they reproduce by hermaphroditism and lay eggs which exit in the faeces with a prepatent period of about 2.5 months. The environmental contamination allows the life cycle to begin again (Manga-Gonzalez *et al*., 2001).

EPIDEMIOLOGICAL ASPECTS: Dicrocoeliosis is present, with various species, worldwide in lowland or mountain pastures. It is considered a typical sheep and goat's parasitosis, with prevalence of up 100% in many European and Eastern countries but is common also in cattle, wild ruminants such as camelids in South America, yaks and buffaloes in India (reviewed by Otranto and Traversa, 2002).

Dicrocoeliosis by *D. dendriticum* is reported as the ovine helminthiasis more widely spread also in Italy even if available bibliographic data are very scanty (Table 1). The most of these data, regarding regions of Central and South Italy, were collected and summarized by

Ambrosi (1995).
As a whole, the regions of Central Italy showed high values of positivity either for animals (74-99% of sheep) or for sheep flocks (65-100%). Higher prevalence was seen in adult animals with a worm burden/liver of 3000-10000 helminthes.
More recent the data reported by Cringoli *et al*. (2002, 2003) in ovine farms of the Southern Italian Apennines and in the Latium region and those referred by Venditti *et al.* (2010) in a survey on sheep endoparasitosis in the regions of Umbria and Marche.
The prevalence was studied also by serological methods: in a work conducted in Sardinia, Sanchez-Andrada *et al*. (2003) detected seropositivity in 86.2% tested sheep whereas a faecal prevalence of 6.7%.
The parasitosis is common also in grazing cattle with values for breeding up to 10% in Sardinia, 61% in Latium, 54% in Umbria, 93% in the Marche, 28% in Apulia, 23% in North Italy (Ambrosi, 1995).
Cringoli *et al.* (2002) reported a prevalence of 53.1% for bovine farm in Southern Italian Apennines.

Table 1. Prevalence of dicrocoeliosis for sheep and flocks in various regions of Central-South Italy (necropsy or coprological examination)

Regions	% sheep positive	% flocks positive	References
Tuscany	74		
Umbria	99		
Marche	91		
Abruzzo	82		Ambrosi 1995
Apulia	57		
Sardinia	53		
Sicily	50		
Southern Apennines		67.5	Cringoli 2002
Latium	30	57.7	Cringoli 2003
Umbria		80	Venditti 2010
Marche		30	

In the past 50 years, many studies have been carried out to clarify the various factors conditioning the epidemiology of dicrocoeliosis.
Eggs passed in the pastures are highly resistant and may over-winter and remain infective for months and years (up to 20 months) sheltered inside of the faeces. The egg elimination in sheep faeces is related to the season with peak during winter (January-March) to favours the spring infection of molluscs after the hibernation period and there is no relationship between infectivity of eggs and their age (Alunda *et al*., 1983).
The environmental and ecological factors are very important because influence the reproduction and survival of the intermediate hosts; in particular, permeable soils (calcareous) with a pH alkaline are favourable biotopes. This fluke is the only parasite showing a marked heterogeneous distribution for the link existing between the parasitic occurrence and the presence of suitable biotopes (alkaline soils) for the terrestrial snails.
The climatic influence and in particular the temperature- humidity binomial can be significant: cold and rainy intermediate seasons decrease the vitality of intermediate hosts and the cycle development.
An higher summer dryness can provoke in the molluscs an aestivation period while short rains can favour the emission of the slime balls.
More than 100 mollusc species have been found receptive to *D. dendriticum* under natural and laboratory conditions. Some of them are present worldwide while others are only regional such as *Cernuella virgata* in Italy (Fasanella *et al*., 1995).
Studies conducted on snails of the genus *Helicella* (Schuster, 1993; Manga-Gonzalez *et al*., 2001) found that the population structure of these molluscs showed fluctuations with young specimens (shell diameter 3-6 mm) more abundant in spring (April-June) while the adults were more so in autumn (shell diameter 9-10 mm) and the largest snails (shell diameter 13-15 mm) in the spring of the following year.
Active snails were recorded in spring, September and October and molluscs withdrawn into their shells, but without an epiphragm, in every month of the year. Molluscs with an epiphragm were observed mainly in summer and winter corresponding with the aestivation and hibernation periods.
Infection prevalence (IR) increased with their age and shell diameter; the young molluscs in the first year were less involved in the epidemiology (IR 3.77%) than the adults (IR 34.77%) perhaps owing to their active metabolism and good nutritional conditions for the developing sporocysts. There is no unanimity amongst the Authors about the dynamics of the mollusc infection either for the different species, age and nutritional state or for the different environmental conditions or for the time in which contamination of the pastures by grazing animals takes place. In areas where animals graze throughout the year, infected molluscs where found in almost every month of the year.
On the whole, all the molluscs found infected from the beginning of the year until the end of the spring were infected the previous year, although at different times according to the degree of the development observed in the parasites.
The molluscs that harboured scarcely developed sporocysts from the end of summer until December must have been infected in spring or at the beginning of summer, while those containing well developed cercariae in the same period could be infected at the beginning of spring or even in the previous year.
Various species of ants act as second intermediate host. Studies con-

ducted (reviewed by Manga Gonzalez *et al.*, 2001) reported that the number of metacercariae per ant varied among the different species and within the same one for various factors: time of the year (higher in summer); different affinity for slime balls; type of vegetation (in general the choice depended on relative abundance); size of the abdomen of the different ants (greater in those with large abdomen) because the number of metacercariae is proportional to the space available; possible ecological and behavioural causes.

The metacercariae can remain in the abdomen of the ants for a year or more without affecting its survival.

The survival of the metacercariae in hibernating ants play an important role in the epidemiology of dicrocoeliosis. Infact, considering an hibernation period from mid-October to February, the first parasitized ants will be present in the pasture beginning from March. The number of the infected ants will increase on the basis of the dynamic of the mollusc infection before reported.

The changes in behaviour of the infected ants, due to the brain worm in the suboesophageal ganglion, are regulated by fluctuation in the ambient temperature, due to which the availability of metacercariae to the grazing animals has a circadian rhythm. The tetania occurs when solar intensity and temperature decrease at the end of the afternoon and disappears in the morning when insolation and temperature increased. However, cloudy or warmer days favour the tetania also at the end of the morning and beginning of the afternoon with temperature of 17-20 °C.

With regard to the cyclic nature of the parasitosis, also the animals show an increase in the parasite burden from September to December (with an egg output reaching the higher values in February-March due to the coming of sexual maturity by the adult forms) and a progressive decline from June to August.

The prevalence increases with the animal age but in greatly contaminated areas young animals put out to first pasture can show an helminth burden very high; there is a correlation between parasite burden and egg output. Stress inducing factors, such as animal transportation and confinement, proved to enhance egg production for the induced immunodepression in animals. Also the migratory period of the transhumance seems to predispose animals to infection either for the high stress or the presence of intermediate hosts in the new pastures (Sotiraki *et al.*, 1999).

CONCLUSIONS: In the past 50 years many biological aspects of this parasite have been broadly investigated included the complex epidemiological model and the economic significance due to the consequent underproduction syndromes have been reported. Even so dicrocoeliosis is still underestimated as showed by the little available data on its diffusion in Italy. This lack of information makes difficult the formulation of suitable surveillance plans either prophylactic or therapeutic.

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Dicrocoeliosis in ruminants: physiopathological aspects

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Dicrocoeliosis is a widespread disease of grazing ruminants caused by *Dicrocoelium dendriticum* in Europe, Asia, North Africa and North America and *D. hospes* in sub-saharan Africa. These parasites live in the bile ducts and gall bladder of domestic and wild ruminants (sheep, goats, cattle, buffaloes, roe-deer…) and occasionally in rabbits, pigs, horses and humans. Compared with fasciolosis, dicrocoeliosis is responsible of mild symptoms in affected animals; however, it causes severe economic losses due to liver function impairment.

It is difficult to determine clearly the pathogenic effects of *D. dendriticum* since it is difficult to reproduce the experimental infections required to define its pathogenicity. Moreover, field studies comparing production traits between anthelmintic treated and non treated animals in the same flock cannot distinguish effects provoked by the small liver fluke alone and effects produced by other internal parasites such as gastro-intestinal nematodes or liver fluke (*F. hepatica*).

The young small liver flukes are migrating directly up the biliary duct system of the liver without penetrating the gut wall, liver capsule or liver parenchyma as in fasciolosis. By this way, the pathogenic effects of *D. dentriticum* are clearly related to the presence of adult parasites in bile ducts only. Establishment of small liver flukes elicits a hyperplasia of biliary epithelium and an infiltration of eosinophils, macrophages and lymphocytes in the vicinity of bile ducts. The presence of a stylet inside the oral sucker could partially explain this hyperplasia. The severity of the microscopic lesions seems to be correlated with the number of parasites. Interestingly, a proliferation of bile ducts as well as atrophy and fibrosis of parenchyma are frequently observed in heavily infected animals likely due to partial obstruction of bile ducts by the accumulation of parasites. Sometimes, a suppurative inflammation of bile ducts is shown consecutive to the “inoculation” or transportation of bacteria until the fine bile ducts during the migration of young *D. dendriticum*. Macroscopic lesions include liver hypertrophy, induration, fibrosis and presence of whitish spots on the surface. Marked distension of bile ducts is also commonly found in sheep and cattle infected by *D. dendriticum*. Blood concentrations of hepatic enzymes such as aspartate aminotransferase, alanine aminotransferase and gamma-glutamyl transpeptidase are commonly increased as well as the values of plasmatic globulins. These changes are related to the parasite burden.

IgG antibody responses to excretory-secretory or somatic antigens of *D. dendriticum* have been detected using the ELISA test in experimentally infected lambs. An early serological detection of small liver fluke infection is possible at least from 30 days post-infection (p.i.), reached a peak at 60 days p.i. and remained high until 180 days p.i. However, no correlation between the antibody level and parasite burden could be established. In the field, lambs and ewes are infected in the same proportion and severity suggesting that no efficient immunity is acquired with age.

Copromicroscopic diagnosis of dicrocoeliosis: what's new?

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Dicrocoelios by *Dicrocoelium dendriticum* is a very important parasitic infection that affects the liver of domestic and wild ruminants, and occasionally rabbits, pigs, dogs, horses and humans. Dicrocoeliosis is often underestimated (undetected or undiagnosed), most likely because of its subclinical nature. *D. dendriticum* diagnosis in animals is mainly performed by recovering adult parasites in the liver at necropsy or by detecting eggs at coprological examination. Faecal egg count techniques, such as the McMaster and the Wisconsin flotation techniques in the veterinary field, and the Kato-Katz technique and the ether-based concentration method in the human field have been available for many years, but their sensitivity and efficiency are very low. New multivalent techniques for quali-quantitative copromicroscopic diagnosis of parasites, including *D. dendriticum*, in animals and humans, are now available, namely the FLOTAC and Mini-FLOTAC techniques. Some preliminary studies to compare, in terms of sensitivity and efficiency, different copromicroscopic techniques for detecting and counting *D. dendriticum* eggs in faecal samples from cattle and sheep, showed that the *Mini-FLOTAC basic technique* is a rapid and promising technique and can be used in place of the FLOTAC techniques, the "Gold standard", in laboratories where the centrifugation step cannot be performed.

Dicrocoeliosis by *Dicrocoelium dendriticum* is an important parasitic infection that affects the liver of domestic and wild ruminants, and occasionally rabbits, pigs, dogs, horses and humans. *D. dendriticum* is reported in America, Asia, North Africa, and Europe; in southern Italy - as well as in other Mediterranean regions - this parasite is the most widespread liver fluke found in cattle, buffaloes, sheep and goats with farm prevalence values up to 100% (Cringoli et al., 2002; Rinaldi et al., 2009; Musella et al., 2011). Its occurrence is related to dry and calcareous or alkaline soils, which represent favorable biotopes for its intermediate hosts (Manga-González et al., 2001). This infection is very important from an economic and health point of view. The economic significance of infection by *D. dendriticum* is due to the direct losses occasioned by the confiscation of altered livers and also to the indirect ones caused by the digestive disorders that influence negatively the animal production, as well as by the cost of anthelminthic treatments (Keiser et al., 2010). Dicrocoeliosis is an underestimated parasitic infection, in fact it often remains clinically undetected or undiagnosed, most likely because of its subclinical nature, but reports of dicrocoeliosis are increasing in animals and humans (Otranto and Traversa, 2003; Jeadron et al., 2010).

D. dendriticum diagnosis in animals is mainly performed by recovering adult parasites in the liver at necropsy or by detecting eggs at coprological examination. Flotation techniques using jodomercurate-based or zinc-sulphate-based solutions (specific gravity = 1.350 and 1.450, respectively) perform better than sedimentation techniques (Rehbein et al., 1999).

Faecal egg count (FEC) techniques are considered relatively straightforward and protocols such as the McMaster techniques and the Wisconsin flotation technique in the veterinary field, and the Kato-Katz technique and the ether-based concentration method in the human field have been available for many years (Cringoli et al., 2010). It is important to note, however, that the afore mentioned techniques have shortcomings, particularly in low-infection intensity settings. For example, the McMaster technique - of which there are at least three variants (MAAF, 1986) - has an analytic sensitivity of 50 EPG for the *modified McMaster method* and the *modified and further improved McMaster method* or 10 EPG in the case of the *special modification of the McMaster method*. Therefore, even the highest analytic sensitivity for McMaster is inadequate for rigorous parasitological diagnosis. Additionally, as reported by Cringoli et al. (2004), the reliability of the McMaster techniques is influenced by the choice of reading area (volume) and so it seems reasonable to say that where larger multiplication factors are needed for extrapolation, for example, under the smaller McMaster slide areas (volumes), the less precise eggs per gram (EPG) of faeces counts will result.

Similarly, in humans, the small amount of faeces examined using the Kato-Katz technique (usually 41.7 mg) underlies its low analytic sensitivity of 24 EPG. In human parasitology, the sensitivity of the Kato-Katz method is further compromised by the time delays from fresh fecal sample production, collection in the field and processing in the laboratories, and rapid over clearing of the eggs. With regard to the Wisconsin technique, when the number of eggs is high, inefficiencies may arise due to the lack of precision in the egg counting procedure sowing to the absence of a grid on the coverslip. Shortcomings of the ether-based concentration method in-

clude fire and explosion hazard, and the method is qualitative rather than quantitative (Cringoli et al., 2010).

New multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites, including *D. dendriticum*, in animals and humans, are now available, namely the FLOTAC and Mini-FLOTAC techniques. The *FLOTAC techniques* are multivalent, copromicroscopic techniques that use the FLOTAC apparatus and are based upon the centrifugal flotation of a faecal suspension and the subsequent translation of the apical portion of the floating suspension. The FLOTAC apparatus is a cylindrical-shaped device which comprises three physical components, namely the base, the translation disc and the reading disc. There are two 5-ml flotation chambers, which are designed for optimal examination of large faecal sample suspensions in each flotation chamber (total volume = 10 ml). There are two versions of the FLOTAC apparatus: FLOTAC-100, which permits a maximum magnification of ×100 and FLOTAC-400, which permits a maximum magnification of ×400. Either FLOTAC-100 or FLOTAC-400 can be used for performing the three *FLOTAC techniques (basic, dual and double*), which are variants of a single technique but have different applications and permit an analytic sensitivity up to 1 egg, larvae, oocysts and cysts/ gram of faeces. The 11 basic operating steps of the three *FLOTAC techniques* are: (1) weigh the sample, (2) add water, (3) homogenize, (4) filter, (5) transfer into the tube, (6) centrifuge the tube at 1,500 r.p.m. × 3 min, (7) discard the supernatant, (8) fill the tube with flotation solution to its previous level, (9) fill the two FLOTAC flotation chambers, (10) centrifuge the FLOTAC apparatus at 1,000 r.p.m. × 5min, (11) translate the apical portion of the flotation chambers and examine under a microscope. Different studies have recently showed a higher sensitivity and efficiency of the *FLOTAC dual technique* than *simple flotation* and *special modification of the McMaster method* for detecting *D. dendriticum* eggs in sheep (Rinaldi et al., 2011). In addition, the *FLOTAC dual technique* showed also higher sensitivity and efficiency than Kato-Katz and ethyl acetate concentration methods to detect *D. dendriticum* eggs in humans (Jeadron et al., 2010; Gualdieri et al., 2011).

Mini-FLOTAC is a new apparatus, a further development and simplification of the FLOTAC, which comprises two physical components, namely the base and the reading disc. There are two 1-ml flotation chambers, which are designed for optimal examination of faecal sample suspensions in each flotation chamber (total volume = 2 ml) and which permits a maximum magnification of ×400. Mini-FLOTAC can be used for performing the three *Mini-FLOTAC techniques (basic, dual and double)*, which are variants of a single technique but have different applications. The seven operating steps of the *Mini-FLOTAC techniques* are: (1) weigh the faecal sample (2 g for dogs, cats and humans + 2 ml of formalin 5%; 10 g for herbivores); (2) add the flotation solution (FS) using a dilution ratio of: (a) 1:20 for dogs, cats and humans with an analytic sensitivity of 10 parasitic elements per grams (PEG = eggs, larvae, oocysts and cysts); (b) 1:10 for herbivores (analytic sensitivity = 5 PEG); (3) homogenize; (4) filter; (5) fill the two Mini-FLOTAC flotation chambers; (6) wait 10 min; (7) translate and examine under a microscope.

Some preliminary studies (Cringoli G, unpublished data) have been conducted in order to compare, in terms of sensitivity and efficiency, different copromicroscopic techniques for detecting *D. dendriticum* eggs in faecal samples from cattle and sheep, using a zinc sulphate based flotation solution (FS7, specific gravity = 1.350) for all the techniques. The results of these studies showed that the *Mini-FLOTAC basic technique* (MFBT) produced very similar values of sensitivity and efficiency compared to the *special modification of the McMaster method.* Furthermore, the MFBT showed higher sensitivity and efficiency than the *simple flotation in tube* and the Wisconsin technique, whereas its sensitivity and efficiency were lower than those resulted by the *FLOTAC dual technique*. This preliminary studies suggest that the MFBT is a rapid and promising technique for detecting and counting *D. dendriticum* eggs in faecal samples from cattle and sheep, and can be used in place of the FLOTAC techniques, the "Gold standard", in laboratories where the centrifugation step cannot be performed.

In recent years, immunological and molecular techniques have been developed for the diagnosis of dicrocoeliosis in animals, as alternatives of coprological examinations and post-mortem inspections of the liver. Immunological tests, such as the enzyme-linked immunosorbent assay (*ELISA*), have been studied thoroughly to increase their stability, sensitivity, specificity and to reduce costs of these indirect immunological diagnostic tools. Several types of tests aimed at the detection of specific antibodies during (or after) a *D. dendriticum* infection are now available, including the indirect *hemagglutination assays (HA), indirect fluorescent antibody tests (IFAT)*, and tests based on the ELISA. These tests use different extracts from parasites, such as adult extracts, secretory substances, or egg antigens, and have the advantage of being applicable during both the acute and the chronic stages of the infection (Keiser and Utzinger, 2009).

In the past few years, *various PCR-based methods* have been also developed and improved to detect *D. dendriticum* DNA in stool samples or metacercariae in the intermediate hosts. These DNA-based techniques offer high sensitivity and specificity, even at low infection intensities, compared to the direct parasitological and in-

direct immunological diagnostic tools. However, *PCR* diagnosis is unlikely to become a tool for routine diagnosis, as investments and the costs involved are high, but the approach remains useful as a research tool (Lovis et al., 2009) in particular for species identification to discriminate between *D. dendriticum* and other similar species as *D. hospes* (Maurelli et al., 2007).

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Control and treatment of *Dicrocoelium dendriticum* in small ruminants

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Netobimin represents an important tool in the control strategy of *Dicrocoelium dendriticum*. In sheep, a single administration of 20 mg/kg bw *per os* results in 96.4% efficacy 14 days after treatment (Veneziano V. et al, SOIPA XXIV, Parassitologia 48, 202-206).
Netobimin is a very effective molecule and also very manageable, since its therapeutic or safety index is 20 (M. Ambrosi, 1995). The pro-benzimidazole molecule of Netobimin is metabolized in the rumen into the active compounds Albendazole (Abz) and Albendazole sulfoxide (Abzo) which are finally inactivated in the liver and intestine in Albendazole sulfone ($ABZO_2$). The mode of action is related to the close link with tubulins in the cells of nematodes, which impairs the glucose transport and leads parasites to death. To be more effective the treatment in the flock should be "strategic", before the lambing period in February-March and during late lactation in July-August, when animals host the *Dicrocoelium*. As a post-treatment precaution, sheep should be housed into the pens for at least 24 hours, the surfaces of the barns should be cleaned and disinfected and then the flock should be moved on a "safe" pasture. A further precaution is the periodic "change" of grazing areas in the pasture.
The treatment, associated with an improvement of flock management, e.g. the restriction of grazing in the early morning, allows an effective parasite control and a significant limitation of the economic losses related to the disease.

SIMPOSIO 4

PROTOZOI DI

INTERESSE MEDICO

New test for the detection and molecular characterization of *Acanthamoeba*

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AIM: *Acanthamoeba* (*A*) provoke eye, brain and skin diseases. Numerous limitations have been reported for the detection of *Acanthamoeba* by classic and real-time PCRs. In addition, the *Acanthamoeba* characterizations by molecular techniques are time consuming and require expensive reactants. We developed a new High Resolution Melting real-time PCR (HRM) to detect *Acanthamoeba* and characterize in one run and without probes *Acanthamoeba* profiles.

MATERIALS AND METHODS: The sequence of the primers selected from the mitochondrion, -out of the gene coding for ribosome-, were: forward 5'GCAGTCGCGGTAATACGA; reverse 5'ACCACCTACGCACCCTTTACA. Serial dilutions of different strains from the American Type Culture Collection and corneal scrapings were tested.

RESULTS: DNA mixed with a solution containing primers and the intercalating SYTO9 (stocheometrically binds to neo-formed double-stranded DNA) allows detection of 0.1 cyst/ml or less (including the genotype T5 and T11), providing simultaneously for each strain consistent molecular signatures according to the GC content and the size of the amplicons. In less than 2.30 h after DNA extraction the melting shape analysis allowed detection and drafting 4 *A* profiles: Type I: genotypes T2 and T4; Type II: T5 and T7; Type III: T8, and Type IV: T1, T3, T6, T9, T11, T12 and T13. High loads of *Bacteria*, *Fungi, Herpes simplex viruses, Adenoviruses* or human cells did not modify HRM performances. Compared to classic PCRs this new HRM has the advantage of minimizing risks for false positives due to cross contamination, because the amplification, signal detection and DNA analyses are carried out in closed tubes. In addition, HRM minimizes risks for false negatives because the yields of extraction of DNA and the potential PCR inhibitors are monitored for all the samples.

CONCLUSIONS: HRM appears as a new, simple, rapid, sensitive, inexpensive and specific tool for the detection and simultaneous molecular characterization of *Acanthamoeba*, which obviates gel electrophoresis, hybridizations and immunoassays. It just requires upgrading the real-time PCR equipment software used routinely in microbiology laboratories.

Identification of novel inhibitors of *Acanthamoeba* species

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Acanthamoeba species can cause severe disease in the eye (*Acanthamoeba* keratitis) in immunocompetent individuals, normally through incorrect contact lens usage. However, to date contact lens solutions do not eliminate *Acanthamoeba* contamination and current treatments for AK are limited and not completely effective. We have been able to examine a number antimicrobial agent targets in. *Acanthamoeba* species. Potential targets were confirmed through PCR-assisted amplification of their genes from trophozoites in combination with information from the *Acanthamoeba* genome projects. Known inhibitors of those targets confirmed were tested for their ability to arrest *Acanthamoeba* growth with the use of a colorimetric assay. We now know that *Acanthamoeba* possess a number of biochemical pathways that are not present in the human host and therefore provide a significant rationale target identification in *Acanthamoeba* species.

Biological characterization of free-living amoebae isolated from cases of amoebic keratitis in Sassari

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The cosmopolitan and ubiquitous free-living amoebae (FLA) are amphizoic protozoa with the ability to exist both as free-living organisms in nature and as parasites within host tissues. Numerous reports indicate that some FLA can cause infections in humans.
Several species of *Acanthamoeba* are the causative agents of amoebic keratitis, a corneal infection mainly associated with contact lens use.

AIM: Given the lack of data on the occurrence of pathologies caused by FLA in our geographical area, we have studied cases of suspected amoebic keratitis in patients admitted to the Institute of Ophthalmology, University of Sassari, Sassari, Italy, from January 2008 to January 2010. Patients with eye infections were classified on the basis of epidemiological data, clinical manifestations, the evolution of the disease, response to pharmacological treatment and recurrence of infection. Axenic cultures obtained from amoebae isolated were used to study their phenotypic and genotypic features.

MATERIALS AND METHODS: Corneal scrapings from non- and soft contact lens wearers with suspected *Acanthamoeba* keratitis at an early stage (presence of dendritic epithelial lesions and/or diffuse punctate epitheliopathy) were plated on Petri plates, containing 1.5% agar in Page's amoeba saline (PAS). Whenever possible, patients' contact lens solutions and samples of the water used for domestic purposes were also analyzed. Amoebic isolates were identified morphologically and by polymerase chain reaction (PCR) analysis with primers P-FLA-F/P-FLA-R (generic for FLA), JDP1/JDP2 (specific for *Acanthamoeba*) and JITS-F/JITS-R (described for vahlkampfiid species).
All patients were treated with PHMB 0.02% (SIFI S.p.A., Catania, Italy) and propamidine isetionate 0.1% eye-drops.

RESULTS: Three genera of FLA (*Acanthamoeba, Hartmannella* and *Vahlkampfia*), were identified by microscopy examination. One patient had mixed *Hartmannella/Vahlkampfia* keratitis. PCR analysis with primers FLA yielded 800 bp amplicons (typical of *Hartmannella*) in seven cases. PCR analysis with primers JDP1/JDP2 yielded 450 bp amplicons (specific for *Acanthamoeba* T4 genotype) in 1 case and 550 bp amplicons in 2 cases (*Acanthamoeba* non T4). Both these PCRs not provided amplification products for *Vahlkampfia* isolates. Works are in progress to identificate they by amplification of ITS and 5.8S rDNA sequences using primers JTS-F/JTS-R.
All patients soft contact lens wearers had amoebic contamination of their care solutions. It is cause for great concern that we found contamination with *Acanthamoeba* in some unopened 10-ml bottles of disinfecting solutions containing polyquaternium-1 (result confirmed by the Istituto Superiore di Sanità, Rome, Italy). Treatment with PHMB 0.02% and propamidine isetionate 0.1% was always successful. Some patients non contact lens wearers had amoebic contamination of their domestic water or frequented swimming pools.

CONCLUSIONS: Our results provided evidence that the ability to cause corneal disease may not be restricted to *Acanthamoeba* genus. Early diagnosis and proper anti-amoebic treatment are crucial to yield a cure. The causes of this cluster of amoebic keratitis in soft contact lens wearers is still under investigation. Preliminary data suggest exposure to contaminated tap water in some cases and contamination of contact lens solutions in others. The comparative evaluation of the pathogenicity of these isolates on human corneal and congiuntival cell lines are in progress. This study will be useful to acquire epidemiological data and promote suitable prophylaxis intervention with regards to these emerging pathogens in our geographic area.

Acanthamoeba castellanii stimulates the production of IL-6, IL-12 and TNF-alpha by murine macrophages

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Acanthamoeba castellanii is an opportunistic, facultative parasitic protozoa known to be the agent of a serious painful potentially blinding keratitis and fatal encephalitis in humans. *Acanthamoeba* is ubiquitous and approximately 85% of humans are seropositive implying that protective immunity is the most common outcome of infection. However, how this is achieved is not currently known. Herein, we establish the kinetics of TNFα, IL-12 and IL-6 production by murine macrophages, following challenge with trophozoites of the *Acanthamoeba castellani* (Neff Strain). The addition of protease inhibitors to cultures increased the levels of cytokines detected. Further studies demonstrated that cytokine levels were considerably lower in macrophage cultures exposed to a clinical isolate of *A. castellanii* (T4 genotype), from a bilateral keratitis than with the Neff strain.

Studies are underway to determine: (i) the molecular mechanism responsible for *Acanthamoeba*-induced cytokine production and (ii) if differences in protease production and therefore proteolytic cleavage of cytokines is responsible for these observed disparity of cytokine levels in macrophage cultures with the 2 *Acanthamoeba* strains.

These results demonstrate that macrophages release pro-inflammatory cytokines, which can be attenuated by *Acanthamoeba*-derived proteases. The balance of production of these cytokines with their digestion by *Acanthamoeba*-produced proteases could determine efficacy of the immune system to resolve infection.

Future studies will focus on the ability of *Acanthamoeba castellanii* to elude the immune response and the identification of the receptors involved in the recognition of *Acanthamoeba castellanii*.

Blastocystis sp.: a world yet to be discovered

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Blastocystis sp.: a polymorphic microorganism presenting an as-yet overlooked life cycle

Blastocystis sp. is an anaerobic parasite that inhabits the intestinal tract of humans and a wide range of animals and is responsible for frequent community infections (Tan 2008). This anaerobic protozoa was only recently unambiguously placed among the Stramenopiles (or heterokonts), a complex and heterogeneous assemblage that includes diatoms and green and brown algae (Silberman et al. 1996; Arisue et al. 2002). Interestingly, *Blastocystis* sp. is the only Stramenopile known to cause infection in humans, which suggests a recent evolutionary adaptation to parasitism in this microorganism. At the morphological level, four major forms of *Blastocystis* sp. have been described in stools and/or *in vitro* cultures: vacuolar, granular, amoeboid, and cyst forms (Tan 2008; Suresh et al. 2009). The infectious vacuolar form is the most common form and allows the identification of the organism by microscopic examination of stool while the environmentally-resistant infective cyst represents the transmissible stage of the parasite. The granular and amoeboid forms likely represent intermediate stages between the two main forms described above. The main mode of transmission of *Blastocystis* sp. is the fecal-oral route by consuming water and food contaminated with cysts.In the hypothetic life cycle proposed by Tan (2008), upon ingestion of cysts, the parasite undergoes excystation in the large intestine and develops into vacuolar forms. Subsequently, the encystation may occur during the crossing along the colon before cyst excretion in the feces and transmission to another host.

Laboratory diagnosis of *Blastocystis* sp.

Blastocystis sp. is mostly diagnosed by direct-light microscopy of fecal smears and formol-ether concentration techniques. However these methods greatly underestimate the prevalence of *Blastocystis* sp. parasites compared to short-term *in vitro* cultures (Leelayova et al. 2002; Suresh et smith 2004). Nevertheless, xenic *in vitro* cultures is a time-consuming diagnostic method, and some STs present a slow growth rate under culture conditions (Stensvold et al. 2007a diagn microbial infect dis). Several nonquantitative PCR assays targeting the SSU rDNA gene to detect and discriminate between *Blastocystis* sp. isolates directly from stool samples have previously been developed (Scicluna et al. 2006). These assays allow both the identification and the genotyping of isolates. After extraction of total DNA from the stool, a fragment of the SSU rDNA gene is amplified by PCR with a pair of primers specific for the genus *Blastocystis*. This amplification product can be directly sequenced or cloned before sequencing of a limited number of positive clones. Recently, Poirier et al. (2011) have reported a highly-sensitive real-time quantitative PCR (qPCR) assay developed to detect *Blastocystis* sp. in stool samples and allowing subtyping of isolates by direct sequencing of qPCR products. Interestingly, in a prospective study including 186 French patients, these authors have shown that direct-light microscopy and xenic *in vitro* stool culture analysis showed only 29% and 52% sensitivity, respectively, compared to the qPCR assay (Poirier et al. 2011).

Blastocystis sp.: the most common intestinal parasite reported in human populations.

Numerous epidemiological surveys carried out in different countries identify *Blastocystis* sp. as the most common eukaryotic organism reported in human fecal samples (Boorom et al. 2008; Tan 2008; Stensvold et al. 2009a). In general, developing countries have higher prevalence of the parasite (30 to 60%) than developed countries (5 to 10%). This difference can be explained by poor hygiene practices, close animal contact, and consumption of contaminated food or water (Eroglu et al. 2010). Overall its prevalence is by far higher than those of other intestinal protozoa such as *Giardia*, *Entamoeba* and *Cryptosporidium* (Boorom et al. 2008).

Clinical significance of *Blastocystis* sp.

The pathogenic potential of *Blastocystis* sp. was widely debated in the literature during the last two decades because the microorgan-

ism can be found in asymptomatic patients (Tan 2008). However, this parasite has been implicated in various gastrointestinal aspecific symptoms such as diarrhea, abdominal pain, flatulence, and vomiting and may also play a significant role in several chronic gastrointestinal illnesses such as irritable bowel syndrome (IBS) (Boorom et al. 2008; Poirier et al. 2012). Numerous cases were also reported regarding the association of *Blastocystis* sp. infection and urticaria (Katsarou-Katsari et al. 2008). In addition, *Blastocystis* sp. has increasingly been implicated for diarrheal illness in immunocompromised individuals including HIV/AIDS and cancer (Tan et al. 2009).

Genetic diversity among *Blastocystis* sp. isolates

Blastocystis sp. isolates from humans and other animals have been reported to be morphologically indistinguishable. However, extensive genetic divergence among *Blastocystis* sp. isolates has been revealed by molecular phylogenies inferred from small subunit (SSU) rDNA gene sequences (Noël et al. 2005). These studies confirmed the low host specificity of the parasite and its zoonotic potential. From these molecular analyses, a consensus on *Blastocystis* sp. terminology was proposed in an international collaborative project (Stensvold et al. 2007b). In this new classification, all human, mammalian and avian isolates should be designated *Blastocystis* sp. and assigned to one of nine STs (ST1-ST9), each of the STs exhibiting sufficient genetic diversity to be classified as separate species. Thereafter, four new STs (ST10 to ST13) were identified from zoo animals (Stensvold et al. 2009b; Parkar et al. 2010). It is reasonable to argue that other STs remain to be identified and the genetic diversity of this genus is still largely underestimated.

STs identification in human and animal populations and zoonotic potential of the parasite

In almost all the epidemiological studies reported so far, a large majority of human infections with *Blastocystis* sp. were attributable to ST3 isolates (worldwide average above 60% according to Souppart et al. 2009 and above 50% according to Forsell et al. 2012). Collectively, these studies suggest that the dominant ST3 was the only ST of human origin as was first proposed by Noël et al. (2005). Consequently, the predominance of this ST might be mainly explained by large-scale human-to-human transmission. Outside the ST3, other STs are of animal origin and for the most able to infect humans because of their zoonotic potential (Noël et al. 2005; Stensvold et al. 2009b; Parkar et al. 2010). The few studies that have addressed ST-dependent differences in pathogenicity of isolates from symptomatic and asymptomatic individuals (Hussein et al. 2008; Souppart et al. 2009; Stensvold et al. 2011) have provided conflicting results mainly due to the limited number of patients examined in each epidemiological survey. Therefore, broad epidemiological studies have to be performed to correlate *Blastocystis* sp. ST with patient symptomatic status. Regarding the animal population, ST6 and ST7 are prevalent in birds, ST5 and ST10 in cattle, ST1 and ST5 in pigs, ST2, ST3 and ST10 in sheep and finally ST4 in rodents. ST10 is the only ST which has never been found so far in humans. A higher risk of *Blastocystis* sp. infection has been found in people with close animal contact including zookeepers and abattoir workers. It is therefore necessary to identify potential sources of environmental and animal contamination to humans. Most of the samples included in published epidemiological surveys represented simple infections with *Blastocystis* sp. However the subtyping methods likely underestimate the prevalence of mixed infections with more than one *Blastocystis* sp. ST. It has been shown in various studies (Tan 2008; Souppart et al. 2009) that the prevalence of mixed infections with two different STs could exceed 10% of samples surveyed. Recently, we have identified several isolates belonging to three STs in a French patient considered at high risk of mixed infection through her lifestyle in rural area and long history of travelling suggesting multiple potential contamination sources (Meloni et al. 2012). In the context of molecular epidemiological studies, patients at risk of mixed infection should be identified in order to optimize the genotyping approach.

Molecular epidemiology of *Blastocystis* sp. in Italy

In Italy several studies have been carried out to identify the prevalence of intestinal parasitic infections in different area and Italian populations. In all the surveys, *Blastocystis* sp. is the most common parasite found in the populations studied (Masucci et al. 2011; Gualdieri et al. 2010; Guidetti et al. 2009) and in some cases can reach 52,5% of the total of infected patients. Due to its prevalence in this country, we determined the *Blastocystis* sp. STs and their relative frequency in symptomatic patients living in the vicinity of two Italian cities (Rome and Sassari) (Meloni et al. 2011). To our knowledge, this is the first large investigation into the molecular genotyping of human *Blastocystis* sp. isolates in Italy. Stool samples were collected from a total of 30 Italian patients classified as symptomatic for the presence of various gastrointestinal troubles. In this analysis, a total of 34 isolates, corresponding to 26 single and 4 mixed infections were subtyped using SSU rDNA gene sequencing. From this molecular approach, the ST distribution in the present Italian population was as follows: ST3 (47,1%), ST2 (20,6%), ST4

(17,7%), ST1 (8,8%) and ST7, and ST8 (2,9%.). In parallel, Mattiuci et al. (2010) had performed the genotyping of 7 human isolates obtained in the Rome area. These isolates were classified as ST1, ST5, and ST7. Consequently, together with our data, a total of seven STs (ST1, ST2, ST3, ST4, ST5, ST7, and ST8) have been identified in Italian symptomatic patients. This wide range of STs in Italy suggests that *Blastocystis* sp. infection is not associated with specific STs even if some subtypes are predominant. Moreover, since most of the STs are zoonotic, our data also raise crucial questions concerning the identification of animal reservoirs for this parasite. Interestingly, the ST distribution in Italy is quite similar to that found in other countries of the Mediterranean area.

Contribution of genomics to understanding the biology of *Blastocystis* sp. and developing new technological tools

One of the significant advances in the knowledge of this parasite is the recent sequencing of the genome of a *Blastocystis* sp. ST7 isolate (Denoeud et al. 2011). Briefly, *in silico* analyses of the predicted proteome and secretome identified proteins potentially involved in the adhesion to host cells and pathogenicity. These genomic data will also enable the development of comparative genomics approaches. Recently, we obtained a financial support from the company Genoscreen (Lille, France) for the genome sequencing of 4 isolates belonging to STs 1, 4, 6 and 8. These four genomes are currently assembled. The goal of this project is to characterize a core set of genes for all STs and to identify genes which are ST-specific and could be involved in transmission to different hosts and/or virulence. These genomics data will also allow the development of microarrays approaches (DNA chips) for transcriptomic analyses as well as the development of molecular genetics tools applied to *Blastocystis* sp. to address functional studies. A strategy for gene silencing may be to use RNA interference (RNAi) since the RNAi machinery involving different components such as DICER and RISC (RNA-induced silencing complex) are present in the *Blastocystis* sp. ST7 genome.

Potential virulence of *Blastocystis* sp.

During the last two decades, it was widely debated in the literature whether *Blastocystis* sp. is a truly pathogenic or commensal organism, since it can also be found in asymptomatic patients. Accumulating recent *in vitro* and *in vivo* studies together with genomic data shed new light on the pathogenic power of this parasite and a hypothetic model for pathogenesis of this parasite was proposed (Poirier et al. 2012). Studies have shown that this parasite can induce apoptosis of epithelial cell in a contact-independent manner, increasing epithelial permeability (Puthia et al. 2006). This alteration in the epithelial barrier function might play an important role in the pathogenesis of this parasitic infection. Cysteine proteases, identified as virulence factors in other parasitic protozoa including *Entamoeba* and *Giardia* (Sajid andMcKerrow 2002), were isolated in *Blastocystis* sp. (Wawrzyniak et al. 2012) and can induce the production of proinflammatory cytokines. They are also able to cleave human-secreted IgA (Puthia et al. 2005). These two latter mechanisms are able to modulate the immune response of the host especially in the first days of infection. Serine proteases may also be involved in the pathogenesis of the parasite by activating PAR-2 receptors on the surface of intestinal cells, inducing a change in intestinal permeability by altering cell junctions and an inflammation by diffusion of bacteria, proteases and antigens. Hydrolases could also damage the intestinal mucosa, which would be a source of nutrients for the parasite. Finally some proteins such as polyketide synthases, known to produce polyketides which may have an anti-microbial activity and protease inhibitors could together induce a modification of lumen microbiota (dysbiosis) and consequently intestinal troubles. It is interesting to note that several pathophysiological mechanisms could be common to blastocystosis and IBS suggesting a link between these two pathologies.

Treatment of blastocystosis

Treatment of *Blastocystis* sp. infection remains a complicated issue. Because there is still a great deal of debate about the true pathogenicity of *Blastocystis* sp., there is still much debate about the need for treatment. Recently Coyle et al. (2012) recommend that individuals who have gastrointestinal or dermatologic signs and symtoms and many cysts in stool specimens may require treatment. To date, a number of antimicrobial agents have been used for treatment of *Blastocystis* sp. infection but metronidazole is considered first-line treatment.

Conclusion

The high prevalence of *Blastocystis* sp. in the human population, its zoonotic character, its potential pathogenicity and its possible link with a chronic intestinal disease such as IBS that affects a large population especially in industrialized countries, have greatly increased interest of the scientific and medical communities for this parasite. This has been emphasized by the recent explosion in the number of publications on this organism, the creation of the *Blastocystis* Research Foundation in the United States, and the addition

of *Blastocystis* sp. in the list of waterborne parasites by the World Health Organization.

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Trichomonas vaginalis pathobiology

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Trichomonas vaginalis is the causative agent of the most widespread non-viral STDs (WHO, 2001; Van der Pol, B., 2007). The infection is associated with higher susceptibility to HIV transmission (Galvin S.R, 2004; Mason P R, 2005), cervical and prostatic cancer (Nanda N, 2006; , 2009), pelvic inflammatory disease, and increased risk for preterm delivery during pregnancy (Cotch MF, 1998). Interestingly, treating *T.vaginalis* reduces HIV mucosal shedding (Kissinger P, 2008), and control of protozoan infection represents one strategy to reduce HIV transmission. Effective treatments of trichomoniasis is limited to the use of metronidazol or related compounds, but resistance has been reported (5%-10% of infections) (Upcroft P, 2001) and is likely to become an increasing problem based on current trends in other pathogens.

Because acute trichomoniasis is often undiagnosed and the eradication of drug-resistant *T.vaginalis* is very difficult, a large number of infections evolves into a sub-acute disease, accompanied by chronic inflammation. Interestingly, it has been established that neoplastic transformation can be promoted or exacerbated by host inflammatory response: indeed, chronic inflammation orchestrates a tumor-supporting microenvironment, leading to the development and progression of cancer, explaining the strict relation between chronic trichomoniasis and tumors.

A major problem in developing innovative therapies and vaccines to control trichomoniasis, is the peculiar ecological niche of infection. Local defences are influenced by hormonal variations during the menstrual cycle and vaginal mucosa and have to face invading pathogens but at the same time, must be tolerant to "good antigens" such as spermatozoa, and foetus. In these conditions, immune responses must be tempered to reduce the risk of surrounding tissues damage. Moreover, immune system relation with *T.vaginalis* is further on gets complicated by the presence of a concomitant microbial flora: even if normal vaginal flora normally does not stimulate local inflammation, co-infecting pathogens can induce a robust inflammation, influencing the fate of *T.vaginalis* infection. We have demonstrated that *Mycoplasma hominis* stably infects *T.vaginalis*, establishing a symbiotic relationship, the only described between two human pathogens. (Rappelli P, 1998; Dessì D, 2005). Interestingly, *M.hominis* lipoprotein is a TLRs ligand, well known to stimulate a massive inflammation (Buwitt-Beckmann U, 2005).

A successful *T.vaginalis* infection could be summarized in three crucial steps: 1. colonization of mucosal surfaces; 2. production of cytolitic molecules; 3. modulation of host pro-inflammatory response. Strategies to design effective therapies should include the control of each single step: the block of a single event could not be sufficient to stimulate a full protection and a total clearance of infecting pathogens.

The early step of infection is adhesion to host cells and/or tissues; by the consequence, the inhibition of this process could protect from infection. So far unique human receptor for Tv, galectin-1, was recently identified when investigating the role of *T.vaginalis* lipophosphoglycan in binding to ectocervical cells lines. The sequencing of the *T.vaginalis* genome has shown that this protist has a very large genome, twice the size of that of its human host (Carlton JM, 2007). Moreover, data from the genome project strongly suggest that the protist possesses a number of candidate surface adhesins (Hirt RP, 2007; Noël CJ, 2010). Among them, our group have focused the attention on the largest gene family (911 entries) encoding surface proteins, known as BspA-like proteins. These proteins display a specific type of leucine rich repeat, a typical domain expressed in mucosal bacteria in which it mediates binding to host tissues, and stimulates innate response (Hirt RP, 2011). There is very little knowledge about modulation of expression of BspA-like, a mechanism known to facilitate maintenance of infections. A second interesting group of surface molecules is represented by M60-like/PF13402 domain; these molecules are also expressed by a number of mucosa-associated prokaryotic and eukaryotic microorganisms. This novel domain is characterized by a typical zinc-metallopeptidase motif, and seems play an important role in colonization of mucosal tissues of mammals (Nakjang S, 2012).

T.vaginalis interactions with the host tissues and its pathogenicity are considered to be complex and multifaceted with adhesion to the various mucosa landmarks (mucus, epithelial cell barrier, extracellular matrix) and modulation of innate and adaptive immune cells. All these aspects are essential to initiate and maintain the infections and leading to *T.vaginalis* cellular differentiation into aggressive amoeboid forms and to the killing of epithelial cells, al-

lowing invasion of the mucosa and access to the extracellular matrix.

The second stage of infection is the production of toxic molecules and the subsequent tissue damage. We hypothesized that toxic effect was mediated functional pore insertion into cell membrane (Fiori PL, 1993; Fiori PL, 1996), associated with disruption of membrane cytoskeleton (Fiori PL, 1997). The same cytolitic strategy has been reported for Entamoeba and Naegleria, able to produce amoeba- and naegleria-pores, molecules belonging to the Saplip (Saposin-like proteins) a protein family that includes molecules well knows for their ability to interact with lipids (Munford RS, 1995; Leippe M2005). Amoebapore has been proposed as putative vaccinogen (27). Recently, a total of 12 so-called TvSaplip genes have been identified in the genome of Tv. Preliminary data showed that *TvSaplip* expression is upregulated upon contact with human RBCs. These data represent a clue to which TvSaplips are more likely to function as trichopores. In addition, we demonstrated that TvSaplip are compartmentalized in intracellular granules, as for pore-forming-proteins produced by other protozoa.

The third step is the modulation of inflammation; even if *T.vaginalis* stimulates maturation of Dendritic Cells (DC), it is also able to kill DCs, downregulating immune response. Notably, when stimulated by *T.vaginalis*, DCs secrete IL10, but not IL-12, indicating the protist counterbalances excessive immune responses, as for other "chronic" microorganisms (28, 29). *T.vaginalis* relation with immune system is complicated by concomitant Mh infection: some data indicate that symbiotic strains stimulate macrophage to secrete proinflammatory cytokines, but, at the same time, Mh-parasitized *T.vaginalis* isolates cope with massive pro-inflammatory response by producing IL-10, which limits collateral damage, but allowing the pathogen to persist. At the same time, other concomitant microorganisms can strongly influence the host immune response, playing an active role in immune protection during *T.vaginalis* infection. Such very complex scenario, seems indicate that the protozoa avoid massive tissue destruction and consequent robust inflammation by establishing of a subtle balance between pro- and anti-inflammatory stimuli. These immunological adjustments are the basis of the evolution to a chronic infection: once established, chronic infections are refractory not only to vaccine-induced immunity, but also to anti-protozoan therapies.

During infection, Trichomonas establish very complex relationship with the human host, mainly focused to escape from immune response: by the consequence the block of a single step of infection by host immune response could not be sufficient to stimulate an effective protective reaction. Hence it is important to fully comprehend host-pathogen interactions and allow the design of new strategies to interfere with virulence, with pathogen adhesion and secreted factors (e.g. toxins) being recognized as important virulence factors in other systems.

This complex picture well explains the difficulties encountered by in studying strategies to control and prevent the infection: only understanding biology of pathogen and mechanisms involved in control of infection, are fundamental to design effective therapies.

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Genotyping of *Cryptosporidium* spp. isolates from different Tanzania areas

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AIM: *Cryptosporidium* spp. are a major cause of diarrhea in depressed areas mainly affecting children and HIV-infected individuals. The infection is self-limiting in immunocompetent hosts, but can be severe in immunocompromised and malnourished persons. Parasite genotyping is a valuable method to assess epidemiological features of *Cryptosporidium* isolates from a wide geographical origins.

MATERIALS AND METHODS: One hundred fifty-seven stool samples were collected from: *i*) 112 HIV-positive children residing at the Mission of Children's Hospital and Research Institute Bambino Gesù (OPBG) of Rome, "Villaggio della Speranza", Dodoma, Tanzania; and *ii*) 45 primary school children living in the district of Chake Chake in the Island of Pemba. Each stool sample was spotted onto FTA ELUTE Micro Card papers and, when possible, also analyzed for *Cryptosporidium* positivity by Ziehl-Neelsen (ZN) microscopy *in loco*. The DNA was extracted from dried fecal spots (DFS) by both automatic (AE) (Biorobot EZ1 DSP, Qiagen) and manual extraction (ME) (QIAamp DNA stool mini kit, Qiagen) and by only ME (QIAamp DNA stool mini kit) from the 112 Dodoma and 45 Pemba samples, respectively. All 157 samples were assayed by GP60-based PCR amplification, sequencing and probing against NCBI non-redundant databases (BLASTN algorithm).

RESULTS: In Dodoma, 85/112 (76%) samples resulted negative, 21/112 (19%) positive and 6/112 uncertainly positive (5%) by ZN microscopy. However, the rate of positivity was reduced to 13/112 by a second round of microscopy in Rome. GP60 amplification yielded products of expected size for 28/112 ME and 15/112 AE Dodoma samples, while for Pemba samples the assay is currently under investigation. Seven/28 amplicons from ME Dodoma samples were successfully genotyped revealing IIaA15G2R1 and IIaA16G2R1 subgenotypes, while for the remaining 21/28 samples the clean-up of PCR products and a sequencing condition upgrading is still in progress to fulfill genotyping list.

CONCLUSIONS: These preliminary GP60-based data show an higher sensitivity of the molecular assay compared to the microscopy. Therefore, the molecular DFS-combined method herein described may represent a valuable point-of-care supporting procedure for the diagnosis of pediatric cryptosporidiosis in depressed areas and an useful survey tool to subgenotype lineages and to elucidate genetic richness of *Cryptosporidium* spp. in children.

Pigs as natural hosts of *Dientamoeba fragilis* genotypes found in humans

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AIM: *Dientamoeba fragilis*, a flagellated protozoan, is one of the most common parasites of the intestinal tract of humans. The infection is highly prevalent in both economically developing regions and industrialized countries. Very little is known about transmission routes and the natural host range of this parasite (Barratt JL et al, 2011, Parasitology, 138:557-572). Other than humans, very few animal hosts of *D. fragilis* have been reported. Surveys of mammals and birds have identified only non-human primates (gorilla, macaque and baboon) as natural hosts. Recently, however, a high prevalence of infection (43.8%) has been reported in breeding and fattening pigs of Italy using microscopy (Crotti D et al, 2007, Vet Parasitol, 145:349-51). In the present study, molecular techniques were employed to detect and characterize the parasite from fecal samples collected from pigs and pig farmers.

MATERIALS AND METHODS: During June-August 2010, a total of 152 fecal samples were collected from the rectum of piglets (age: 1-3 months, weight: 6-25 kg), fattening pigs (3-4 months, 25-50 kg) and sows (1-2 years, 180-250 kg) raised in 6 farrow-to-finish, 2 fattening and 1 weaner indoor farms of central Italy (7 in the Umbria region and 2 in the Marche region). Pig samples from 7 of the 9 farms were available for molecular analysis. Twenty-one fecal samples from pig farmers were collected in 5 of the 9 farms, and 17 samples were available for molecular analysis. Microscopical diagnosis of *D. fragilis* was based on visualization of pleomorphic trophozoites, ranging in size from 4 to 20 µm, with fragmented chromatin and pale grey-blue finely vacuolated cytoplasm after Giemsa staining. DNA was extracted directly from fecal material using a commercial kit. A TaqMan real-time PCR assay was used as a diagnostic tool. Next, a fragment of the 18S rRNA gene, as well as the internal transcribed spacer 1 (ITS1) region, were amplified by PCR and sequenced. The sequences were assembled using SeqMan II, and compared with those available in public databases using BLAST.

RESULTS: The microscopic examination revealed that 52 of the 74 piglets, 11 of the 14 fattening pigs, and 8 of the 64 sows were positive for *D. fragilis*, whereas of the 21 samples from pig farmers, 4 from farmers working on two farms, were positive. Molecular techniques were applied to 38 pig fecal samples, namely 24 microscopically positive samples from 6 farms and 14 microscopically negative samples from 2 farms, and to 17 human fecal samples from 5 farms of which 4 were microscopically positive. Using a TaqMan real-time PCR assay that targets the 5.8S rRNA gene, all 24 microscopically positive pig samples were amplified, with cycle threshold (Ct) values ranging from 30 to 34, whereas none of the microscopically negative samples were positive to this assay. Of the 17 human fecal samples, 13 were positive with Ct values ranging from 29 to 40. The sequence analysis of the 5.8S rRNA gene from 15 amplified products (11 from pigs and 4 from humans) revealed 100% homology with *D. fragilis* genotype 1. Genotype 2 was not found in any of the samples from pigs or humans. Amplification and sequencing of a 366 bp fragment of the 18S rRNA gene confirmed the presence of genotype 1 in 6 pig samples and in 8 human samples, and indicate a very limited genetic polymorphism in this gene. Finally, the analysis of the more variable ITS1 region indicate that the genotypes found in 2 pig samples are identical to genotypes previously found in humans. A direct comparison of parasite isolates from pigs and pig farmers from the same farm was, unfortunately, not possible.

CONCLUSIONS: Considering the size of the world's pig population (more than 1 billion), the close contact between pigs and humans in many parts of the world, and the difficulties in the proper management of pig fecal waste, the role of these animals as reservoirs of zoonotic pathogens must be carefully evaluated. Here, we demonstrate that pigs are host of *Dientamoeba fragilis* based on molecular analysis of three fragments in the ribosomal cluster. Sequencing of fragments of the 18S and 5.8S DNA revealed genotype 1 in both human and pig isolates collected in the same farm, suggesting the potential for zoonotic transmission of this parasite. Characterization of the more polymorphic ITS1 locus also revealed that pigs harbor genotypes previously found in humans, but the specificity of this assay is limited, particularly when other flagellates are present in stools. If a transmittable cyst stage of *D. fragilis* exists, then environmental contamination with pig feces should be considered as an important factor in the transmission of this parasite. This work was supported by a research grant from the Ministry of Health (IZSUM 16/09 RC).

Trypanosoma cruzi in blood donors: a study started at the Umberto 1° teaching Policlinic in Rome

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Chagas disease (the "hidden" disease) is an anthropozoonosis due to the protozoon *Trypanosoma cruzi.* The parasite is endemic to portions of Mexico, Central and Southern America, where it is estimated to infect 16-18-million people. In rural areas human infections are acquired primarily by exposure to feces released by a triatomine insect vector during the course of a blood meal, whereas in urban areas human-to-human transmission by transfusion, organ transplant, or vertically, from mother to child, represent the most important ways to become infected.
The migration of millions of people from *T. cruzi*-endemic countries in non-endemic areas, occurred during the past decades, focused the attention of the *T. cruzi*-free countries (where triatomid vectors are absent) on the possible "urban" transmission routes, particularly by transfusion. In fact mainly apheresis or whole blood–derived platelets, including leukoreduced and irradiated products, have been recognized at the origin of infection for recipients from serologically positive Latino-American donors. There is no evidence of transmission by red blood cells (RBCs) or frozen products, whereas transmission by whole blood transfusion remains a possibility (Benjamin et al, Transfusion 2012, doi: 10.1111/j.1537-2995.2011.03554.x).

AIM: In Italy, in order to avoid nosocomial infections, at the Umberto 1° Policlinic in Rome we started selective for *T. cruzi* infection testing of Latino-American blood donors and, recently, also of Italian people whose anamnesis reports travels in endemic areas.

MATERIALS AND METHODS: All blood donors are screened for past history of Chagas disease through medical history questioning before donation. Donors who respond positively are deferred from donation; those ones who respond negatively are enrolled in programmed controls.
A blood sample (7 mL) is drawn from each subject: it is partly additioned to heparin and partly used to obtain serum. Sera are submitted to two (three in case of conflicting results) immunological tests: a rapid immunocromatografic test (ICT Chagas Ab, effegiemme diagnostici, Nerviano, Italy) and an ELISA (Bioelisa Chagas assay, Biokit, Lliçà d'Amunt, Spain and/or NovaLisa Chagas ELISA test, Nova Tec Immunodiagnostica, GmbH, Dietzenbach, Germany). Positive results are deeply investigated by means of molecular diagnostics applied to the donor blood (nested PCR: primers TCZ 1-2/TCZ 3-4 and sequencing of the amplicons).

RESULTS: A total of 96 people have been to date examined (mean age 45 years, 87 of them coming mainly from Perù, Argentina, Venezuela and Colombia, whereas the remaining 9 were Italian voyagers). Overall seroprevalence to date evidenced is 3.1%, with 3 donors confirmed positive among the 96 at-risk donors studied; one is coming from Colombia, and 2 are from Rome donors: one of them travelled in Messico (during year 2009), the other worked in Messico and Brazil (till 2011). The blood of the last donor proved PCR-positive and presented a very low parasitemia.

CONCLUSIONS: Preliminary results, based on the analysis of few Latino-American donors and, what's more, mainly coming from countries where Chagas disease has -currently- low prevalence due to the donor control programme performed, are quite alarming. In fact, serological findings overcome that reported in USA (0.026%: Custer et al, Transfusion 2012, doi: 10.1111/j.15-37-2995.2012.03569x) and in Spain (0.62%: Piron et al, Transfusion 2008; 48:1862-1868), and Italian voyagers proved source of possible transmission even more important. Therefore, in non-endemic countries these results emphasize the need of *T. cruzi* screenings in at-risk blood donors, including in this category people who have resided in (but were not necessarily born in) an endemic region. Finally, an accurate pre-donation interview for the proper identification of at-risk donors, have to be considered of fundamental importance.

Sporadic cases of cryptic malaria (2009-2011) and the risk of local vector-borne transmission in Italy

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AIM: The malaria outbreak occurred in Greece in the summer 2011 is paradigmatic of the increasing public health threat about malaria re-emergence, that mainly involves the south Mediterranean European countries where the disease was endemic up the 1960s' (Takken W, Knols BGJ. Emerging pests and vector-borne diseases in Europe, Wageningen Academic Publishers; 2007). Aim of this paper is to prevent about the possibility that isolated cases of autochthonous malaria, as well as of other emerging mosquito borne diseases, may reappear in areas where potential vectors and environmental condition favourable to the event exist. Hereby, we report two cases of "cryptic" malaria occurred in the last three years in Central-Southern Italy that, despite the incomplete result of the epidemiological inquiries, are strongly suspected to have been transmitted by our main potential malaria vector *Anopheles labranchiae.*

MATERIALS AND METHODS: Mainly aimed to find the source of infection, the epidemiological inquiries were carried out by the Deartments of Diseases Prevention of the local health unit. Clinical suspicion of *Plasmodium vivax* malaria was confirmed at the ISS by parasitological and molecular diagnosis. Entomological investigations, targeted to collect resting anophelines and to detect their possible breeding sites, were conducted by the Istituto Superiore di Sanità (ISS) team in a radius of 1 km around both the houses of residence and those of summer holidays. Mosquitoes were identified by both morphological observation and molecular assay.

RESULTS: The cases involved 2 Italian citizens, males, Caucasian, 39 and 41 years old respectively, living in the outskirt of Rome the first (Case 1) and in the midtown of Rende (Cosenza Province, Calabria) the second one (Case 2). They presented no history of recent travelling abroad, blood transfusions or tissue/organ transplantation, use of drugs or other risk factors for malaria, and no remarkable febrile attacks during the last 3 years. Both patients were admitted to the local hospitals, where they recovered after treatment with chloroquine. In July 2009, Case 1 spent 2 weekends in two different holiday farms close to Terracina and Pontinia town (Southern Lazio) both located in the former "Pontine marshes", where malaria was hyperendemic until 1946. The inquiry revealed the presence of a number of potential malaria reservoirs (immigrants from India and Sri Lanka) but not that of the potential main vector. Case 2 spent August 2011 in the village of Scalea, where some streams historically recognized as productive of both *An. labranchiae* and *An. superpictus* larvae, flow into the sea (Romi R. et al. 2001. Emerg. Infect. Dis. 7 (6): 915-919). The surveys revealed the presence of the main vector *An. labranchiae* in both Rende and Scalea, but no possible parasite reservoirs were detected.

CONCLUSIONS: Among the 13 autochthonous malaria cases reported in the last decade (Boccolini et al 2012, this volume), the two cases described above, were classified as cryptic, because even if they are likely to be considered transmitted by indigenous vectors the source of infection remains undefined. However, recent entomological surveys carried out throughout Italy, showed the presence (and somewhere an abundance) of the main indigenous vector *An labranchiae* more remarkable than the expected (Di Luca M. et al. 2009. Vector Borne Zoonotic Dis, 9: 703-717) and an unknown number of irregular seasonapl workers, part of which could come from malaria endemic countries (Romi R et al. 2012 Mal. J. 11: 98-in press). Finally, it should be mentioned that the occurrence of autochthonous transmission cases of exotic mosquito borne infections in Europe involves also infections other than malaria. In the last 2 years, sporadic cases of Dengue and Chikungunya have been detected in France (Medlock JM et al, 2012, Vector Borne Zoonotic Dis, 12: 1-13). In particular, the identification of sporadic autochthonous cases, suggests that the combination of two factors, such as the presence of a competent vector and imported cases of disease may determine the occurrence of several sporadic cases that will not necessarily result in a long chain of transmission.

Development of a new molecular approach for the diagnosis of *Trichomonas vaginalis* in its natural human host

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AIM: The protozoan parasite *Trichomonas vaginalis* is the causative agent of human trichomoniasis, the most common non-viral sexually transmitted disease. The infection may be asymptomatic but can also lead to serious consequences; in women, trichomoniasis has been associated with adverse outcomes in pregnancy (Hardy PH et al,1984 Lancet 2(8398): 333-7; Cotch MF et al, 1997, Sex Transm Dis 24: 353-60) and increased susceptibility and transmission of HIV-1 and cervical cancer (McClelland RS et al, 2007, J. Infect. Dis. 195(5), 698–702; Yap EH et al, 1995 Genitourin Med 71: 402-4). To date, the most common method for *T. vaginalis* diagnosis remains microscopic evaluation of vaginal/urethral wet smears due to its low cost and simplicity. Unfortunately, the sensitivity of this method is poor (60%–70%) and can dramatically decrease if microscopic examination occurs late after collection; this condition is not uncommon in busy clinical settings (Kingston MA et al, 2003. Int J STD AIDS, 14:28-9). The aim of this study was to develop a quick and cheap protocol based on molecular techniques to diagnose *T. vaginalis* infection that is more sensitive than conventional microscopic examination and that provides an internal control for each test.

MATERIALS AND METHODS: A Touch-Down Multiplex-PCR molecular protocol, with simultaneous identification of both human and parasite DNA fragments, was developed and tested on 500 samples (481 vaginal swabs; 16 urethral swabs and 3 seminal fluids) collected from the Gynecology and Urology Units of the Sant'Andrea Hospital in Rome for detection of *T.vaginalis* by microscopic examination. The DNA extraction was carried out by Chelex® 100 Resin. For the amplification of *T. vaginalis* DNA we designed a primers pair for -tubulin 1 gene while the human DNA was amplified with a primers pair for -actin gene (Diaz N et al, 2010, Diagn Microbiol Infect Dis 67(1):30-6) (Fig1).

RESULTS: The diagnostic protocol we developed confirmed the outcome of microscopic examination for all 18 positive samples; furthermore it allowed us to diagnose trichomoniasis in two patients who were evaluated as negative by microscopic examination. Therefore, the sensitivity of the molecular diagnosis appears to be greater than that of microscopic examination. The amplification of human DNA within each PCR reaction provides an effective internal control which allows to safely exclude false negative results.

CONCLUSIONS: In this work we describe a cheap Touch-down Multiplex-PCR to rapidly and effectively diagnose *T. vaginalis* infection which could be a promising method to support the microscopic examination, especially in busy clinical settings, and/or to conduct large scale epidemiological studies in order to generate more accurate estimates of the prevalence and incidence of trichomoniasis in Italy.

Zoonotic transmission of *Cryptosporidium parvum* in Italy caused by an uncommon genotype

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AIM: Worldwide, human cryptosporidiosis is caused mainly by two species, *Cryptosporidium hominis*, a parasite that predominantly infects humans, and *C. parvum*, a parasite highly prevalent in young ruminants that has an established zoonotic potential (Chalmers RM, Davies AP, 2010, Exp Parasitol, 124:138-146). In particular, zoonotic transmission from lambs has been demonstrated by a case-control study (Casemore DP, 1989, J Infect, 19: 101-104) and by investigation of school children who visited petting farms in the United Kingdom (Gomley JF et al, 2011, Emerg Infect Dis, 17:151-152).
In Italy, cryptosporidiosis is not included in the list of notifiable diseases, therefore little is known on the epidemiology of human infections. On the other hand, a number of investigations in livestock have shown that the infection is widespread and can be associated with significant morbidity and mortality (Duranti A et al, 2008, Zoonoses Public Health, 56: 176, 182). In this work, we describe a case of infection in a children that was linked to an outbreak of cryptosporidiosis in young lambs.

MATERIALS AND METHODS: Diagnosis of animal cryptosporidiosis was based on microscopic examination of fecal smears stained by the Kynioun method and confirmed by an ELISA test. Diagnosis of the human case was done by microscopic examination of fecal smear after auramine staining and by an immunochromatographic card test. Samples for molecular analysis were available from seven lambs (one sample collected at the time of the outbreak and available as a microscopic slide, and six samples collected about one month after the onset of the outbreak), and from the human case. DNA was extracted using the QiAmp DNA Stool kit. PCR and sequencing of fragments of the CpA135 and of the GP60 genes was performed following standard procedures. Sequences were compared with those available in public databases using BLAST.

RESULTS: An outbreak of cryptosporidiosis occurred in early October 2011 in a farm of the Grosseto province (Tuscany region, Central Italy). The farm hosts about 200 adult sheep and 130 adult cows, that are raised in two separate units. The outbreak involved about 100 of 200 lambs, with a high mortality rate (80%) of infected animals. Shortly after the outbreak, the son of the farm's owner, a young children aged 18 months, experienced an acute enteritis. The patient was hospitalized because of persistent diarrhea, fever, vomiting and lack of appetite. Coprological analysis revealed the presence of *Cryptosporidium* oocysts but excluded other common cause of enteritis, including *Salmonella* spp., *Campylobacter* spp., *Shigella* spp., Rotavirus and Adenovirus. The patient received rehydration therapy and was discharged 3 days after admission, as symptoms have regressed.
Molecular analysis was conducted on DNA from fecal samples of lambs and of the children. The parasite was identified as *C. parvum* by sequence analysis of a CpA135 gene fragment. Genotyping by sequence analysis of a GP60 gene fragment revealed that the lamb sample from the outbreak and the human sample harbored the same and uncommon genotype, IIaA20G2R1. The same genotype was detected in fecal samples from 3 lambs collected after the outbreak, whereas another and common genotype, IIaA15G2R1, was detected in another lamb isolate collected after the outbreak. One lamb isolate tested negative in PCR reactions. The genotype IIaA20G2R1 has been previously identified only in calves in Northern Ireland (Thompson HP et al, 2007, Parasitol Res, 100: 619-624), but not in humans. Of note, this genotype was not detected in a recent genetic study of 173 *C. parvum* isolates from livestock and humans in Italy (Drumo R et al, 2012, Appl Environ Microbiol, in press).

CONCLUSIONS: The epidemiologic and molecular data presented here are the first demonstration of zoonotic transmission of *C. parvum* in Italy, and parallel the situation reported in many other countries of the world. Interestingly, an uncommon GP60 genotype was associated with a high mortality in young lambs and was then identified in the feces of a children who acquired the infection. The father was the most likely source of infection for the son, who had no direct contact with farmed animals, underlining the ease of spread of this parasite to a susceptible host.

Pneumocystis jirovecii: genotypes and molecular analysis at mtLSU rRNA and DHPS loci

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Pneumocystis jirovecii (formerly *P. carinii* f. sp. *hominis*) is an ascomycetous fungus that causes opportunistic infections, particularly pneumonia (PCP), in the lower respiratory tract of patients with impaired immunity. Human pneumocystosis remains the most prevalent opportunistic infection in patients with AIDS. Genome analysis has led to major advances in the understanding of the genetic diversity of this pathogen, showing that specific polymorphisms could determine epidemiological profiles of the pathogen, including geographical distribution, drug resistance, virulence and modes of transmission (Esteves et al., Clin Microbiol Infect 2009). The goal of this investigation was to characterize *P. jirovecii* at two different genetic targets: the mitochondrial large subunit ribosomal RNA (*mtLSU rRNA*), which is involved in basic metabolic functions, and the dihydropteroate synthase (*DHPS*), the target protein of the first-line treatment and prophylaxis of PCP based on sulfamethoxazole. Indeed several studies have evidenced that the most common mutations identified in the DHPS protein are amino acid substitutions at positions 55 and 57, which confer resistance to sulfonamides.

From May 2011 to July 2011, 52 *Pneumocystis* isolates from patients admitted to the Istituto Nazionale Malattie Infettive L.Spallanzani were analysed by molecular assays. Genotyping was based on the sequence analysis of the two independent loci *mtLSU rRNA* and *DHPS.*

At *mtLSU rRNA* locus, 4 genotypes were distinguished on the basis of polymorphisms at the position 85 and 248: genotype 1 (85:C/248:C), genotype 2 (85:A/248:C), genotype 3 (85:T/248:C) and genotype 4 (85:C/248:T). No mutations at 55 and 57 residues of DHPS protein have been founded.

PCP remains an important disease associated with AIDS and in immunosupressed patients as well. PCR assay is confirmed as a suitable method to validate and improve the diagnosis of PCP in high-risk patients. Our results allow to a major knowledge of rate mutations in the *mtLSU rRNA* and *DHPS* genes, suggesting the utility of these loci in epidemiologic studies for the detection of genetic diversity and to investigate the relationship between genotype and drug resistance.

Reactivation of *Toxoplasma gondii* in HIV-infected population from rural area of Zimbabwe

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Toxoplasmosis is an emerging disease because of the increasing number of immunocompromised people in which the opportunistic etiological agent *Toxoplasma gondii* causes severe diseases (Weiss and Dubey, Int J Parasitol, 2009, 39: 895-901), In fact, in immunocompromised individuals, as in AIDS patients, bradyzoite tissue cysts can convert to tachyzoite and cause reactivated toxoplasmosis, either in form of local tissue cyst reactivation or parasite blood spread (Di Cristina et al, 2008, Infect Immun, 76: 3491-501). The consequent opportunistic disease can be life-threatening.

AIM: Our aim was to evaluate the frequency of the bradyzoite reactivation (or the persistence of tachyzoites in peripheral blood) and the tachyzoite dissemination in peripheral blood of HIV-infected patients, in which this biological phenomenon should be more evident.

MATERIALS AND METHODS: We examined 113 patients (79 female) with HIV infection living in a rural area of Zimbabwe. Between December 2011 to January 2012, blood peripheral samples were collected on dried blood spots (Whatman 903®). To detect anti-*T. gondii* IgG and IgM antibodies, automatic chemioluminescence test (Advia Centaur XP) was performed, and to evidence *T. gondii* DNA molecular diagnostics (Fast nested PCR, Medical System S.p.A.) were applied.

RESULTS: Positive *T. gondii* PCR results were found in 19 (16.8%) pa-tients, whose only 1 (5.2%) proved positive for IgG anti-*T. gondii*, and no one for IgM that were absent in all patients. Two more patients were IgG-positive (one at very low level) but they didn't have in blood DNA of circulating tachyzoites. The CD4 count was below 100/mm^3 in 6/19 (31%) PCR positive patients, and above 400/mm^3 in 13/19 (69%) patients; moreover, 6/19 (31%) had AIDS defining diseases but only 4 of them (21%) had CD4 count below 100/mm^3.

CONCLUSIONS: These findings demonstrate that *T. gondii* can have a haematological spread, in HIV positive patients, as a result of reactivation and that the parasite can persist even in absence of the corresponding antibody and without any clinical evidence. Finally, the degree of immunological impairment, as indicate by CD4 count, does not seem to influence *T. gondii* reactivation. Unfortunately, CD8 counts of the patients are not available to confirm the recent finding that exposure to a persistent pathogen despite initial control of parasitemia can lead to CD8+ T-cell dysfunction and parasite reactivation (Bhadra et al, 2011, PNAS USA, 108: 9196-201). However, our results give a new insight to understand the pathogenesis and the mechanisms of persistence and latency of *T. gondii* infection, In addition, tachyzoite permanence/return in peripheral blood of the host is a biological detail of great interest in public health: how long and how many infected donors are a possible source of infection for transfused people? What about pregnant women? Does the parasite genotype play a key role in this biological phenomenon?

Visceral leishmaniosis/HIV co-infection: first case detected in Nis (southeastern Serbia), and diagnostic observations on further five Italian cases

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Visceral leishmaniasis (VL) is an important opportunistic parasitosis sometime associated with human immunodeficiency virus (HIV) infection.

AIM: To report the first case of *Leishmania*/HIV co-infection observed in a patient living in Niš (Southeastern Serbia), and further cases occurred in Italy, identified following different diagnostic pathways.

MATERIALS AND METHODS: The first patient was a 58-year-old AIDS patient resident in the city of Nis (had travelled as a construction worker in Iraq in 1992 and Ukraine in 2002), with severe malaise, weight loss (40 kg weight loss over a period of 12 months), anemia, hypergammaglobulinemia, pancytopenia, splenomegalia and asthenia. Further 5 patients were Italian (25-47 year-old) and were admitted to the Umberto 1° teaching Policlinic in Rome; 3 of them were HIV-positive and the remaining 2 subjects had AIDS. Clinical signs of immunodeficiency were more or less marked, and pancytopenia, splenomegalia and fever were present in all.
For haematological investigations, bone marrow (BM) and peripheral blood were taken from the Serbian patient. BM was prepared as smear, Giemsa stained and microscopically examined (40-100X magnification). Blood was partly used to prepare a serum sample, and then submitted to a Rapid Dipstick rK39 test (ICT). The remaining blood (200 µL) was examined by molecular diagnostics (PCR with specific primers LEI 1-2, which amplify a fragment of 116bp of the kinetoplast DNA, followed by sequencing of the amplicon and submission of the sequences obtained to Blast Identity Search to identify the species of *Leshmania* involved in the infection. Italian patients were only submitted to serological tests (ICT, ELISA and Western Blotting) and to PCR applied to blood samples.

RESULTS: Microscopical examination of Giemsa stained BM smears proved the presence of leishmania amastigotes and allowed to diagnose the visceral form of leshmaniosis for the Serbian patient, whereas ICT serological test gave negative results. Serology applied to Italian patients gave the following results: 1/5 was positive to ICT, 5/5 negative to ELISA (but showing optical densities greater than expected for negative samples), 4/5 positive to WB (protein pattern of 14 and/or 16 kda were weaks but present). As for molecular analyses, they confirmed the infection in all 6 patients and enabled us to identify leishmania species as *L. infantum* (100% identity).

CONCLUSIONS: VL-HIV co-infection has important clinical, diagnostic and epidemiological implications. His occurrence in Nis (Southern Serbia) has been here firstly reported, and represents an alarming finding. In fact, in this condition, the failure of serological tests is expected, even if serological tests applied to Italian patients show that some of them can help to enforce clinical suspicions even in immunocompromised hosts. Their application followed by the molecular analysis of the blood can confirm the infection (giving also the identification of the leishmania species involved in the infection) and may offer, apart from an easy and non-invasive diagnostic chance, the opportunity of warning about the risk of possible nosocomial infections (Dey et al, 2006, Indian J Microbiol, 24: 165-70; Gregory et al, 2011, Acta Tropica, 119: 69-75).

TAVOLA ROTONDA

L'ECHINOCOCCOSI CISTICA IN SARDEGNA: QUALI POSSIBILITÀ DI CONTROLLO?

Evaluation of new control options for cystic echinococcosis

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Following the 1950-70s, during which there was intense interest in control of cystic echinococcosis, a marked decline has occurred in active efforts to control the diseases despite the infection remaining highly prevalent in large areas of Africa, Asia and South America. This decline may be because many attempts to control the disease in continental situations were poorly effective. Until now, the only control methods available have been the treatment of dogs with anthelmintics, other aspects of dog control, control of livestock slaughter and disposal of offal, and public education. Limitations inherent in these methods, particularly in continental situations, have restricted the effectiveness of disease control efforts.

The EG95 vaccine for use in livestock animals is a new tool for control of cystic echinococcosis transmission. Numerous experimental trials of the vaccine have been undertaken in a variety of countries and all have found the vaccine to be highly effective and reliable. The vaccine has not yet been incorporated into any widescale control program, although there may have been no new programs initiated since the vaccine's development. The EG95 vaccine has been, and is being, evaluated as part of field trials being undertaken in China, Argentina and Italy. However, limitations inherent in all of the existing field trial activities are such that none will provide clear scientific evidence for the value, if any, of EG95 vaccination for the control of cystic echinococcosis. The principal limitations with existing field work relate to the accuracy of pre-control disease prevalence and intensity and the methods being used to evaluate disease transmission levels during and following the intervention activities.

There is an urgent need to undertake a scientifically rigorous evaluation of potential new regimes for control of cystic echinococcosis incorporating livestock vaccination. Data from such an evaluation would provide a clear, evidence-based plan for future cystic echinococcosis control activities and, hopefully, encourage renewed investment in cystic echinococcosis control. The selection of which control options are to be evaluated would take into account aspects including effectiveness, cost, feasibility and sustainability. Choices would be guided by existing data from mathematical models.

Four scenarios should be evaluated, representing different levels of investment in control activities:

1. EG95+PZQ - Vaccination of all young animals (x2), annual booster immunization (x1) of all previously vaccinated livestock, 6-monthly treatment of all dogs with praziquantel (PZQ);
2. EG95 ONLY - Vaccination of all young animals (x2), annual booster immunization (x1) of all previously vaccinated animals;
3. PZQ ONLY - 6-monthly treatment of all dogs with praziquantel
4. No intervention control

Methods of assessment of pre- and post-control levels of cystic echinococcosis transmission would form critical aspects of the design of an evaluation trial and these will be discussed.

Historical and current perspectives for CE control

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Several national and provincial control programs for cystic echinococcosis have been undertaken from the 1950s. The island programs (New Zealand, Tasmania, Falklands, Cyprus) were largely a success after >20 years, and reduced human cases dramatically, though the parasite remains at low transmission in some areas. Control efforts in non-island regions were more difficult but significant reductions in human CE cases occurred (Chile, Argentina, Uruguay, Spain). Some programs were of limited or little impact on transmission (Wales, Turkana, Datangma). Successful features included- medical prioritisation, sufficient funding, dosing dogs with PZQ >4 times per year, good abattoir surveillance in sheep, good use of veterinary services. Additional tools now available to facilitate CE control include- coproELISA for dog surveillance, EG95 sheep vaccine, ultrasound screening in communities, and predictive transmission models. However no intervention has properly assessed application and sustainability of a modern integrated CE program. The largest CE control campaign is currently underway in western China (from 2006) and is based on PZQ dosing/coproELISA for dog populations and ultrasound for human surveillance.

Human cystic echinococcosis: WHO-IWGE ultrasound classification of cysts allows tailored clinical management

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Cystic echinococcosis (CE), an infection by the larval stage of the dog tapeworm *Echinococcus granulosus* , remains an important public health issue in many countries around the world, and is specifically targeted by WHO as one of the Neglected Zoonotic Diseases (NZD). Since many decades clinical issues were mainly pushed forward by surgeons, or more recently, by other interventional medical disciplines, and obviously, primary attention was paid to best care for patients with defined cysts as well as with complications of the disease. On the other side, sole or adjunctive medical therapy with benzimidazoles became fashioned during the past 30 years, and was applied at large to this very heterogeneous population of patients. Unfortunately, an evidence-based concept of best treatment is still lacking despite exhaustive experiences, many of them reported in the literature. The *E.granulosus* larva forms a single or multiple, fluid-filled cyst(s) - since ancient times named as hydatid(s) – which are always surrounded by a well-organized, compact capsule of host origin. Larval growth occurs inside the cyst (endogenous budding). This strong enclosure of the viable parasitic endocyst by the host capsule is the key to understand how to diagnose, and to design appropriate management of the disease. In the past decade, the 2003 WHO-ultrasound classification of liver and abdominal cysts became an international standard, and may now serve as the benchmark for structured treatment decisions for human CE. Four management procedures, surgery, percutaneous sterilization techniques, anti-parasitic treatment, and watch & wait, are ready to be applied. However, there is need to for an adequate comparative evaluation of efficacy, effectiveness, rate of adverse events, relapse rates, and cost. Parallel to the initiation of a control program, it would be desirable to create a network of clinicians and surgeons in order to prepare prospective-possibly randomized- structured treatment of CE, as well as to exchange and standardize epidemiological, clinical and diagnostic data.

Neglected Zoonotic Diseases (NZDs) with special focus on cystic echinococcosis

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Neglected zoonotic diseases (NZDs) mostly affect poor and disadvantaged populations with low visibility and little political voice and do not easily spread beyond national borders. These diseases cause significant morbidity and mortality and are relatively neglected by research with expertise waning in developed, and lacking in developing countries. Some NZDs can be controlled, prevented and possibly eliminated with currently available tools. Many NZDs are not notifiable and/or not reported. Because of this lack of data there is a general lack of information and awareness about the extent of the problem and consequently the burden and impact on society is perceived as low so that research and health resources needed are not available to control them. A number of improvements have however occurred recently in the NZDs field. For example, the burden of these diseases is much better known; sub-regional, regional and global NZD-specific networks and public–private partnerships have been established; information has been collated to develop global situation analyses and identify research priorities; field projects to study and control diseases as well as research and development projects have been undertaken and, for the first time, target dates have been set for eliminating an endemic zoonotic disease, human and dog rabies, in Latin America and ASEAN countries. Participants in a WHO consultation on echinococcosis held in June 2011 in Geneva argued that the success of highly intensive intervention programmes in developed countries may not work in low or middle income countries, where much of the disease burden occurs. The consultation assessed results obtained from the use of the recently developed EG95 vaccine against cystic echinococcosis/hydatidosis (CE) in sheep and identified potential countries where well-controlled efficacy and feasibility studies could be conducted. This consultation has recommended field trials of the EG95 vaccine to control and prevent transmission of CE from animals to humans, with the aim of demonstrating that the vaccine can be used in an effective, simple, inexpensive and sustainable way. Today NZDs are an integral component of the WHO Department of Control of Neglected Tropical Diseases. They are included in the WHO Global Plan to combat Neglected Tropical Diseases (NTDs) for the years 2008-2015 and three of them, including CE, are described in the first WHO report "Working to overcome the global impact of NTDs" launched by DG in October 2010. CE is also one of the 6 priority packages identified by the Interagency (FAO/OIE/WHO) in its plan for prevention and control of NZDs elaborated in July 2011. More recently, these NZDs have been included in the roadmap for "Accelerating work to overcome the global impact of NTDs" released in January 2012. As part of this document, the 2012-2020 roadmap for CE prevention and control will be described in the presentation.

SESSIONE 1

TERAPIA E FARMACORESISTENZA

Control of strongyles in captive equines by using the nematophagous fungus *Duddingtonia flagrans*

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AIM: The role of zoological parks has changed significantly in the last few decades. The former objective consisting of displaying wild animals for visitor entertainment has been replaced by the conservation of endangered species, education, and research. The current investigation deals on the need for improving and progressing in the control of strongyles affecting equids maintained in Zoological Parks. The main goal in this research was to determine the beneficial effect of incorporating biological procedures to the parasite control programs applied on equids from Zoological Parks; this will allow preventing the reinfection of the equids.

MATERIAL AND METHODS: Four equid species are maintained in Marcelle Natureza zoological park located in NW Spain (Outeiro de Rei, Lugo). Collection animals live in fenced semi-free ranging exhibits of various sizes. Two trials have been developed on *Equus quagga* (3 zebras), *E. asinus* (6 European donkeys) and *E. africanus asinus* (6 African Wild Ass). The first consisted of chemotherapy (ivermectin + praziquantel at a dosage of 1.07 g gel / 100 kg body weight) only, and the second in the administration of chemotherapy and chlamydospores of the nematophagous fungus *Duddingtonia flagrans*. The total quantity of spores required for each individual was 2 x 10^6 *D. flagrans* chlamydospores kg bw. This was delivered orally by carefully dissolving the spores into 50 mL of water and then mixing with the feedstuff immediately prior to feeding the equids. Collection of feces was done early in the morning, prior to the daily cleaning of the paddocks by the animal keepers. The effect of these measures was evaluated by the estimation of the reduction in the fecal egg-counts (FECR) and in the number of equids positive to the coprology (PER).

RESULTS: Chemotherapy provided FECR values of 100% fifteen days after treatment in all the animals. In the first trial, an Egg Reappearance Period of 2 months for the donkeys, and 3 months for the zebras was observed. In the second experiment, the ERP was of 3 months for the European donkeys and 4 months for the African asses; no strongyle egg-output was observed in the zebras.

CONCLUSIONS: The incorporation of chlamydospores of nematophagous fungus as *Duddingtonia flagrans* reveals a very efficient measure to contribute for the infective stages of the strongyles diminish. It is concluded that biological control measures provide a very useful way of integration of ecological and sustainable processes for the care of captive animals by preventing their infection.

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The maintenance of anthelmintic efficacy in sheep and goats in southern Italy

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AIM: Anthelmintic resistance (AR) has become a worldwide issue in the control of nematodes of sheep and goats in the major small ruminant producing regions of the world. In central Italy, AR was found on a number of farms involving imidazothiazole and macrocyclic lactone resistance in trichostrongylids in sheep (Traversa D et al, 2007, Parasitol Res, 101: 1713-1716). In addition, one case of benzimidazole resistance has been reported in *Trichostrongylus colubriformis* on a goat farm (Cringoli G et al, 2007, Parasitol Res, 101: 577-581). In the present study the presence of AR was investigated on 54 sheep farms and 7 goat farms using the faecal egg count reduction test (FECRT) following the recommendations of Coles et al. (1992) and using four groups of anthelmintics (benzimidazoles/probenzimidazoles, levamisole, ivermectin/moxidectin and monepantel) and the FLOTAC technique (Cringoli G et al, 2010, Nat Prot, 5: 503-515). The data collected also permitted a re-examination of the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines, which suggested that resistance should be diagnosed if efficacy was less than 95% with the 95% lower confidence limit below 90% (Coles G C et al, 1992, Vet Parasitol, 44: 35-44).

MATERIALS AND METHODS: Trials were conducted over the period 2008 to 2011 in the Campania region of southern Italy. On each farm all the experimental animals were weighed and given the correct dose. With ivermectin a half dose was also given to indicate whether resistance to the macrocyclic lactones might be developing (Palmer D G et al, 2000, AVA Conference Perth, 124-131). Fresh faecal samples were collected rectally at the time of treatment and 14 days later and FEC were made using the FLOTAC double technique (using a salt flotation solution s.g. = 1.200) with a sensitivity of 2 eggs per gram (EPG) of faeces. Tests were run with flocks of sheep (12 to 20 animals per group) using six anthelmintics administered orally, namely levamisole (LEV, 7.5 mg/kg) in 8 farms, ivermectin (IVM, 0.1 and 0.2 mg/kg) in 8 farms, moxidectin (MOX, 0.2 mg/kg) in 3 farms, monepantel (MON, 2.5 mg/kg) in 8 farms, netobimin (NET, 7.5 mg/kg) in 22 farms (composite samples) and albendazole (ALB, 3.8 mg/kg) in 5 farms (composite samples). In goat farms (12 animals per group) four anthelmintics administered orally were used, namely levamisole (LEV, 12 mg/kg) in 2 farms, ivermectin (IVM, 0.2 and 0.4 mg/kg) in 2 farms, monepantel (MON, 2.5 mg/kg) in 2 farms and netobimin (NET, 15 mg/kg) in 1 farm (composite samples). On each faecal sampling day, arithmetic mean EPG was calculated as recommended by the WAAVP guidelines for evaluating the efficacy of anthelmintics in ruminants and, for each treatment group, percent efficacy (%) was calculated in terms of FECR on the different days using different formulae based on the presence of a control group and on the use of single or composite samples.

RESULTS: A very high efficacy was obtained with all anthelmintics tested (and using the different formulae) both in sheep and goats. In ovine farms, the following efficacy were found: LEV 99.3% (98-100%), IVM 0.1 mg/kg 99.5% (98.0-100%), IVM 0.2 mg/kg 99.9% (99.3-100%), MOX 100%, MON 99.4% (range 97-100%), NET 99.1% (range 92-100%) and ALB 100%. Regarding goats, the following efficacy values were found: LEV 99.3% (98.7-99.9%), IVM 0.2 mg/kg 99.9% (99.8-100%), IVM 0.4 mg/kg 100% (99.9-100%), MON 98.9% (98.2-99.5%) and NET 99.9%.

CONCLUSIONS: Nematode infection remains one of the main constraints to small ruminant production in southern Italy (Rinaldi L et al, 2007, Trans R Soc Trop Med Hyg, 101: 745-746) and maintenance of anthelmintic efficacy is important to ensure maximal production and animal welfare. The present data suggest that AR is rare in southern Italy and supports the idea that with correct management the development of resistance can be greatly reduced. The efficacies found using the sensitive FLOTAC technique suggest that the definition of anthelmintic resistance as less than 95% efficacy is too low and that values closer to 100% need to be agreed. This will enable the first signs of resistance to be detected earlier.

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Efficacy of fenbendazole and moxidectin in horse stables with previous history of resistance to benzimidazoles in cyathostomin populations

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The control of horse Cyathostominae relies on the use of benzimidazoles - BZs (e.g. fenbendazole - FBZ), tetrahydropyrimidines - THP or macrocyclic lactones - MLs (i.e. ivermectin-IVM and moxidectin-MOX) (von Samson-Himmelstjerna G, 2012, Vet Parasitol, 185: 2-8). Nonetheless, resistance to BZs is now ubiquitous and resistance to THP is increasing worldwide (Traversa D et al, 2009, Parasit Vectors, Suppl2: S2; von Samson-Himmelstjerna G, 2012, Vet Parasitol, 185: 2-8). Additionally, the failure of MLs to provide control of cyathostomins has been recently reported in Europe and the Americas (von Samson-Himmelstjerna G et al, 2007, Vet Parasitol, 144: 74-80; Molento MB et al, 2008, Vet Rec, 162: 384-385; Lyons ET et al, 2011, Parasitol Res, 108: 1315-1319). Hence, a continuous monitoring on the susceptibility of cyathostomines to different anthelmintics is pivotal for planning worm control programs in horse stables.

AIM: The aim of this study was to evaluate the persistency of reduced efficacy of FBZ in two horse stables with a known history of BZ-resistant cyathostomines since 2008 and to evaluate the efficacy of MOX versus these populations.

MATERIALS AND METHODS: The study was carried out by the Faecal Egg Count Reduction Test (FECRT) (Coles GC et al, 2006, Vet Parasitol, 136: 167-185). Two stables (Site A and Site B) in which the presence of BZs-resistant cyathostomins was shown in 2008 (Traversa D et al, 2009, Parasit Vectors, Suppl2: S2), were enrolled in Friuli Venezia Giulia region. Pre-treatment screening was performed with FECs on all horses present on site. In both stables the ten horses with the highest values of strongylid eggs per gram (EPG) of faeces were selected and randomly assigned to 2 groups of five horses each. Animals were orally treated either with MOX (Equest®, Pfizer Animal Health) or FBZ (Panacur®, Intervet) at the dosages recommended by the manufacturers. Individual faecal samples collected from each animal at the treatment day (Day 0) and two weeks later (Day 14) were subjected to quantitative copromicroscopical analysis to determine pre and post-treatment EPG values. The calculation of the FECR percentages was performed according to the formula FECR=100 × (1 - FEC post-treatment/FEC pre-treatment). Pre-and post-treatment larval cultures were performed using pool faecal samples collected from each treatment group. Third-stage larvae (L3) were identified using morphological keys (MAFF, 1986, Manual of Veterinary Parasitological Laboratory Techniques, Technical Bulletin 18, HMSO, London, UK).

RESULTS: Horses treated with FBZ showed pre-treatment EPG ranges of 800-3250 and 750-2050 in Sites A and B respectively . The same animals showed post-treatment EPG values from 50 to 1600 (Site A) and from 450 to 850 (Site B). Therefore, the FECR percentage varied from 11.6% to 97.7% after treatment with FBZ in individual animals in Site A (mean efficacy 56.7%), and, individually from 25% to 63.4% in Site B (mean efficacy 43.7%). Horses treated with MOX showed pre-treatment EPG values from 650 to 2750 in Site A and from 100 to 1100 in Site B. All MOX-treated horses in both sites were negative on Day 14 post treatment (efficacy 100%). Larvae collected from pre-treatment and post FBZ-treatment samples belonged exclusively to the Cyathostominae Subfamily.

CONCLUSIONS: this study confirmed the presence of FBZ-resistant cyathostomine populations in Italy (Traversa D et al, 2009, Parasit Vectors, Suppl2: S2) and confirmed the efficacy of MOX against these parasites. Moreover, it is shown that anthelmintic resistance may persist over different years in the same sites, likely due different drivers, such as inadequate worm control programs, absence of drug resistance monitoring and erroneous use of parasiticides. Owners, farmers and veterinary practitioners must have a leading role in planning and monitoring effective control measures, which should be always based on regular testing for resistance in all equine establishments and operations.

Milbemax® (milbemyicin oxime/praziquantel) flavored tablets in cats naturally infected by helminths: a clinical trial.

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AIM: Milbemax® Cat (milbemycin oxime / praziquantel) film-coated tablets, is a pharmaceutical formulation which allows vets and cat owners to easily treat intestinal helminthic infections administering a single tablet. The purpose of this field study was to confirm efficacy of this anthelminthic drug, as well as safety and palatability of Milbemax® on naturally infected cats.

MATERIALS AND METHODS: The main inclusion criterium to enroll cats in this study was a natural intestinal helminthosis confirmed by fecal analysis. No other restrictions were imposed about age, gender, breeding condition and health status. The treatment, at D0, consisted in a single tablet administrated according to the patient weight as recommended by the manufacturer (Novartis Animal Health). The available formulations were Milbemax film-coated tablets for cats containing 16 mg Milbemycin Oxime and 40 mg praziquantel and Milbemax film-coated for small cats and kittens containing 4mg milbemycin oxime and 10 mg praziquantel. The minimum dose is 2mg milbemycin oxime and 5 mg praziquantel per kilogram of body weight.
In order to confirm the drug efficacy, copromicroscopic tests were executed two and four weeks (D14 and D28) after treatment (Jacobs DE et al, 1994). Fecal floatation was performed using a 1.30 specific gravity sodium nitrate solution (MAFF, 1986, Manual of Veterinary Parasitological Laboratory Techniques, HMSO, London, UK). In case of positive results at D14, a further treatment was planned in the study protocol. For each treated cat, drug intake way (e.g., spontaneous or forced) was recorded.

RESULTS: A total of 30 cats were enrolled including kittens (n=9), adults (n=17) and pregnant queens (n=4). Regarding the feline population health condition, it is possible to define two groups: Apparently healthy cats (n=25) and cats affected by other concomitant diseases (5). The most frequently diagnosed parasites were *Toxocara cati* (65%) followed by *Dipylidium caninum* (23%) and *Ancylostoma tubaeforme* (12%). Among helminthosis, both single (84%) and multiple parasitic infections were observed. The most common parasites association was represented by *T. cati* and *D. caninum*. At D14, 29 out of 30 (96.6%) treated cats were negative. The cat found still coprologically positive for *T.cati* eggs at D14 was treated again. All copromicroscopic analysis scored negative at D28. Any side effect was observed and pregnant queens delivered healthy full term kittens. A spontaneous tablet intake was observed in 83.3% of the cats whilst for 17.7% of them, represented by cats in poor general health conditions impair normal vegetative functions such as olfactory and gustatory sensitivity, a forced administration was needed.

CONCLUSIONS: According to high efficacy results, safety and palatability of this drug is possible to confirm the definite advantages treating feline intestinal helminthosis using Milbemax tablets for cat. The single treatment and the flavored tablets make this therapy more feasible for practitioners, for owners and for cats avoiding their routinely stressful behavior that normally follows forced and repeated treatments.

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Effectiveness of Decoquinate on control of subclinical coccidiosis in naturally infected beef cattle: preliminary results

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AIM: At the arrival in Italy, beef cattle are challenged by many stressing factors (e.g. marking, transport, and location into a feedlot environment) increasing incidence of subclinical and clinical coccidiosis, and thereby reducing cattle performance and profits. Decoquinate is a synthetic feed additive approved for use in the control of coccidiosis in cattle, veal calves, goats, sheep and poultry. Its coccidiostat effect has been demonstrated in cattle when fed at 0.5 mg/kg body weight (bw) daily, for at least 28 days, and no adverse reaction have been detected with levels up to 6.25 mg/kg bw (Fox J, 1983, Agri-Practice, 4: 19; Penzhorn B, Swan G, 1993, Coccidiosis. Current Veterinary Therapy 3: Food Animal Practice. W.B. Saunders Co, Philadelphia, PA, USA). This study aimed to evaluate the effectiveness of Decoquinate in reducing oocyst shedding and prevalence of coccidiosis in naturally infected beef cattle in a stable located in Veneto Region, North-eastern Italy.

MATERIALS AND METHODS: A field trial was carried out from November 2011 to January 2012 on 92 newly-imported Charolaise yearling bulls. Animals were reared in covered boxes with littered floors (for about 2-3 weeks) and then allocated on slatted floors. They were vaccinated for Infectious Bovine Rhinotracheitis (IBR), Parainfluenza3 (PI3), Bovine Virus Diarrhoea (BVD), and dewormed with the association ivermectin and clorsulon. After six days from their arrival, bulls were randomly allotted to either control (n=46) or Decoquinate treated (n=46) groups. In the latter, at Day 0 a medicated feed supplement was daily incorporated into the diet giving the recommended rate of 0.5 mg Decoquinate/kg bw for up to 28 days. At least 23 individual faecal samples, corresponding to 50% of the animals present in each experimental group, were collected at Day 0 (pre-treatment: PreT), Days 7,14,28 (during treatment: DT), and Days 42,60 (post-treatment: PostT). Faecal samples were tested by McMaster (McM) technique adding 5g of faeces in a sodium nitrate solution (density 1.3) to obtain a sensitivity value of 20 oocysts per gram (opg). Differences in coccidiosis prevalence and oocyst shedding between treated and untreated bulls were evaluated considering datasets obtained in PreT, DT, and PostT. Differences in prevalence and opg values were evaluated by Pearson's chi squared Test and Mann-Whitney U tests, respectively, choosing a significance level $p<0.05$. The software used was SPSS for Windows, version 16.0.

RESULTS: A total of 297 individual faecal samples were examined by McMaster technique (Table). At Day 0, the experimental groups showed no significant differences in prevalence ($p>0.05$) and oocyst shedding ($p>0.05$), while prevalence and opg values decreased significantly ($p<0.001$) in treated animals during Decoquinate administration. Prevalence and opg values increased again in treated bulls during the PostT period, and these values did not significantly deviate ($p>0.05$) from data obtained in untreated bulls. No clinical signs of coccidiosis developed in the animals during the entire experimental period.

Table 1. Prevalence and opg values detected in treated (T) and untreated (U) bulls at different sampling periods.

Period of sampling	Samples (n.)		Prevalence (%)		Mean opg values (min-max)	
	T	U	T	U	T	U
PreT (Day0)	24	24	95.8	95.8	328.3 (20-2280)	294.2 (20-2600)
DT (Days 7,14,28)	76	76	18.4	82.9	6.6 (20-100)	165.8 (20-2140)
PostT (Days 42,60)	48	49	60.4	69.4	135.8 (20-2080)	50.2 (20-500)

CONCLUSIONS: This preliminary study confirm the effectiveness of Decoquinate on control of subclinical coccidiosis by a significant reduction in prevalence, opg values and, consequently, environmental contamination by oocysts during its administration. The resumption in prevalence and opg values detected in treated bulls during the PostT period could be explained by a partial reactivation of coccidial sporozoites due to the end of coccidiostat effect of Decoquinate, as previously demonstrate with daily doses of 0.36 mg/kg bw for up to 28 days, very similar to the rate (0.5 mg/kg bw) applied in this trial (Miner ML, 1976, Am J Vet Res, 37: 1043-1045). Higher dosage of 1.5 mg/kg bw, daily administered for the same period, showed an increased killing effect of Decoquinate against coccidia (Fitzgerald PR, Mansfield ME, 1986, Am J Vet Res, 47: 130-133).

Influence of a nutritional addition of Oregano essential oil (OEO) on *Eimeria* spp. infection prevalence and oocyst shedding dynamics in dairy cattle calves.

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AIM: Bovine coccidiosis is an enteric disease mainly affecting calves between 1 and 6 months of age. It is caused by several protozoan parasites belonging to the Family Eimeriidae, each characterized by a certain degree of pathogenicity. Severe, bloody diarrhea can follow massive infection by species like *Eimeria zuernii* end *E. bovis*, but usually bovine coccidiosis is asymptomatic (Daugschies A, Najdrowski M, 2005, J. Vet. Med. B 52, 417–427, Muirhead S, 1989, Feedstuffs, 15, 87). Some activity of Oregano essential oil (OEO) on *Eimeria* spp. has been demonstrated in broilers (Giannenas I et al, 2003, Arch Anim Nutr, 57:99-106). The aim of the present study was to evaluate the effect of an OEO nutritional addition on the excretion pattern of bovine coccidia (*Eimeria* spp.) in calves and heifers in a large dairy cattle farm in the Province of Piacenza.

MATERIALS AND METHODS: A total of 52 calves of 1 month of age were divided, according to the date of birth, into 2 groups, Control (C, n = 25) and Experimental (E, n = 27), the latter receiving a concentrated feed containing *Origanum heracleoticum L.* EO at the concentration of 100,00 ppm. Calves were fed dairy milk from birth to 60 days of age (starting from 4 l/d, gradually increasing to 8 l/d), then with hay (42.0% NDF, 14.3% CP) and concentrate (21.6% CP) ad libitum. Concentrate was administered at a maximum of 2 kg/d starting at 60 days of age. Individual fecal samples were collected at 30, 45, 60, 90, 120 and 150 days of age and analysed in blind for *Eimeria* spp oocysts through copromicroscopic analysis (NaCl flotation) and McMaster quantification of oocyst shedding. Statistical analysis was performed using ANOVA technique (PASW Statistics 18, vers. 18.0.0).

RESULTS: During the trial, the calves' health status and growth were consistent with the nutritional plan and fell within normal ranges. The overall prevalence of infection (% of animals positive at least once) was of 83%. Overall prevalence within the groups was of 92.59% (group E) and of 72.00% (group C). Prevalence within groups at the different intervals is reported in Table 1:

Prevalence/ interval	d30	d45	d60	d90	d120	d150	Average
Group E	7.41	37.04	22.22	22.22	33.33	15.38	22.93
Group C	16.67	36.00	20.00	20.00	20.83	20.00	22.25

Oocyst shedding at different intervals is reported in Table 2:

Mean OPG (n. of calves)/ interval	d30	d45	d60	d90	d120	d150
Group E	11625.00(2)	6890.00(10)	3095.83(6)	479.17(6)	480.56(9)	106.25(4)
Group C	16025.00(4)	4708.33(9)	10365.00(5)	485.00(5)	320.00(5)	706.25(4)
P	0.804	0.701	0.383	0.99	0.681	0.179

CONCLUSIONS: Results demonstrate *Eimeria* spp. circulation in the farm studied here and a progressive decrease of oocyst shedding during the first months of calves' growth, confirming the self-limiting nature of the infection. No influence of OEO addition on prevalence and oocyst shedding was observed. Peaks of prevalence were observed at d45 and at d120. The second peak may be related to the immunodeficiency window that usually takes place in calves (fading of passive immunity) and is present only in group E. OPG values numerically showed a slightly higher infection rate in group C. Despite the reduced clinical significance and self-limiting nature of coccidiosis observed in the present study, further studies are needed to assess the potential protective role of OEO in terms of growth performance and feed efficiency in replacement animals.

Efficacy of milbemycin oxime/praziquantel tablets (Milbemax®- Novartis Animal Health) against *Thelazia callipaeda* in naturally infested dogs

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AIM: Over the last decades, canine and feline thelaziosis caused by *Thelazia callipaeda* eye worms gained the attention of the scientific community due to the spread of this ocular infestation in geographical areas previously regarded as non endemic, such as Switzerland (Malacrida F et al, 2008, Vet Parasitol, 157: 321 -327), France (Dorchies F et al, 2007, Vet Parasitol, 149: 294 -297), and Spain (Mirò G et al, 2011, Parasit Vectors, 4: 148). In Europe, the zoonotic role of this nematode has also been ascertained (Otranto O, Dutto M, 2008, Emerg Infect Dis, 14: 647 -649). Antiparasitic drugs, such as macrocyclic lactones (e.g., moxidectin or milbemycin oxime in spot-on and oral formulations, respectively) have been proven efficacious in treating thelaziosis (Bianciardi P, Otranto D, 2005, Vet Parasitol 129: 89 -93; Ferroglio E et al, 2008, Vet Parasitol 154: 351 -353). The aim of this work was to evaluate the therapeutic efficacy of a commercial formulation of milbemycin oxime/praziquantel tablets (Milbemax®- Novartis Animal Health) at the minimal dose of 0.5 mg/kg b.w. in dogs naturally infested with *T. callipaeda*.

MATERIALS AND METHODS: From January 2009 to July 2011, the efficacy of milbemycin oxime/praziquantel tablets against *T. callipaeda* was evaluated in a placebo controlled, blinded and randomized field study in an endemic area of the Basilicata region, southern Italy. Naturally infested client-owned dogs (n = 37) were physically examined by the veterinarian at the enrolment (D0) and then twice (D7 and D14). At each visit, both eyes were examined for the presence of eye worms by clinical inspection of the conjunctival pouch. Infested animals were orally treated with Milbemax® or with placebo tablets (administered with food as advised by the producer) on D0 and, if an animal was still infested with *T. callipaeda*, also on D7. On D14 (final visit) nematodes were flushed from the conjunctiva, identified and counted. Data were statistically examined using SAS® Version 9.2 (SAS Institute, Cary, NC, USA).

RESULTS: On D0, dogs treated with Milbemax® (n = 19) and dogs of the placebo group (n = 18) harboured a mean (arithmetic) of 9.63 (range: 1 – 22 worms), and of 13.28 (range: 1 – 22 worms) worms, respectively. At D7 a total of 9 dogs treated with Milbemax® were still positive for *T. callipaeda* and were re-treated. Thus at D7, the cure rate in the dogs treated with Milbemax® was 52.6%, which was significantly higher (p = 0.0030) than in the placebo group (5.6%). At D14, the cure rate in the dogs treated with Milbemax® (63.2%) increased and was significantly higher (p = 0.0004) than the placebo group result (5.6%). At D7 and D14, the mean percentage worm count reduction for treated group 1 was 73.8% and 83.2%, respectively. Both results were significantly higher (p = 0.0001) than the placebo group percentages for D7 and D14 (10.9% and 17.9%) and the worm counts for treated group 1 significantly lower than group 2 at D7 and D14 (p = 0.0011 and 0.0003).

CONCLUSIONS: The commercial formulation of milbemycin oxime/praziquantel tablets at the minimal dose of 0.5 mg/kg b.w. for dogs showed a high therapeutic efficacy in curing *T. callipaeda* naturally infested animals with an efficacy against eye worms from 73.8% to 83.2% after a single or two treatments at a weekly interval.

Effects of condensed tannin on natural coccidian infection in goat kids

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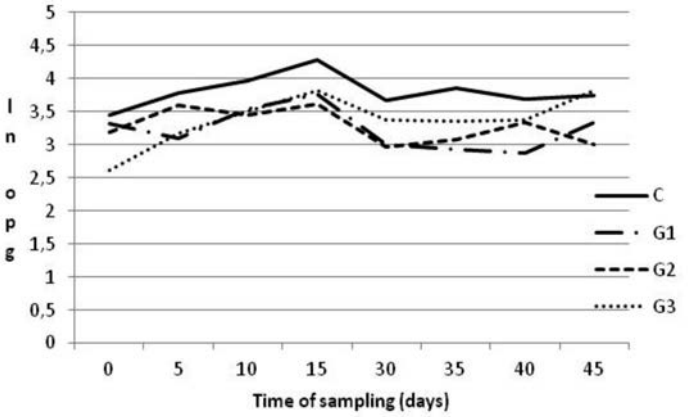
Coccidiosis is one of most ubiquitous and spread enteric diseases of goats worldwide. Infections by *Eimeria* can have serious clinical signs in kids and loss of animals have been recorded. Currently, chemotherapy is used extensively to control coccidiosis but the worldwide and rapid development of drug resistance coupled with the public's concern on drug-treated meats show the need to explore alternative methods of controlling this disease. In the last years, the antiparasite activity of condensed tannins (CT) against trichostrongylid infections in goats (Min et al, 2005, Vet Parasitol 130: 105- 113; Osoro et al, 2009, Rangeland Ecol Manage 62: 127-135) and in sheep (Athanasiadou et al, 2000, Int J Parasitol, 30: 1025-1033) have been shown. Further, Hur et al (2005, Asian Australas. J. Anim. Sci., 18 (9): 1262-1266) showed that feeding pine needles and oak leaves to goats naturally infected with coccidian parasites significantly reduced the number of oocysts.

AIM: To investigate the possible effects of condensed tannins (Quebracho) on coccidian infection and growth performances of kids.

MATERIALS AND METHODS: Thirty nine male kids nine weeks old of Alpine breed, naturally infected with *Eimeria* were allocated in a control (C) or tannin treated group (TG) by weight and opg counts. Kids of TG were offered the Quebracho extract powder ready-mix with food. Cold soluble Quebracho extract commercially available (SILVAFEED BY PROQ) containing 70% tannins of the condensed type was used. Finally, the food contained 5% of condensed tannins per kg dry matter. The study lasted 45 days: G1 kids received treated food twice once a day for a week (Day 0-Day 6 and Day 30-36), G2 kits once a day every 5 days (9 daily treatments) and G3 kits for 3 days every 15 days (3 treatments of 3 days). Faecal samples were collected at T0, T4, T9, T15 and T45. Oocyst counts were carried out using FLOTAC DOUBLE technique (flotation solution MgSO4, s.g.: 1.280). A mixed model of variance analysis for repeated measures was applied to evaluate the effect of several factors (time, treatment, body weight as fixed effects and goat as random effect) after log transformation of opg counts. A autoregressive covariance structure was fitted. Body weight was pooled in 3 classes: <18 kg, 19 – 21 kg, >21 The time*treatment interaction was dropped from the model because not significant.



RESULTS: At T0 opg mean counts were 14320 (±26115), 9796 (±17124), 16764 (±46524) and 1352 (±2099) for C and G1, G2 and G3, respectively. Different opg values were found at 10 and 15 days between C and TGs. From 30 day the differences become lower and at day 45 no differences were found (p>0.05). Kids of control group showed opg values higher than treated groups (p<0.05). No difference of opg mean counts was observed between treated groups. The opg counts decreased from day 15 with the increasing of kid weight but in G3 from day 35.

CONCLUSIONS: The kit daily weight gain (DWG), on days T0, T15, T30 and T45, significantly differed between treated and untreated animals (Mann Whitney test, p<0.05). DWG of G1 kids were highest than C at T30 and T45; G2 was different from C at T15, T30 e T45. No difference was observed for G3. The TC administered every 5 days (G2) showed the best performance in terms of oocyst reduction (77% at T10 and T15) and daily weight gain.

Acaricide residues in laying hens naturally infested by red mite *Dermanyssus gallinae*

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In poultry industry, the control of the red mite *Dermanyssus gallinae* (De Geer, 1778) (Mesostigmata: Dermanyssidae) (PRM) primarily relies worldwide on acaricides; the most widely used are carbamates, followed by amidines, pyrethoids and organophosphorus. Despite their proved efficacy against *D. gallinae*, none of these compounds is specifically registered in Italy for use against red mites, except for the very recently labeled organophosphate and spinosad-based products. This means that farmers have always used -and continue to use - acaricides registered for use in agriculture or for other farm animal species. In the last few years, some acaricides have become worldwide less effective; thus, to control mite infestations farmers are keen to use chemicals at higher concentrations, more frequently and repeatedly. This improper use of acaricides to control PRM could lead firstly to the development of acaricide-resistant *D. gallinae* populations, as previously observed also in Italy (Marangi M et al, 2009, Exp Appl Acarol, 48:11–18), and, more importantly, to the accumulation of acaricides in chickens' organs, in tissues and in eggs.

AIM: To highlight some concealed situations of risk for human health, we investigated three farms (denoted A, B and C) in a southern Italian region where *D. gallinae* populations were found to be significantly tolerant to carbammates and permethrins (Marangi M et al, o.c.), likely due to the high chemical pressure.

MATERIALS AND METHODS: Fifteen laying hens at the end of their production cycle and destined to the slaughterhouse were taken from each farm. In the necropsy lab, all 45 hens were euthanized, and a total of 225 samples were taken from skin, fat, liver, muscle, hearth, and kidney. Feed samples were also collected from each farm. Analysis was carried out using a HPLC coupled to a QQQ Triple Quadrupole Mass Detector for the residual contents of carbaryl and permethrin from all matrices and detection limits and the average pesticide recoveries were performed. Statistical analysis (one-way variance analysis, Duncan's test and one-tailed t-student test) was applied and the Statistica 6.0 software package was used.

RESULTS: Thirty-seven (82.2%) laying hens were positive for carbaryl residues and 4 (8.8%) for permethrin. Ninety-one (40.4%) samples resulted positive for carbaryl (25 skin, 27 fat, 8 liver, 16 muscle, 15 heart and kidney samples), showing a mean concentration of 5 ppm, 0.04 ppm, 0.05 ppm, 0.14 ppm, 0.04 ppm, respectively. Four samples (1.7%) resulted positive for permethrin (2 fat and 2 liver samples), with a mean concentration of 0.012 ppm and 0.006 ppm, respectively. On one farm (Farm B), all investigated hens were found to be contaminated by carbaryl, and 80% of their organs and tissues contained residues of the compound, with the highest concentrations in the skin (16 ppm), fat (0.11 ppm) and muscle (0.3 ppm). Concentrations of carbaryl exceeding the detection limit (0.005 ppm) were registered in the skin and fat of birds from two farms ($p<0.01$), although these concentrations remained below the maximum residue limit (MRLs) (0.05 ppm) ($p<0.01$). All organs/tissues of hens from a third farm were significantly more contaminated, with skin and muscle samples exceeding the MRL (0.05 ppm) ($p<0.01$).

CONCLUSIONS: This study shows that most laying hens (37 out of 45) from all three investigated farms were contaminated by carbaryl, and that the hens of one farm also contained permethrin. Furthermore, with different accumulation levels among animals - possibly due to differences in factors like management strategies and unpredictable individual predispositions - all organs/tissues were contaminated by carbaryl, with the highest levels found in skin, fat, and muscle. These data are all worrying, because: a) carbaryl was banned by the EU in 2007 (Allegato I, Direttiva 91/414, 1376/07, 07/355); b) no carbaryl-based products specifically labeled for use against *D. gallinae* infestation were available on the Italian market before the ban; c) no registered permethrin-based products are available on the market for use against red mite infestation; and d) tissues and organs from laying hens can be consumed as food. The detection of acaricide residues in tissues/organs of laying hens seems to confirm - at least in the studied area - their extensive and improper use by farmers against *D. gallinae*. It also shows up the illegal use and persistent commercialization of pesti-

cides banned years ago (carbaryl), and more importantly, it indicates that some specific/restricted situations of misuse or abuse of chemicals may remain undetectable. The current availability on the Italian market of licensed products against *D. gallinae* may help farmers to better manage infestation and to limit the consequences of misuse of chemicals.

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Control of trematode infection can be improved by means of soil fungi

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AIM: To assess the possibility for improving the success of control measures against the infection by trematodes by using soil fungi developing activity against the parasitic eggs.

MATERIALS AND METHODS: Sixteen fungi specimens directly from the soil (5) and the faeces of grazing sheep (11) were collected and isolated after their serial culture onto Petri dishes with corn enriched agar. Once the isolation of each of the fungi was reached, two probes for determining their activity against different parasitic stages were conducted. Firstly, the action against *Calicophoron daubneyi* eggs was evaluated; secondly, the ability for reducing the presence of cyathostomin larvae was probed.
Among the fungi showing ovicide effect, the F11Ot was used for conducting a field trial. This test consisted of adding different doses of fungal spores to faecal pats collected from cattle passing *C. daubneyi*-eggs by faeces (average= 481 ± 67 eggs per gram of faeces). Five doses ($2.5 \cdot 10^4$, $5 \cdot 10^4$, $1 \cdot 10^5$, $2 \cdot 10^5$, $4 \cdot 10^5$) were assayed in quadruplicate during 52 days. A total of 120 5-g faecal pats were added the fungus in the current investigation and maintained under field conditions. The number of control faecal pats was 24.
The effect of the biological measure was studied by the copromicroscopical sedimentation technique. In each pat, the trematode eggs were counted and classified into viable, non-viable and empty. Four repetitions were examined under the microscope for each of the faecal pats.

RESULTS: Eight of the isolated fungi showed ovicide effect while four did it against larval stages. In the control pats, a percentage of viable eggs ranging from 63% to 81% throughout the study was observed. After adding the fungal spores to the stools, the *C. daubneyi* egg-viability oscillated between 80% and 28% for the D1 dose, 80%-13% for D2, 57%-10% for D3, 79%-9% for D4 and 56% and 19% for D5. The earliest reduction in the egg viability when using the highest dose of spores (D5) was reached. The percentages of egg-viability reduced to 50% by 18 days after the treatment of the faeces.
Statistically significant difference regarding the percentages of *C. daubneyi* eggs viability by using the Friedman test was demonstrated ($\chi^2 = 11.731$, $P = 0.039$). These differences were established among the control and the treated groups excluding the D1.

CONCLUSIONS: The possibilities for the control of trematode infection among grazing livestock could be improved by employing naturally-occurring soil fungi. In this way, successful results as early as 18-21 days after the addition of fungal spores to stools from *C. daubneyi*-infected cattle can be achieved. It is strongly encouraged the implementation of biological measures for helping to reduce the risk of infection in grazing livestock. Further investigations are on course for the molecular identification of the F11Ot fungus and also to establish the most appropriate methods for spreading it in the soil.

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Efficacy and safety of topical eprinomectin to control *Myocoptes musculinus* infestation in mice

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AIM: To assess the safety and efficacy of a high single dosage of pour-on eprinomectin in the control of the fur mite, *Myocoptes musculinus,* which is frequently occurring in laboratory mice. An effective single topical treatment would be practical, hence welcome.

MATERIALS AND METHODS: In January 2012, mice exhibiting excessive pruritus as the only clinical sign were signaled at a laboratory animal facility of the University of Turin. At that time, the facility housed approximately 2700 adult valuable transgenic mice. *M. musculinus* was identified as the single ectoparasitic agent involved. In the effort to develop a simple and rapid one-time treatment protocol, topical administration of eprinomectin (a last generation avermectin registered for use in cattle) was tested. A preliminary safety trial was conducted on 20 gravid females (term delivery at 24 h-10 days), which were isolated in individual cages and randomly assigned to one of two experimental groups of 10. Group A received pour-on eprinomectin (Eprinex® Pour-on, Merial Italia SpA, containing 0,5% active ingredient) at the approximate dosage of 5 mg/kg BW (27 l/mouse); Eprinex® Pour-on was topically applied to the skin in a single spot at the base of the neck. Control mice (Group B) received 27 l mineral oil on the same day. Mice were examined daily for any signs of illness or toxicity, including neurological abnormalities. Nests were individually weighted at 15 and 21 days post partum. In parallel, an efficacy trial was carried out on 20 naturally infected non-gravid females, which were randomly assigned to one of two experimental groups of 10, based on detection of fur mites and/or their eggs in a double (dorsal and ventral) pelage tape test. On Day 0, Group C received pour-on eprinomectin (same formulation, application and dosage as in the safety trial). Positive control mice (Group D) received 27 l mineral oil on the same day. Each group was observed 5 days/week during three weeks (corresponding to two life cycles of *M. musculinus*) for presence of pruritus. During each observation period of 15 min, all scratching, gnawing and muzzle cleaning acts were recorded and a "pruritus index" (acts/mouse/min) was calculated. The presence/absence of mites and/or their eggs was estimated of all mice by double (ventral and dorsal) pelage tape test on experimental days 7, 21 and 50.

RESULTS: In the safety trial, no acute toxicity was observed in 10 treated mothers and the corresponding 58 neonates. Gross behavior and appetite were not affected. All treated females gave full term delivery, and the number and weight of newborns were in the normal range of the corresponding lines. In the efficacy trial, treatment resulted in clinical improvement since the first week following Eprinex® Pour-on administration. The "pruritus index" was significantly (from <.01 to <.001) and remarkably (from 2.4 to 3.8 folds) lower in the treated group in each of the three experimental weeks. Pelage tape tests showed that all mites were eliminated. Actually, only empty and unviable desiccated eggs were observed in the treated group on days 7 and 21 (in 7 and 9 mice, respectively), and no egg or mite on day 50. Mites and/or viable eggs were observed in all untreated controls along the trial. No side effects or signs of ill health were observed in any of the treated animals.

CONCLUSIONS: A single topical administration of eprinomectin at the (high) dosage of 5 mg/kg BW was safe, efficacious, and rapidly relieved from pruritus a group of mice with natural infestation by the fur mite, *M. musculinus*. Under the conditions of this study, the tested single-dosage had similar efficacy as the single-dosage topical treatment with moxidectin (Pullium et al., 2005, *Contemp Top Lab Anim Sci* 44: 26-28), which is a well cognized operator-friendly option for control and eradication of the fur mite in large colonies of laboratory mice.

Efficiency and persistence of anthelmintics against intestinal strongyles of the horse

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AIM: To evaluate the field efficacy and persistency of five anthelmintics for the control of strongyles infection (SI) in horses.

MATERIALS AND METHODS: The study was carried out from February 2009 to August 2010 on 122 horses bred in Northern Sardinia. Inclusion criteria for the study were 1) ≥6 months of age 2) no anthelmintic treatment in the previous 10 weeks and 3) faecal egg per gram counts (FEC) ≥300 at preliminary examinations carried out 7 days (D) before the start of the study. Eighty four horses of various races (48 AAS, 11 SI, PSI 9 and 6 ponies), acknowledging such a criteria, were included in the study. Forty five were male horses, 39 female horses, the age ranged 6 months-26 years. Throughout the study, horses were managed in the same conditions. Based on FECs, age, sex and race, horses were divided into 5 treatment groups (TG) and one control group (CG): TG1, 15 horses treated with Eqvalan® (Merial), Product A – ivermectin (IVM); TG2, 17 horses treated with Eraquell® (Virbac), Product B, IVM; TG3, 10 horses treated with Equest® (Fort Dodge), Product C, moxidectin (MOX); TG4, 19 horses treated with Strike® (Acme), Product D, pyrantel pamoate (PYR); TG5, 13 horses treated with Panacur® (Intervet), fenbendazole (FBZ); CG, 10 untreated horses. Faecal samples were taken directly from rectum and FECs were carried out with McMaster technique using a flotation solution of NaCl (sd 1,150). Faecal examinations were carried out weekly throughout the first month of the study (on D 0, treatment day, and on D 7, D 14, D 21, D 28 post treatment) and each 15 days throughout the following 4 months (on D 45, D 60, D 75, D 90, D 105, D 120, D 135, D 150). Coprocoltures were carried out on pooled faces (each for treatment grop) On D 0, and then on all faecal samples with FECs ≥150. The efficacy of each anthelmintic was assessed by FEC reduction (FECR%) as suggested by Coles GC et al (1992, Vet Parasitol, 44: 35-44).

RESULTS: Accepting ≥90% FECR as effective (Kaplan RM et al., 2004, Am J Vet Med Assoc, 225: 903-910), IVM product A lasted effective until D 75 (FECR 93%), IVM product B until D 135 (FECR 91%), MOX until D 150 (FECR 94%), PYR until D 75 (FECR 91%) and FBZ until D 45 (FECR 97%). Higst FECRs were found on D 7, 14 and 45 (100% efficacy) for IVM A, on D 28 and 45 (100% efficacy) for IVM B, from D 7 to D 75 for MOX (100% efficacy), from D 7 to D 28 for PYR (98-99%) and from D 7 to D 28 for FBZ (99.5-99%), respectively. On D 135, 4.5 months from tretament, IVM A showed an efficacy of 76%, IVM B of 91%, MOX of 96%, PYR of 78% and FBZ of 14.5%, respectively. On D 150, 5 months from treatment, the efficacy of IVM A was 57%, of IVM B 88% and of MOX 94%, respectively. PYR and FBZ were no more effective.
Mean FEC of untreated horses ranged throughout the study between 802 and 1197, the latter value reached at the end of the test. Faecal cultures carried out on D 0 showed larvae of Cyathostominae (96.4%), *Oesophagodontus* spp. (1.5%), *Strongylus vulgaris* (1.3%), *Strongylus equinus* (0.1%), *Triodontophorus* spp. and *Strongylus edentatus* (0.01%), *of Gyalocephalus capitatus* (0.4%) and of *Trichostrongylus axei* (0.3%). Only Cyathostominae larvae were found in coprocultures carried out after treatment.

DISCUSSIONS: No evidence of anthelmintic resistance against commonly used anthelmintics for horses was found in this study. Among the molecules used the MOX showed significantly higher performance with an efficacy> 90% until the end of the trial (5 months).

Cattle Paramphistomosis: taxonomical, epidemiological updates in Sardinia and therapeutic field trials

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AIM: To give a contribute for the control of rumen fluke infection, we carried out a survey to update the tassonomical and epidemiological data in Sardinia and to evaluate the effectiveness of two molecules, such as albendazole and Oxyclozanide against this trematoda.

MATERIALS AND METHODS: Twelve adult specimens of Paramaphimstomidae were collected at 3 abattoirs in Sardinia from slaughetered cattle, in order to perform morphological and molecular classification, by DNA extraction and sequencing of the ITS-2 region according to Rinaldi L, 2005 (Vet Parasitol, 131(3-4):247-53). Faecal pools samples coming from 27 cattle herds bred at pasture (including various breeds as Sarda, Brown, Limousine, Charolais and their crosses) were examined in Sardinia during the year 2011, with sedimentation and flotation with zinc sulphate solution (s.g. 1,350) after centrifugation (2000g X 10 minutes).
For the field trial were used 27 cattle Brown x Sarda breed from 9 months to 10 years of age, naturally infected by paramphistomidae and bred at pasture in the province of Sassari.
Animals, according to the results of the copromicroscopic investigation carried out with the FLOTAC technique using a zinc sulphate solution (s.g. 1,350) at D0, were divided into two groups: Group A, with 14 cattle treated orally with a dose of 15 mg/kg BW of Albendazole (equal to 7,5ml/50kg of Valbazen® 10%- Pfizer) and Group O, with 13 cattle treated orally with a combination of Oxyclozanide/Levamisole, with a dose of 15mg/kg and 7.5mg/kg (equal to 25ml/50kg of Toloxan® - Intervet). Drug efficacy was evaluated using the Faecal egg count reduction test (FECRT), monitoring the field trial animals during D0, D14, D21, D45 and D75.

RESULTS: The molecular analyses of all the rumen flukes identified them as *Calicophoron daubneyi*. Paramphistomidae eggs were detected in faecal pools in the 66.7% of the monitored herds (18/27). Table 1 shows the average levels of eggs per gram of faeces (EPG) of Paramphistomidae detected in the two trial groups for the evaluation of the effectiveness of anthelmintics:

Table 1	D0 - EPG	D14 - EPG	D21 - EPG	D45- EPG	D75 - EPG
Group A	151.4	67.4	131.1	113.5	249.9
Group O	154.5	37.7	33.0	30.3	83.1

The Mann-Whitney test showed no significant difference between the means of EPG between the two groups at D0, as well as in Group A between D0 and the other days of monitoring (P> 0.05), whereas in the Group O highly significant differences were found (P <0.001) between D0 and all the other days of monitoring. The level of effectiveness (FECR) of the two drugs in both groups of animals is shown in Table 2.

Table 2	D14	D21	D45	D75
Group A	55.5%	13.4%	18.6%	0.0%
Group O	75.6%	78.6%	85.6%	46.2%

CONCLUSIONS: Our molecular results have shown for the first time in Sardinia the evidence of *C. daubneyi.* Results have confirmed the high prevalence of Paramphistomosis in cattle bred extensively in Sardinia, that in recent years seems to be significant increased compared with data reported by Scala A et al in 1997 (Praxis Veterinaria, 18(3): 10-13) (19.6% vs. 66.7% - 2 = 28.65, P <0.001). No therapeutic benefit was observed in animals treated with Albendazole, in contrast to what reported by other authors (Urquhart GM et al, 1998, Parassitologia Veterinaria, UTET, Torino).
Even with levels of efficacy that never have reached 90%, the association Oxyclozanide/Levamisole seems at present in Italy the only drug available on the market able to ensure a degree of effectiveness for at least 45 days after its administration against Paramphistomosis in cattle.

The influence of anthelmintic treatments on quali-quantitative milk production during post partum period in Sarda breed ewes

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AIMS: To evaluate the effects of anthelmintic treatments in sheep, we report the results of a field trial carried out on Sarda ewes treated against gastro-intestinal nematodes (GIN) during post-partum.

MATERIALS AND METHODS: The study was carried out on 106 sheep of Sarda breed ranging from 3 to 4 years of age, pluriparous, treated subcutaneously with 200mcg of moxidectin/kg bw (0.1 ml / 5 kg bw Cydectin® injection of 1%) in August 20, 2010. Approximately 10 days after the birth (10 ± 7 days), in November 8, 2010, the animals were divided into three groups: 1) Group T1, which was orally treated with 10ml of Cydectin®; 2) Group T2, which was orally treated with 10ml of Valbazen®; 3) Group C, without any treatment, as control. Faecal samples were examined, with McMaster technique, at the day of drenching (D0) and subsequently at D15, D45, D75, D105, D135, D165 and D195. Coprocoltures of pooled feces sampled at D0 were also performed in order to identify nematode genera. Efficacy of treatments was evaluated in each group through the following formula: *% efficacy = 100 x ((mean epg D0- mean epg Dn)/ mean epg. D0).*
From 10-20 days (about 50 days after birth) milk yield and contents were examined bi-monthly for 13 times. Fat, lactose and protein contents were determined by infrared method by Milkoscan (Foss Electric, Hillerød, Denmark). The statistical analysis on production data was performed with ANOVA test, according to the different groups of ewes and sampling dates.

RESULTS: Copromicroscopic analysis carried out during the test showed the following results:

Group	D0 epg Mean (ds)	D15 epg	D45 epg	D75 epg	D105 epg	D135 epg	D165 epg	D195 epg
C	274.2	347.4	581.8	710	460.9	429.1	583.9	252.9
T1	377.3	4	189	294	368	374	386.5	216
efficacy		*98.94%*	*49.91%*	*44.07%*	*3.16%*	*0.90%*	*0.00%*	*41.73%*
T2	305.2	16.2	221	324.2	304.6	302.4	341	195.6
efficacy		*94.69%*	*27.59%*	*0.00%*	*0.19%*	*0.92%*	*0.00%*	*32.14%*

No significant difference were recorded between EPG means of the three trial groups of sheep at D0. Coprocultures performed at D0 showed the presence of the following nematodes genera: *Teladorsagia* spp. 56%; *Trichostrongylus* spp. 15%; *Cooperia* spp. 20%; *Chabertia/Oesophagostomum* spp. 6%; *Haemonchus* spp. 3%. The average amount of milk measured in each sampling did not show any significant differences between the groups throughout the trial. In the following table are reported the mean values of fat, protein and lactose (*).

Group	mean % fat	mean % proteins	mean % lactose
C	5.84[a]	5.48[a]	4.72[b]
T1	6.11[c]	5.57[b]	4.64[a]
T2	6.00[b]	5.60[b]	4.65[a]
significance level - P	*0.000*	*0.003*	*0.027*

(*) The means in the columns with different letters indicate significant differences at P< 0.05.

CONCLUSIONS: The post-partum anthelmintic treatment following that given in the dry period, resulted a significant increase in milk yield in Sarda ewes. Further, a significant increase of the percentage of fat and protein in the treated groups was observed. Unfortunately, the overall results obtained confirmed the difficulty to predict the outcomes of these treatments in the presence of "border line" parasites burden and hence difficult to estimate the effects at these levels on productions in the post partum period, when ewes there were also treated before birth.

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Efficacy and safety of Imidacloprid 10 %/Moxidectin 1 % spot on formulation in the treatment of the feline infection by *Eucoleus aerophilus*

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Eucoleus aerophilus (syn. *Capillaria aerophila*) is a cosmopolitan trichuroid lungworm affecting domestic (e.g. cats, dogs) and wild (e.g. mustelids and foxes) carnivores. Adult stages live embedded in the respiratory epithelium of bronchioles, bronchi and trachea and cause damages to lung parenchyma. The infection induces a chronic bronchitis characterized by symptoms ranging from minimal bronchovesicular sounds to sneezing, wheezing, and chronic dry or productive cough. In the case of heavy parasite burden, the infection can be life-threatening due to bronchopneumonia and respiratory failure (Burgess H et al, 2008, Can Vet J, 49: 389-392; Traversa D et al, 2010, Parasit Vectors, 3: 62). Recently, *E. aerophilus* has been repeatedly reported in symptomatic and asymptomatic cats from different regions of Italy (Traversa D et al, 2008, Vet Par, 153: 182-186; Traversa D et al, 2009, Res Vet Sci, 87: 270-272). Although *E. aerophilus* is of veterinary concern and has also a certain zoonotic role, information on treatment protocols in cats are lacking.

AIM: The present work evaluated the efficacy and safety of a spot on formulation containing Imidacloprid 10 %/Moxidectin 1 % (Advocate®, Bayer Animal Health) in the treatment of cats naturally infected with *E. aerophilus.*

MATERIALS AND METHODS: The study has been conducted in 2009-2011 in seven sites from Central Italy, as a controlled, randomised, multicentric field trial according to VICH GL9 Good Clinical Practice. All cats were included according to pre-defined inclusion and exclusion criteria and allocated in a ratio of 1:1 to one of two study groups, i.e. Advocate® (i.e. treatment group) and untreated control group. Efficacy assessment was based on eggs per gram of faeces (epg) counts measured on two counts at Days 7±1 and 11±1 following treatment at Day 0, and compared to pre-treatment counts on Days -6±1 and -2±1. The highest epg value was used as baseline value from the egg counts performed in the pre-treatment assessment period 0 (two examinations for the sample collected on day -6±1 and two examinations for the sample collected on day-2±1). The analysis of the efficacy criterion was performed on the basis of reduction of post-baseline faecal epg counts using an analysis of covariance adjusted for baseline counts. Geometric means were calculated as: Gmean = $e^{AML} - 1$ where AML = the arithmetic mean of the epg counts. The difference between the geometric mean (GM) of epg before and after treatment was determined and expressed as an efficacy value using the following formula: efficacy % = 100 x (GM pre-treatment– GM post-treatment) / GM pre-treatment. The percentage decrease was considered effective if efficacy was at least 90%.

RESULTS: Thirty-six cats treated either with Advocate® (n=17) or left untreated (n=19) were included in the trial and no adverse events were observed. Geometric means of epg were 124.03 (baseline) and 0.26 (post-treatment) in the Advocate® and 107.03 (baseline) and 123.94 (post-treatment) in the untreated control group. Relative change from baseline was 99.79% in the treatment group, showing superiority of Advocate® spot-on compared to the untreated control group. Such superiority was proven by statistically significant differences in change of log-transformed counts from baseline ($P<0.0001$).

CONCLUSIONS: This study demonstrated the statistically significant superiority of the group treated with Advocate® spot-on compared to the untreated control group. Given that Advocate® spot-on is highly efficacious and safe in the treatment of lung capillariosis in cats under field conditions new avenues are now open for the treatment of feline infection by *E. aerophilus.* Other than the therapeutic efficacy, this spot-on formulation presents the major advantage of the possibility of a single dose and the easy-to-apply dermal administration.

Field efficacy study of alphacypermethrin pour-on against natural *Haematopinus tuberculatus* infestation on buffalo (*Bubalus bubalis*) and influence of the treatment on milk production.

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AIM: *Haematopinus tuberculatus*, is a specific louse of buffalo (*Bubalus bubalis*), being the principal ectoparasite which attaches this species (Bastianetto E et al, 2002, 1st Buffalo Symposium of Americas, 357-359), louse infestation often leads to skin irritation, anemia, anorexia, restlessness and loss of body condition. The itch caused by *H. tuberculatus* is responsible for the low milk and meat productivity (Bastianetto and Leite, 2005, Rev Bras Reprod Anim, 29: 118-121). The infestation needs to be controlled, particularly if the general condition of animals is affected. On buffalo, several formulations marketed for cattle have been tested in field trials against *H. tuberculatus*, mainly macrocyclic lactones such as ivermectin, avermectin, doramectin and eprinomectin (Veneziano V et al, 2004, Bubalus bubalis, 2: 56-65). Alphacypermethrin (ACYP) is a synthetic pyrethroid insecticide effective against a wide range of pests of many crops and is used for the control of various veterinary insects, including lice. In Italy ACYP is marketed as a pour-on formulation for use in cattle, with zero milk-withdrawal time. Therefore, the aim of this study was to assess the efficacy and safety of ACYP pour-on against *H. tuberculatus* on naturally infested buffaloes and the influence of the treatment on milk production.

MATERIALS AND METHODS: The study was performed on 56 adult buffaloes, naturally infested by *H. tuberculatus,* at 86.8±60.9 days in milk (DIM) bred in a commercial farm in Southern Italy. One day before the treatment (day -1) all animals were divided into two Groups (28 buffaloes in each Group), according to DIM, total milk production recorded in the previous year, milk production in the last seven days and louse counts. On day 0, ACYP-group received ACYP pour-on formulation at the manufacturer's recommended dose rate (Renegade™, 1.5%, Pfizer Animal Health). Control (C-Group) received pour-on saline solution. The parasitological investigations were performed on 20 buffaloes (10 in each Group). Louse counts were performed on days -1, 7, 14, 21, 28, 35, 42, 49 and 56 at eight predilection sites on the skin of each buffalo, all according to the procedures described in the WAAVP guidelines for evaluating the efficacy of ectoparasiticides in ruminants (Holdsworth PA et al, 2006, Vet Parasitol, 136: 45-54). Milk production was daily recorded by software connected with an automatized milking machine throughout the experimental period. Statistical analysis of the data was performed by ANOVA for repeated measures.

RESULTS: On day -1 an average of 77.9 ±46 and 66.7±33 lice per buffalo were counted in ACYP and C groups, respectively. During the trial, ACYP was well tolerated by all the animals since there were no adverse reactions following the treatment. ACYP was completely effective (100%) at day 7, highly effective (99.7%) at day 14, and completely effective (100%) from day 21 until the end of study, day 56 after treatment. Total milk production throughout the experimental period was not significantly different between Groups, although buffaloes in ACYP-Group showed an increase of about 0.2 kg/day compared to C-group (10.4±1.2 vs 10.2±1.1, respectively). A significantly higher ($P<0.05$) milk yield was recorded in ACYP Group from day 14 to day 35 of the trial, when the animals produced meanly 0.3 kg/day of milk more than those in C-Group (11.1±1.7 vs 10.8±1.4, respectively). A further statistical analysis was carried out dividing the buffaloes according to their DIM. In this case, in the animals that were at less than 75 DIM at the beginning of the trial, a higher ($P<0.01$) milk yield was recorded from day 14 to day 42 of the trial in ACYP Group (12.2±1.4 vs. 11.7±1.4, ACYP and C-Groups, respectively). No differences were found among buffaloes with more than 75 DIM.

CONCLUSIONS: The results of this field trial suggest that ACYP is effective, safe and user-friendly compound suitable for the treatment of buffaloes with natural louse infestation. Furthermore, the higher milk production recorded, particularly in animals at the beginning of lactation, justifies the cost of the treatment.

Treatment of natural infestation of the chewing louse (*Werneckiella equi*) on donkeys using alphacypermethrin pour-on.

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AIM: Donkeys and horses may be infested with two species of louse, the chewing louse, *Werneckiella equi* and the sucking louse *Haematopinus asini*. *W. equi* feeds on the most superficial layers of the skin and louse infestation tends to be seen in unkempt animals and often leads to hyperkeratosis, alopecia, anorexia, restlessness and loss of body condition (Wright, 1999, Can Vet J 40: 590-591). *W. equi* has been found on horses worldwide, however, there is a paucity of literature on the presence of *W. equi* on donkeys. Alphacypermethrin (ACYP) is a synthetic pyrethroid insecticide effective against a wide range of pests of many crops and is used for the control of several veterinary insects, including lice. In Italy, ACYP is marketed for cattle as a pour-on formulation. Therapeutics, such as antiparasitic compounds, are often administered to donkeys based on dosage and intervals recommended for horses and cattle, because very few drugs have donkey-specific label indications (Grosenbaugh et al, 2011, Equine Vet Educ, 23: 523-530). The literature lacks information on the use and the efficacy for donkeys of most insecticides. Therefore, the aim of the present study was to evaluate the field efficacy of ACYP pour-on against naturally occurring infestation of *W. equi* on donkeys.

MATERIALS AND METHODS: The trial was performed in a donkey farm, consisted of 40 donkeys, located in Southern Italy. In the absence of standardized guidelines for the quantification of lice on equids, the WAAVP guidelines used to evaluate the efficacy of ectoparasiticides in ruminants (Holdsworth PA et al, 2006, Vet Parasitol, 136: 45-54) and the louse counting procedures described for horses (Lowden S et al, 2007, Vet Parasitol, 148: 295-300) were used. On the day before treatment, louse counts were performed on 13 naturally infested donkeys by recording the individual louse count at seven louse predilection sites: head, neck/mane, shoulders/withers, foreleg, back, hindleg and tailhead/rump. For each count, the hair at the site (about 10 x 20 cm area) was parted with a comb and, the part inspected for a length of approximately 10 cm for live (motile) lice. On day 0 the study animals received ACYP pour-on (Renegade 1.5%, Pfizer Animal Health) at the manufacturer's recommended cattle dose rate. The formulation was applied topically along the midline of the back from the withers to the tailhead. There was no untreated control group for animal welfare reasons. Louse counts were performed weekly (day – 1, 7, 14, 21, 28, 35, 42, 49 and 56) by summing all predilection site counts. The efficacy (%) of ACYP was determined in terms of percent louse reduction using Abbott's formula as proposed by WAAVP guidelines: Efficacy = 100 x [(C-T)/C], where T = louse count after the treatment and C = louse count before the treatment. The Body Condition Score (BCS) of each animal was determined prior to treatment (day -1) and at the end of study (day 56) using the BCS chart (The Donkey Sanctuary, 2003). Significant differences between sets of data were carried out using ANOVA.

RESULTS: In total 1,140 *W. equi* were recorded from the inspection sites on 13 study donkeys on day -1 (mean 87.7±72.9). On the majority of the animals (76.9%) more than 40 lice were found prior to treatment. The infestations were variable between the study donkeys with counts ranging from 25 to 240 lice. More than the half of the louse burden was found in the area along the neck/mane (21.7%) and shoulders/withers (29.4%). The back, foreleg, head and hindleg sites contained 16.7, 14.0, 10.7 and 7.5% of the counted lice, respectively. No lice were found on tailhead/rump. For all post-treatment days of inspection, no lice were counted at the inspection sites or during whole body inspections; resulting in an efficacy of 100% for days 7-56. No abnormal animal health conditions related to treatment were observed during the study. The BCS values were in the normal range for donkeys and did not show a significant ($P > 0.05$) difference before and after treatment.

CONCLUSIONS: This field trial demonstrates that ACYP applied pour-on at the manufacturer's recommended cattle dose rate was completely effective, safe and user-friendly for the treatment of *W. equi* on donkeys.

SESSIONE 2

ENTOMOLOGIA MEDICA E VETERINARIA

Sand fly-borne diseases: sand fly seasonal dynamics and search for *Leishmania* and Phleboviruses in two different areas of Rome province, Italy

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Sand fly distribution has changed in European countries in the last decades (Ready PD, 2010, Euro Surveill, 15: 19505) causing an increase of human and canine leishmaniasis, as well as a spread of human infections by known and/or novel phleboviruses (Depaquit J et al, 2010, Euro Surveill, 15: 19507; Maroli M et al, 2012, Med Vet Entomol, in press). *Leishmania infantum* associated to *Phlebotomus (Larroussius)* vector species still represents the main risk for human infections in Europe. Recent investigations have indicated that Phlebovirus genetic variability within the Mediterranean is higher than initially suspected. In this scene a collaborative FP7 EU project has launched the mapping of phlebotomine sand fly-borne diseases as one of the main objectives.

AIM: To evaluate the population dynamics and the presence of natural *Leishmania* and Phlebovirus infections in *Phlebotomus perniciosus*, the main Italian leishmaniasis vector species, in a rural site at east of Rome. Search for these agents was also performed in a different sand fly species, *Phlebotomus perfiliewi*, a proven vector of *L. infantum* and Toscana virus in Italy, in a rural area at west of Rome 35 km from the first site. Both sites have long been investigated for sand fly monitoring and found to be *Larroussius* monospecific depending on collection methods employed (unpublished data).

MATERIALS AND METHODS: For *P. perniciosus* population dynamics, collections were performed monthly from April through November 2011 by CDC light traps and sticky papers (SP). CDC traps were operated for two consecutive nights whereas SP were left for 48 hours inside drainage holes. Sand fly number and species, sex, habitat, min and max temperature and humidity, mean precipitations and wind speed registered during the days of collection, were entered in a standardized database for further analyses. *Leishmania* infections in *P. perniciosus* were searched in specimens collected from August through October, whereas Phleboviruses detection was investigated in specimens collected from July through September. As regards the search for natural *P. perfiliewi* infections, samples were collected by CDC light traps set one night inside animal shelters in July, September and October. For both sand fly species, PCR analyses were performed to detect *Leishmania* DNA (Alcover MM, 2012, Parasitol Res, doi: 10.1007/s00436-012-2863-4) and Phlebovirus RNA (Sànchez-Seco MP et al, 2003, J Med Virol, 71: 140-149) in females or in both sexes, respectively. Phleboviruses isolation was also performed in VERO-cell culture isolation (Verani P et al, 1988, Am J Trop Med Hyg, 38: 433-439).

RESULTS: Collections performed in the "*P. perniciosus* site" gave a total of 2949 phlebotomine specimens (58.7% males). Presence of 2 species only was confirmed, *Sergentomyia minuta* being the prevalent one (84.9%). The target *Larroussius* species showed a typical bimodal density peaks in June and August (170 and 193 specimens, respectively). In June most of *P. perniciosus* specimens were collected by SP (138) whereas in August by CDC traps (160). Peaks where characterized by absence of precipitation and low wind speed (1.2-1.5 m/s); temperature and relative humidity were in a range of 23-28.5°C and 65.7-43.3%, respectively. Both ranges may explain different yields obtained by catching methods during the two peaks. Forty-four females were analysed for *Leishmania* and 162 specimens (67.9% males) for Phleboviruses. For *Leishmania* analysis insects were pooled by collection date (3-10 specimens/pool). One pool of 6 specimens out of a total of 6 pools examined was *Leishmania* positive, for an estimated general infection rate of 2.3%. For Phleboviruses detection, sand flies were grouped in 10 pools (3-36 specimens/pool) which tested negative by both methods employed. Investigations in "*P. perfiliewi* site" confirmed its monospecific nature when CDC collections are employed. A total of 161 specimens (16.1% males) were collected. For natural *Leishmania* and Phlebovirus infections, 25 (3 pools) and 136 specimens (8 pools) were analysed, respectively. Neither *Leishmania* nor Phleboviruses were detected.

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Antifeeding activity of *Azadirachta indica* (Neem) extract against sand fly bites on dogs

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Antifeeding agents are important tools for host's protection from sand fly bites and hence prevention from sand fly-borne diseases. Chemical repellents or insecticides are the mainstay for the protection of hosts because of elevated and/or durable efficacy. However, in many communities there is a decreasing compliance with chemicals exhibiting potential adverse effects, that may represent a drawback for the control programs against sand fly-borne diseases.

AIM: to evaluate the antifeeding efficacy of a natural compound, *Azadirachta indica* (Neem) extract (RP03™, 2400 ppm azadirachtin) against *Phlebotomus perniciosus* in dogs. We report herein preliminary results suggesting a potential use of this product as an additional measure for dog protection from sand fly bites and hence from *Leishmania* infections.

MATERIALS AND METHODS: The experiments were performed on 5 beagle dogs after owner's informed consent. They were designed to determine a minimal effective dose of RP03™ and to evaluate its anti-feeding efficacy after different treatment regimens, taking into account the low stability of the product under natural conditions. Data from preliminary studies indicated that dog's attractiveness to colonized *P. perniciosus* may vary considerably when experiments are performed in field conditions; for example, in one experiment the same batch of flies used in parallel on 2 dogs resulted in 10.0% and 51.7% blood-feeding rate, respectively. Therefore, each dog served as own control through a sand fly feeding test performed before treatment. Topical administration of the compound was made by spraying the head. The dog was sedated and the head inserted into a cage containing about fifty 3-7 day-old unfed *P. perniciosus* females, which were recollected after 1-hour exposure. Protection from sand fly bites was estimated considering the rate of blood-fed flies compared with the pre-treatment rate. If less than 15% of flies took a blood meal on the dog before treatment, the test was considered invalid.

RESULTS: In dog 1, a low dose of 1.75 ml of 1000 ppm azadirachtin was given once and the sand-fly test performed at 72 h from treatment, resulting in no protection. Dog 2 received a sub-dose (1.25 ml of 2400 ppm azadirachtin) during 7 consecutive days ("conditioning treatment") plus a full dose (2.5 ml) on day 8, the sand fly test being performed at 48 h from last treatment. A significant 74.9% protection from bites ($p=0.044$) was recorded. To verify whether the treatment could be administered intermittently without loss of protection, dog 3 received the same "conditioning treatment" as above, plus full doses on day 8 and 10, and sand fly test at 48 h from last treatment. Results showed similarly high activity (67.6%, $p=0.008$) suggesting that intermittent treatments could maintain over time elevated protection against *P. perniciosus* bites. To verify if protection could last longer than 48 h after last treatment, dog 4 was treated as dog 2 and sand fly tests were performed at 24 h and 7 days from last dose. Surprisingly, a significant protection detected at 7 days (89.2%, $p<0.001$) was even higher than at 24 h from treatment (63.0%, $p=0.023$). Hence, RP03™ efficacy seems to increase after a "conditioning treatment" independently from the length of the treatment gaps. Finally, dog 5 was excluded from the trial because only 12.2% of flies fed in pre-treatment test. By grouping all blood-feeding data in pre- and post-treatment exposures of dogs 2-4, the proportion of fed *P. perniciosus* before treatment was 30.7%, with a wide SE range (± 7.8%) reflecting the individual dog variability in sand fly attractiveness. After treatments, the mean percentage of fed females decreased to 8.1%, with a much narrow SE range (± 2.6%) suggesting a homogeneous response of *P. perniciosus* to the natural compound. These values gave an estimate of 73.6% protection against sand fly bites. The statistical analysis made considering each sand fly as an independent variable resulted very robust, giving an estimate of 81.3% maximum protection ($p<0.0001$) and 0.28 (95% CI 0.1686-0.4650) relative risk.

Altogether our results suggest that the repeated use of RP03™ (2400 ppm azadirachtin) during the sand fly season can be a valid tool for protection of dogs against *P. perniciosus* bites.

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Argas transgariepinus, White 1846 (Acari: Argasidae) and *Ixodes festai* Rondelli, 1926 (Acari: Ixodidae): two novel ticks for the Apulia region

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AIM: To update the checklist of the tick fauna for the Apulia region by reporting the finding of two novel tick species. The ectoparasites were identified respectively as *Argas (A.) transgariepinus* and *Ixodes (I.) festai,* using morphological keys (Manilla G, 1998, Acari Ixodida In: Fauna d'Italia, Calderini Edizioni, Bologna; Iori A et al, 2005, Zecche d'Italia, Mappe parassitologiche, Rolando Editore, Italy) and description of morphological characters (Gilot B, Pérez C, 1978, Revue Suisse Zool, 85: 143-149; Manilla G, 1991, Parassitologia, 70: 197-206).

Argas transgariepinus (Argasidae, *Argas*). In August 2006, the Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata was contacted to identify a parasite collected in an apartment in Ortanova (Foggia, Apulia region). It was identified as a male specimen of *Argas transgariepinus* and preserved in a vial in 70° alchool. Anamnesis revealed the removal of a bat colony from the roller blind head box in the infested room, several months before. *A. transgariepinus* was first originally described by White in 1846 who observed two females from the Gariep region, South Africa. In Italy, female specimens were first collected by Berlese in 1913 in a building in Florence (Tuscany) and on the dress of a girl in Riposto (Catania, Sicily), respectively (Berlese A, 1913, Redia, 9: 118-119). In the period from 1956 to 1962, immature stages of this tick were captured on microchiroptera in Sicily, Tuscany, Liguria, Latium whereas free-living larvae were recorded in Piedmont (Sobrero L, Manilla G, 1988, Bonifica, 2: 63; Manilla G, 1998, ibid.) The last finding of *A. transgariepinus* consisted of three free-living female specimens collected in dwellings in Perugia (Umbria) where quite interestingly dermatological skin disorders occurred in the human inhabitants (Principato M, 2002, Atti XIX Congresso Nazionale Italiano di Entomologia, 1153-1157). Previous to this report, three Argasidae tick species have been recorded in the Apulia region (Manilla G, 1998, ibid.); Iori A, Di Giulio A, De Felici S, 2005, Zecche d'Italia, Mappe parassitologiche, Rolando Editore, Italy). Considering the present note, their number has now risen to 4 for the region. To the best of our knowledge, this is the first record of a male specimen of *A. transgariepinus* in Italy.

Ixodes festai (Ixodidae, *Ixodes*). Two female ticks collected in the Bosco Incoronata Regional Park, Foggia by dragging, respectively in December 2011 and January 2012, were identified as belonging to the *Ixodes festai* tick species (Raele D *et al*, 2012, this issue). *I. festai* is a bird tick originally described by Rondelli in 1926 from a female specimen on *Alectoris barbara* (Phasianidae) in Derna (Libya). The species was confused for a long time with *I. ventalloi*, Gil Corrado, 1936 which is usually a rabbit ectoparasite at each development stage. The species were definitively split by Gilot and Pérez in 1978 (Gilot B, Pérez C, 1978, Revue Suisse Zool, 85: 143-149). *I. festai* mainly occurs in Western Mediterranean (Tunisia, Morocco, Southern France, Corsica, Libya). In Italy, it has previously been collected only on Tyrrhenian islands (Sardinia, Ventotene, Montecristo) from avian hosts (Contini C, 1998, Parassitologia, 40: 37; Iori A et al, 2004, Parassitologia, 46: 134; Contini C et al, 2011, Parasite 18: 235-240) and from mammal hosts (Garippa G et al, 1998, Parassitologia, 40: 70), respectively. This note report for the first time the presence of *I. festai* in Apulia region. Considering this finding, the number of Ixodidae tick species has now risen to 21 for the Apulia region.

Entomological survey of phlebotomine sand flies (Diptera: Psychodidae) in Emilia-Romagna region, northern Italy, 2005-2011

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Emilia-Romagna region, northern Italy, is known to be endemic for human visceral (VL) and canine leishmaniasis (CanL). In early seventies of the last century, a VL outbreak occurred in the region with 11 fatal cases (Pampiglione S et al, 1974, Trans R Soc Trop Med Hyg, 68: 349-359). The recent occurrence of VL and CanL cases (Maroli M et al, 2008, Trop Med & Int Health, 13: 256-264; Baldelli R et al, 2001, Parassitologia, 43: 151-153) as well as of summer meningitis cases due to the Toscana Phlebovirus (TOSV), have stimulated new entomological surveys aimed to ascertain the current status of the competent vectors of both the diseases.

AIM: In this paper we report the preliminary results of the sand fly monitoring carried out from 2005 through 2011 in the frame of the regional surveillance for leishmaniasis, along with results from dedicated collections performed in sites where VL, CanL and TOSV meningitis cases have occurred.
The Emilia-Romagna region consists of nine provinces and nearly half of the region (48%) is lowland while 27% is hilly and 25% mountainous. The region's section of the Apennines is marked by areas of "calanques" and caves. The plain was formed by the gradual retreat of the sea from the Po river basin and by the detritus deposited by the rivers. With the exception of Ferrara province, all provinces are bordering with the Apennines.

MATERIALS AND METHODS: During the period of sand fly collection, a total of 133 sites were monitored in the whole region, of which 74 sites (55.6 %) were kennels enrolled in the frame of regional leishmaniasis surveillance for CanL control; 28 sites (21.1%) were human dwellings monitored after the occurrence of VL, CanL and TOSV cases; the remaining 31 sites (23.3%) consisted of farms. Different collecting methods were employed: (i) from June to October, oiled sticky papers and CDC miniature light and CO_2 baited traps were used bi-monthly in the kennels enrolled; (ii) CDC miniature light traps were employed weekly for 3 consecutive weeks in human dwellings and their surroundings; (iii) CDC miniature light or CO_2 baited traps were used once in the surroundings of TOSV summer meningitis cases; (iv) in 2006, samplings were made in farms by using black light traps; (v) in 2009 and 2010, a site was monitored using different types of traps with the aim to assess their efficiency.

RESULTS: During the whole surveyed period a total of 75,927 sand fly specimens were collected of which 5,221 (6.8%) have been identified so far at species level. By sorting data according to collecting site categories, 44/79 kennels (59.5%) resulted positive for sand flies and produced 4,026 sand flies, all identified at species level. *Phlebotomus perfiliewi* was the most abundant species recorded (98.2%) followed by *P. perniciosus* (1.7%) and *Sergentomyia minuta* (0.1%). Nineteen out of 28 collecting sites (67.8%) located in human dwellings and their surroundings, were positive for sand flies. Out of 7,656 specimens caught, 967 (12.6%) have been identified so far. *P. perfiliewi* was found to be very abundant (99.3%), only 6 *P. perniciosus* being found. Most of the phlebotomines caught (64,245 = 76.8%) was from farms, particularly from two sites in Forli-Cesena province. The presence of cows in great number could have favored elevated sand fly densities in this sites, particularly *P. perfiliewi* (Maroli M et al., Parassitologia, 36: 251-264, 1994). The species identification of this huge number of sand flies is still in progress.

CONCLUSIONS: The results from a small sample (228 specimens) show that in this particular habitat only two species are present, namely *P. perfiliewi* (86%) and *P. perniciosus* (14%). As regards the geographical distribution of sand flies, all provinces were found positive. Of particular interest are the sand fly specimens caught in Ferrara and Ravenna provinces, respectively 2 and 108 *P. perfiliewi*, being the first time that phlebotomine sand flies are recorded in these lowland territories. Moreover it should be pointed out that 36/67 (53.7%) collecting sites located at low altitudes (<80 m a.s.l) were positive for sand flies (743 specimens). Our preliminary data show that two competent vectors, *P. perfiliewi* and *P. perniciosus* are widely distributed in Emilia-Romagna, probably both supporting the transmission of leishmaniasis and summer meningitis due to TOSV.

The "auto-dissemination" approach: a novel concept to fight *Aedes albopictus* in urban areas

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AIM: The main constraint to the fight against container-breeding mosquitoes vectors of human arboviruses is the difficulty in targeting a multiplicity of larval sources, mostly represented by small man-made water containers. The aim of this work is to assess the feasibility of the "auto-dissemination" approach, already tested for *Aedes aegypti* (Devine GJ et al, 2009, Proc Natl Acad Sci USA, 106: 11530-11534), as a possible alternative to traditional and scarcely efficient control approaches for *Aedes albopictus* in urban areas. The approach is based on the possibility that wild adult females exposed to artificial resting sites contaminated with pyriproxyfen disseminate this juvenile hormone analogue into larval habitats, thus interfering with adult emergence.

MATERIALS AND METHODS: We carried out four field experiments in two *Ae. albopictus* areas of Rome that are typically highly infested, i.e. the main cemetery (Site1) and a small green area within a highly urbanised neighbourhood (Site2). In each experiment, 10 pyriproxyfen "dissemination" stations (DS), and 10 "sentinel" (SS) plus 10 control (CS) sites, each containing 25 III-instar larvae, were located in the study areas. Mortality was monitored until completion of adult emergence.

RESULTS: We observed 20% and 50-70% mortality at the pupal stage in SS when a 0.5% and 5% pyriproxyfen formulations were used to contaminate the DSs, respectively, while mortality in CS was <2.5%. In Site1, no significant differences in mortality in SSs *vs.* CSs were observed in the first experiment, using a 0.5% concentration (K-S, p=0.164). However, at 5% concentration mortality in SSs was higher than that observed in CSs (K-S, p<<0.001). In Site2, in which a 5% pyriproxyfen concentration was used in both experiments, the mortality was higher in SS *vs*. CS (K-S: first replicate, p=0.055; second replicate, p=0.015). The results of the mixed-effect logistic regression model showed that mortality was always significantly higher in SSs than in CSs: 9- and 66.5-fold higher in Site1 with 0.5% and 5% pyriproxyfen, respectively, and 49- and 37-fold higher in the two replicates carried out in Site2. In the replicate carried out in this site mortality was mostly concentrated in 3 out of 10 SSs, while in the second one it ranged between 40 and 100%. In both experiments carried out in Site2, a mortality higher than 76% was observed in 5 out of 10 SSs, 4 of which were located in the same position in both replicates.

CONCLUSIONS: A number of evidences support the working hypothesis and suggest that auto-dissemination could represent a valid novel approach to reduce *Ae. albopictus* densities in urban temperate areas. First, we observed significantly higher mortality in our SSs than in CSs, showing that pyriproxyfen was actually transferred by mosquitoes into sentinel sites and elicited a lethal effect. Second, the observed mortality was not uniformly distributed among sentinel sites, strongly suggesting that this was due to pyriproxyfen contamination by mosquitoes visiting some sites, but not others. Third, at the cemetery, a 10-fold increase of the pyriproxyfen concentration resulted in a 3-fold increase in mortality in sentinel sites. Overall, the results strongly support the potential feasibility of the auto-dissemination approach to control *Ae. albopictus* in urban areas. Further studies will be carried out to optimize the method in order to provide an effective tool to reduce the nuisance of this aggressive species and the risk of transmission of arboviruses, such as Dengue and Chikungunya, recently transmitted by endemic *Ae. albopictus* populations also in Europe.

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Prevalence, species composition and detection of piroplasms in ixodid ticks of cattle and sheep in Oromia Regional State (Ethiopia)

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AIM: Ethiopia possesses huge population of domestic ruminants, which constitutes an important source of food and income for rural communities. Besides, hides and skins represent one of the major export items of the Country. Nevertheless, animal productivity is poor and parasitic diseases are one of the major constrains. Ticks are widely distributed in Ethiopia and affect ruminants, particularly because of losses in productivity, damages to hides and skins and transmission of diseases. The aim of the present study was to determine tick species affecting cattle and sheep in Bako District, western Oromia. Tick burden and transmitted piroplasms were also investigated.

MATERIALS AND METHODS: Ticks were collected from October 2010 to April 2011, from cattle and sheep kept in extensive or semi-intensive management systems. Collected ticks were preserved in 70% ethanol and identified to species level according to the standard identification keys (Walker AR et al, 2003, Tick of domestic animals in Africa: A guide to identification of species, Bioscience report, 42, Edinburgh, UK). Differences in prevalence values between host species were evaluated using Pearson's chi-square test. Biomolecular analyses were conducted on 127 *Rhipicephalus* (*Boophilus*) *decoloratus* and 91 *Rh. evertsi evertsi*, in order to detect the presence of piroplasm species. DNA from ticks was extracted using NucleoSpin® Tissue (Macheray-Nagel, Düren, Germany) and samples were screened by PCR for the presence of piroplasms as previously described (Centero-Lima S et al, 2003, Trop Med Int Health, 8: 760-764; Herwaldt BL et al, 2003, Emerg Infect Dis, 9: 942–948). Samples positive for piroplasms were sequenced on both strands by BMR Genomics (Padova, Italy). The consensus sequences were obtained using ChromasPro (Version: 1.42) and compared with sequences available in GenBank™.

Table 1. Prevalence and number of collected ticks

Host species	Tick species	*Pos. (host)*	*Prev. (%)*	*N (ticks)*
cattle (N=238)	*Am. variegatum*	46	19,3%	75
	Am. cohaerens	176	74,4%	518
	Rh. decoloratus	102	42,9%	236
	Rh. e. evertsi	69	29,0%	170
sheep (N=40)	*Am. variegatum*	6	15,0%	10
	Am. cohaerens	20	50,0%	59
	Rh. decoloratus	17	42,5%	51
	Rh. e. evertsi	23	57,5%	120

RESULTS: Overall, 1,246 ticks were collected from 238 cattle and 40 sheep. A total of 8 tick species were encountered: *Amblyomma variegatum* (n=85), *Am. cohaerens* (n=577), *Rh.* (*Bo.*) *decoloratus* (n=287) and *Rh. eversti eversti* (n=290) in cattle and sheep, whereas *Am. gemma* (n=3), *Hyalomma marginatum rufipes* (n=1), *Rh. pulchellus* (n=2) and *Rh. praetextatus* (n=1) only in cattle. Tick burden ranged from 1 to 22 ticks, with an average of 3.98 per animal. Prevalence and number of prevalent tick species are reported in Table 1. *Am. cohaerens* prevalence in cattle was significantly higher than in sheep ($p<0.01$), whereas *Rh. eversti eversti* showed host preference for sheep ($p<0.001$).
Piroplasm species were identified in 6 ticks. In particular 4 ticks (3 *Rh. decoloratus* and 1 *Rh. e. eversti*) from 2 cattle were found positive for *Theileria buffeli/sergenti/orientalis* (ID ranging from 95% to 99%; GenBank a.n. FJ225391, HM538209), 1 *Rh. decoloratus* from a cattle for *Theileria velifera* (ID 96%; GenBank a.n. FJ869897) and 1 *Rh. e. eversti* from a sheep was positive for *Theileria ovis* (ID 95%; GenBank a.n. FJ603460).

CONCLUSIONS: Results of the present study showed generally low tick burden probably due to collection of ticks during the dry season. Biomolecular investigation suggests a low circulation of mildly pathogenic species of piroplasm among cattle of the study area. Similarly, only one *Rh. e. eversti*, out of 26 specimens collected from sheep and examined, was found positive for *Th. ovis*.

Comparative evaluation of two different sampling methods for *Culex pipiens* and other mosquitoes species

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AIM: Mosquito traps are designed to capture insects both for control and monitoring systems. Each category of trap has different advantages and disadvantages, according to the aim of collection, i.e. species of mosquitoes to capture and/or analyses to perform in vector-borne disease surveillance programs. The purpose of this study was to compare the field efficacy of BG-Sentinel (BG-S) traps and CDC traps in catching different species of mosquitoes.

MATERIALS AND METHODS: BG-Sentinel traps and CDC traps, both baited with CO_2, were deployed in four rural sites, located in the province of Rovigo, Veneto region, North Eastern Italy. Sampling with BG-S was performed in the frame of the European Project FP7- Health- 2010-Eurowestnile, while sampling with the CDC trap was performed during the Veneto surveillance activities for West Nile virus, in place since 2008 (Busani L et al, 2011, Epidemiol Infect, 139: 818-25). The two trap types were simultaneously placed at each site every two weeks from June through September. The traps were activated from sunset to the early next morning. Mosquitoes collected were counted and identified, using standard morphological keys (Severini F et al, 2009, Fragmenta entomologica, 41: 213-372). The differences among the collected mosquitoes/site for each species were compared by ANOVA, after $\log_e$ transformation of the data.

RESULTS: Overall, 3445 mosquitoes were captured using BG-Sentinel traps and 8835 mosquitoes using CDC traps, belonging to 7 species (*Culex pipiens, Ochlerotatus caspius, Aedes albopictus, Culex modestus, Anopheles maculipennis, Culiseta annulata* and *Adedes vexans*). The CDC trap collected significantly more *Cx. pipiens* than BG-Sentinel trap in the four sites. As expected BG-S demonstrated to be more specific for collecting *Ae. albopictus* (Farajollahi A et al, 2009, 46: 919-25). Furthermore BG-Sentinel trap caught more *Oc. caspius* than CDC although this difference was not significant (p=0.094). The table below shows a comparison between mosquitoes captured by the two trap types.

Species	CDC-CO_2 mean mosquitoes/site	BG-S mean mosquitoes/site	P value
Culex pipiens	237.97	38.13	<0.01
Aedes albopictus	1.00	10.78	<0.01
Ocherotatus caspius	14.75	55.19	0.094

CONCLUSIONS: The CDC traps proved to be more efficient to collect *Cx. pipiens* confirming to be suitable for WNV surveillance in this area, where *Cx. pipiens* is the only known WNV vector. The BG-S trap collected *Ae. albopictus* more efficiently than CDC trap, confirming previous data reported in literature. Notably, BG-S trap seems to be more useful for the collection of *Oc. caspius*, an other important vector species of other arboviruses and filariae.

Preliminary results on *Rattus norvegicus* as a host for immature stages of *Rhipicephalus sanguineus*

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AIM: The brown dog tick *Rhipicephalus sanguineus* is widespread all over the world and is implicated as a vector of numerous pathogens of dogs, occasionally affecting other mammals, including humans (Dantas-Torres F, 2010, Parasit Vectors, 3: 26). A parasite primarily associated with dogs, *R. sanguineus* has been found on different host species, although the role of these animals in the maintenance of natural populations of this tick is poorly known. In this regard, cases of natural infestation by *R. sanguineus* on Norwegian rats (*Rattus norvegicus*) have been reported in the literature (Abd El-Halim et al, 2009, J Egypt Soc Parasitol, 39: 617-624), but little is known about the role of this rodent in maintaining populations of this tick in urban settlements. This study was aimed to evaluate the role of this common synanthropic rodent as a possible host for *R. sanguineus*.

MATERIALS AND METHODS: Two tick-naïve Norwegian rats were experimentally infested with immature stages of *R. sanguineus*. In particular, the first tick-naïve rat was infested with 270 larvae. The second tick-naïve rat was infested with 80 nymphs obtained from larvae collected from the rat used before. Rats were restricted in a cage over waste trays covered with paper towels and with double-side tape around the edge of the tray to prevent tick escape. Engorged ticks dropped from the host were collected on the paper towels and removed with a brush. All the developmental stage of ticks were kept in a climatic chamber at 26°C, 80% RH and a 16:8 light-dark photoperiod.

RESULTS: After the first infestation with 270 larvae, 180 (67%) successfully engorged. However, only 85 of them (47%) moulted to nymph. After infestation of the second rat with 80 nymphs, the rate of recovery of engorged nymphs was very low (4%) and only three nymphs successfully moulted to adult. Considering the initial load of larvae, the overall rate of moulting was 31.5% for nymphs and 1.1% for adults.

CONCLUSIONS: The low number of ticks recovered in this study suggests that Norwegian rats might not be suitable hosts for the completion of the life cycle of *R. sanguineus*. However, a third of the initial population could be spread by the rats in the environment and possibly these nymphs could complete the cycle on other suitable hosts. Further research with a large number of rats and ticks are needed to confirm this hypothesis. Moreover, it would be necessary to evaluate different *R. sanguineus* populations considering that ticks currently identified as *R. sanguineus* might actually represent more than one species (Szabó MP et al, 2005, Vet Parasitol, 130: 131-140).

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Seasonal dynamics of *Ixodes ricinus* in a protected wooded area in southern Europe

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The tick *Ixodes ricinus* is a triphasic telotropic species associated with wooded areas and renewed for its role as a major vector of pathogens to animals and humans. Its distribution includes nearly all of Europe, to the east as far as the Volga River; Caucasus, Turkmenistan, Turkey, and Iran; North Africa: Morocco, Algeria, Tunisia, and Madeira Island. In spite of its wide distribution range, data on the ecology of *I. ricinus* in some areas is meagre. Such information might be relevant to understand the epidemiology of certain tick-borne diseases associated with this tick.

AIM: The objective of this study was to assess the seasonal dynamics of *I. ricinus* in a protected wooded area located in southern Europe.

MATERIALS AND METHODS: From March 2010 to December 2011, ticks were collected by dragging and flagging in three sites (S1, animal enclosure within a wooded area; S2, high-altitude wooded area; S3, picnic area near a house) located within the boundaries of the Gallipoli Cognato Forest. This forest is part of the municipalities of Accettura, Calciano, and Oliveto Lucano (Matera province, Basilicata region, southern Italy). It covers a surface of 4,159 ha. Each collection section lasted for 30 min, and involved three collectors; two of them dragged the lower vegetation stratum (usually formed by grasses and bushes) whereas the third one flagged the higher vegetation stratum (>50 cm). Ticks collected were immediately placed in vials containing 70% ethanol and later on identified morphologically.

RESULTS: A total of 5,755 (2,372 larvae, 2,829 nymphs, 272 females, and 282 males) *I. ricinus* ticks were collected during the whole study period. Most of the ticks were collected from S1 (85.7%), followed by S2 (10.7%). The number of ticks collected during 2011 increased over 4-fold in relation to 2010. Immature ticks were mostly responsible for this difference, as their number was over 5 times higher in the second year of collections. In 2010, ticks predominated during spring, autumn and winter. In 2011, the number of ticks collected during spring was quite similar to 2010, whereas a marked increase was observed in the number of ticks collected from July to December.

CONCLUSIONS: This study shows that *I. ricinus* is present in southern Italy during all seasons, being less abundant during some summer months. The different seasonal patterns observed during the two years of study suggest that *I. ricinus* might be particularly sensitive to weather changes. Further studies to assess this hypothesis and whether this southern Italian population of *I. ricinus* is infected by relevant pathogens including tick-borne encephalitis virus and *Borrelia* spirochetes associated with human disease in Europe are needed.

Tick bionomics and detection of pathogens potentially transmitted by ticks in a park of Rome

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The Insugherata park (740 ha) is localized in the north-western outskirts of Rome and is an important protected area of city, characterized by different biotopes and a significant biodiversity. This park is also an important area for human recreational activity, but also interested by agriculture and pasture of sheep flocks.

AIM: an acarological and bacteriological research was performed during 2011 in this natural park to investigate the tick distribution and the species composition and the possible presence of pathogens potentially circulating in an urban ecosystem. The study was planned after two preliminary surveys carried out in 2010 and followed by a bacteriological analysis.

MATERIALS AND METHODS: Besides two surveys carried out in June and July 2010, biweekly tick collections were conducted from January to December 2011 in three selected sites, by flagging and dragging for 15 minutes/operator. During each sampling, temperature (T) and relative humidity (RH) were recorded. The samples were identified according to morphological characters (Manilla G, 1998, Acari Ixodida, Fauna d'Italia, Calderini Bologna), and stored at –80°C. For bacteriological analysis, a first Real-time PCR was performed using *glt*A gene as molecular marker to distinguish *Rickettsiae* species belonging to both Spotted Fever Group (SFG) and to Typhus Group (TG) (Paris DH et al, 2008, Trans R Soc Trop Med Hyg, 102: 186-193). A following Real-time PCR using *omp*B gene allowed discriminating the species within SFG (Parola et al, 2003, Emerg Infect Dis, 9: 592–595; Blair et al, 2004, J Clin Microbiol, 42**:** 4961-4967). To identify among 15 rickettsie of the SFG, the *omp*A gene was amplified and sequenced (Simser et al, 2001, Appl Environ Microbiol, 67: 546-552). For *Borrelia* spp. were used as specific molecular marker *osp*A gene (Bunikis J et al, 2004, Microbiology, 150: 1741-1755). To detect *Coxiella burnetii* a TaqMan-based real-time PCR was performed using the targeted the singular *icd* (isocitratedehydrogenase) gene (Klee SR et al, 2006, BMC Microbiol, 19: 2). For *Ehrlichiae* genera was used as specific molecular marker the 16S rRNA gene (Parola et al, 2000, Trans R Soc Trop Med Hyg, 94: 707-708). Statistical analysis was performed to evaluate differences in species composition and abundance per site and during the year by using χ^2 test.

RESULTS: During 2010 and 2011 surveys, a total of 325 ticks were collected in the selected sites. *Rhipicephalus turanicus* was the most abundant species (72.3%), followed by *Ixodes ricinus* (19.7%), *Dermacentor marginatus* (6.5%), *Haemaphysalis punctata* (1.2%) and *Rhipicephalus bursa* (0.3%), with only 1 female. *R. turanicus* occurred mainly in pasture and showed a mono-modal seasonal pattern from spring to early summer with one peak of abundance in April. As expected, *I. ricinus* resulted prevalent in woodland from October to May and the seasonal trend of specimens showed a peak in winter. Although *D. marginatus* exhibited a similar seasonal dynamic in comparison with the wood tick, resulting active from October to April, this species occurred in a different environment (pasture) and with densities considerably less abundant. *H. punctata* and *R. bursa* were rare, with an apparent autumn and autumn-winter seasonal activity, respectively. Statistical analyses to evaluate influences in seasonal dynamics in relation to climatic parameters are ongoing. Eight *R. turanicus* females of the whole 2010 sample (32%) were processed for pathogen investigation. Only one resulted positive for *Rickettsia massiliae*, showing 100% of identity with the homologous sequences available in GenBank. The pathogen analysis of about 40% of the 2011 tick sample is in progress. Until now one *H. punctata* and nine *R. turanicus* specimens were found positive for *C. burnetii*; one *I. ricinus* resulted positive for *Ehrlichia* sp; seven *I. ricinus* and eight *R. turanicus* specimens were found positive for Rickettsiae belonging to the SFG. The pathogen identification at species level is ongoing.

CONCLUSIONS: In the light of our acarological and bacteriological results, this research represents not only a significant contribution to the knowledge of the tick fauna of Rome, but a clear evidence of occurrence of tick-borne pathogens in a park, driving us to extend this approach toward other natural areas of city. In fact the detection of these pathogens together with a contemporary and promiscuous presence of different human activities in natural zones of city could lead to high risk of tick-borne diseases for human population of the whole urban area.

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Genetic differentiation of mosquito populations within the *Culex pipiens* complex in Italy

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Several species of *Culex pipiens* complex are considered to be involved in the West Nile virus (WNV) transmission (Hubálek Z, 2000, Viral Immunol, 13: 415-26). In Europe WNV circulation is confined both in rural and urban ecosystems. In sylvatic cycle the virus circulates between wild and usually wetland birds and ornithophilic mosquito species. In urban cycle virus transmission involves domestic birds and mosquitoes of the *Cx. pipiens* complex as possible bridge-vectors, feeding on both birds and humans (Hubálek Z, Halouzka J, 1999, Emerg Infect Dis, 5: 643-50). The composition of *Cx. pipiens* complex is still debated among taxonomists due to the presence of taxa with distinctive physiological and behavioural traits without a clear morphological discrimination. The nominal species of the complex, *Culex pipiens* Linnaeus 1758, comprises two different entities or biotypes: *pipiens* form that lives and mates in open spaces (eurygamous), requiring a blood meal for egg development (anautogenous) by biting birds (ornitophilic) and hibernates in diapause as gravid females (heterodynamic), and *molestus* form Forskäl that generally breeds in underground habitats without diapause (homodynamic), mating in confined spaces (stenogamous) and laying a first batch of eggs without a blood meals (autogenous), although it can bite humans readily (anthropophagous). Consequently, the distinction between ornithophilic and anthropophilic entities becomes a focal point not only for ecological and epidemiological studies, but also for vector control and public health strategies.

AIM: This study aims to genetically discriminate both forms, exploring two promising molecular methods, in order to assess the distribution of *Cx. pipiens* complex in Italy.

MATERIALS AND METHODS: *Cx. pipiens* sensu lato populations from different Italian localities were collected in different years and stored at -20°C until processing. Several populations were reared in Insectary (25±2°C; 70±10% RH) to evaluate their bio-ecological characters. For molecular analysis, eight populations were assayed by Shaikevich's method (Shaikevich EV, 2007, Euro Mosq Bull, 23: 25-30), a RFLP-PCR of the COI gene that discriminates the two forms by one-nucleotide difference in a 603bp amplicon, displayed after the next digestion with *Hae*III or *Bcl*I endonucleases. About fifteen populations were processed by using CQ11-assay (Bahnck CM, Fonseca DM, 2006, Am J Trop Med Hyg, 75: 251-255), that detects a size polymorphism in the 5' flanking region of the CQ11 microsatellite, allowing to differentiate not only *pipiens* f. (200bp amplicon), from *molestus* f. (250bp), but also hybrids by displaying of both PCR products (200bp/250bp).

RESULTS: Our preliminary results suggest that Shaikevich's method is able to discriminate the two biological forms, but this identification (*molestus/pipiens*) is rarely in accordance with the ecological and physiological aspects of the population (euri/stenogamy, anauto/autogeny, above/belowground habitat). Moreover, several samples were sequenced and the results confirmed this inconsistency. The results from CQ11-assay show in each population different frequencies of three genotypes, *pipiens* and *molestus* forms and hybrids. In particular, in rural areas of North-East Italy, *pipiens* component is prevalent (72-100%), drastically reducing in an urban belowground habitat of Rome, where *molestus* fraction predominates (89%). The Shaikevich's method did not reveal any consistently association between genotyping and etho-ecological characteristics of the populations considered. Conversely, CQ11-assay provides evidence of a molecular and genetic basis for the observable phenotypes, not among, but within populations. Each population includes specimens with genetic and behavioural features previously referred to *molestus* and/or *pipiens* forms and/or hybrids. Differences in the composition of three fractions seem to be in accordance with the ecological and physiological features of each population so far examined. Our findings show that in Italy autogenous and anautogenous individuals can co-occur in aboveground habitats, showing a degree of gene flow between two forms and resulting in hybrid females with intermediate physiological and behavioural traits, such as the opportunistic biting behaviour. This could eventually elucidate the role of *Cx. pipiens* as WNV bridge-vector in southern Europe.

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First signalling of *Ornithodoros maritimus* (Ixodida: Argasidae) in the coastal mainland of southern Sardinia, and its potential role as vector of pathogenic arboviruses

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AIM: *Ornithodoros* (*Alectorobius*) *maritimus* (Vermeil & Marguet, 1967) is an Argasidae that belongs to the subfamily Ornithodorinae and is included in the *Ornithodoros* (*A.*) *capensis* complex. As an ornithophilic tick species, it is known to colonize uninhabited islands and reefs where seabirds preferentially nest and also deemed to be very aggressive towards humans who approach to seabird hosts or their nests (Manilla G, 1998, Fauna d'Italia, vol. 36, Acari Ixodida. Ed Calderini, Bologna). Even though *O. maritimus* is considered a Mediterranean-Atlantic species (sensu La Greca, 1964), there have been evidences that the reports of *O. capensis* in the Aral Sea and the Black Sea (Filippova HA, 1966 Fauna USSR, Paukoobraznye) could be rather attributed to *O. maritimus* (Hoogstraal H, 1985, Adv Parasitol, 24: 135-238), thereby amplifying the geographical distribution of the latter. In Italy, three species belonging to the genus *Ornithodoros* have been reported so far: *O. coniceps* (Canestrini, 1890), that is broadly distributed from north to south, including Sicily, and has been recently reported in Latium (Khoury C et al, 2011, Exp Appl Acarol, 54: 205-209); *O. erraticus* (Lucas, 1840), reported in Tuscany, near Grosseto (Starkoff O, 1958, Ixodoidea d'Italia, Studio Monografico. Il Pensiero Scientifico, Roma) and in the island of Pantelleria in Sicily (Iori A, 2005); *O. maritimus*, that was reported in several islands along the coast of Sardinia and in the island of Pianosa in Puglia (Manilla G, 1998, ibid.). During a research campaign *O. maritimus* was found for the first time in 1988 in a nesting colony of *Larus cachinnans* (Pallas, 1811) in the Isola della Vacca, off the island of St. Antioco,. Here, the researchers, drawn by a large number of corpses of seagulls, caught several ticks under the rocks surrounding the nests, identifying them as *O. maritimus* (Manilla G, 1990, Parassitologia, 32: 265-274). Later, the species was further reported in other Sardinian islands: Tavolara, Catalano and Poveri (Manilla G, 1998, ibid.). This paper reports the first signalling in the mainland of two specimens of *O. maritimus* captured in the southern coast of Sardinia.

MATERIALS AND METHODS: A female was captured in August 2010 near Capo Malfatano (Teulada, Cagliari), while it was trying to parasitize one of the authors. This stretch of coastline consists of metamorphic rocks and it is rough and uneven, rich in cracks, crevices, and ledges, with a typical Mediterranean vegetation where herring gulls (*Larus michaellis* Naumann, 1840), Adouin's gulls (*Ichthyaetus audouinii* Payraudeau, 1826), various other small passerines and, sporadically, also shags (*Phalacrocorax aristotelis desmarestii* Payraudeau, 1826) are used to nest. The second specimen, a male, was found in July 2011 in a site very close to the place of the first sampling, into a crack of the rocks. Both specimens were captured at approximately 10.30 am, consistently with the known period of maximum activity for adults (Cringoli G et al, 2005, Mappe parassitologiche, 6 - Zecche. Series Editor. Napoli). They have been caught on sight, fixed in 70% ethanol and morphologically determined according to analytical keys (Manilla G, 1990, ibid., 1998, ibid.).

RESULTS AND CONCLUSIONS: These findings extend the current knowledge on the distribution of *O. maritimus*, showing that it is not limited to small islands, but also to sections of the mainland coast which possess ideal conditions in order to settle. Noteworthy, this first report of *O. maritimus* in a coastal area occurs fifteen years after the last finding of the species in Sardinia. Furthermore, *O. maritimus* is recognized as competent vector of several tick-borne Flaviviruses, including West Nile virus (WNV) (Lawrie CH et al, 2004, Med Vet Entomol 18: 268-274; Mumcuoglu KY et al, 2005, Vector Borne Zoonotic Dis, 5: 65-71) and other arboviruses pathogenic to humans (Charrel RN et al, 2004, Clin Microbiol Infect, 10: 1040-1055; Nicoletti L et al, 2008, Emerg Infect Dis 14: 177-178). Hence, given the recent emergence of human fatalities due to WNV disease occurred in Sardinia in 2011 (unpublished data), concern rises about the potential role of this and other species of Argasids in transmitting the virus. Therefore, the overall importance of either seabirds as reservoir hosts, either their parasites as amplifiers of viral pathogens should be not underestimated, and long-term surveys, aimed to detect the presence of *O. maritimus* and other similar species on small islands and coastal areas of Sardinia, may represent a valuable strategy to prevent the spreading of such tick-borne zoonotic viral diseases.

Myiasis by *Wohlfahrtia magnifica* (Diptera: Sarcophagidae) in different mammals in Sardinia

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AIM: Myiasis are infestations caused by the larvae of some Diptera Cyclorrhapha, belonging to different families, carrying out part of their life cycle feeding on host tissues, both in humans and in domestic and wild vertebrate animals. They can be classified into accidental, optional and obligatory myiasis. Larvae of *Wohlfahrtia magnifica* (Schiner, 1862) (Diptera, Sarcophagidae) parasitize several warm-blooded vertebrates and are responsible for traumatic myasis of living tissues, often invading wounds and the anus or urogenital system (Colerbrook E, Wall R, 2004, Vet Parasitol, 120: 251-274). Throughout the Mediterranean basin and eastern Europe extending eastward into China, this species is commonly a much more prevalent and clinical important agent of myasis in livestock (Hall M J R, 1997, Parassitologia, 39: 409-413; Hall MJR, Farkas R, 2000, Manual of Paleartic Diptera: Science Herald, Budapest, Hungary, 978 pp). This fly has been a particular problem in livestock in central and eastern Europe, and high prevalence of wohlfahrtiosis have been reported for example in Hungary, in Greece and Crete, in Bulgaria, in Romania and in the former USSR. The primary agent of myasis in southern Spain also appears to be *W. magnifica* (Martinez R I, 1987, Isr Vet Med, 43: 34-41). Reports of wohlfahrtiosis in Italy are scant and myasis caused by this fly have been recorded in grazing animals in Tuscany and Abruzzo Regions (Martinez R, Lecquercq M, 1994, Notes fauniques de Gembloux, 28: 53-60), in one human being (Iori A et al, 1999, Parassitologia, 41: 583-585) and in sheeps from Central Italy (Giangaspero A et al, 2010, Parassitologia, 52: 171). Given the limited number of *W. magnifica* infection reported in Italy, the aim of this work was to describe cases of myasis in different mammals in Sardinia.

MATERIALS AND METHODS: In the following note four cases of myiasis by *W. magnifica* are reported, between 2010 and 2011, on three live animals and on a dead one. Part of the samples analyzed were found during the diagnostic activities of the IZS of Sardinia. Larvae extracted from their hosts were immersed in water at 80°C for 30 seconds, fixed in 70% ethanol and subsequently included in slides, after preparation, for microscopic analysis. The animals from which the larvae were extracted were a cat, a dog, a goat and a wild boar.

RESULTS: The first case concerns a young stray cat found in the town of Cagliari (on 23.VIII.2010), that presented an imposing nose, palate and tongue myiasis, with numerous II instar larvae of *W. magnifica*. The second case concerns a young goat (*Capra hircus*) with evident neck abscess due to *Staphylococcus aureus* subsp. *anaerobius*, isolated for the first time in Sardinia in 2004 (Cabras PA et al, 2011, 19th International Congress FeMeSPRum, Belgrade, Serbia), from a herd in Talana, from which numerous III instar of *W. magnifica* larvae were extracted (on 16.IX.2011). Almost all the individuals in the herd presented such abscesses probably aggravated by myiasis, as in this case. The third case involved a dog living in the village of Castiadas, on which an important post surgical myiasis on the tail, with numerous III instar larvae was found (on 2.X.2011). The last case was a wild boar (*Sus scrofa meridionalis*) found dead (on 4.X.2011) in the territory of Tortoli, with a traumatic myiasis from which one III instar larvae of *W. magnifica* was extracted and which presented a incipient colonization of Diptera Calliphoridae I instar larvae.

CONCLUSIONS: By these data it emerges that: the presence of *W. magnifica* in Sardinia, already known also for a case of human auricular myiasis (Panu F et al, 2000, J Laryngol Otol, 114: 450-452) is confirmed; that this Sarcophagid can parasitize livestock, domestic and wild animals both in urban settings (Cagliari and Castiadas) and in rural areas and pastures (Talana), and in forests and natural areas (Tortolì). Moreover, the possibility that *W. magnifica* can parasitize man also makes it a species of sanitary interest for public health too. The importance of this species in the forensic field should not be underestimated since the discovery of larvae of *W. magnifica* in human sores or wounds or in animals died due to mistreatment could provide useful information to investigators in cases of criminal investigations.

Contribution to the knowledge of Diptera Hippoboscidae in Sardinia

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AIM: Once Hippoboscidae were included in the group of Diptera Pupiparia, together with Streblidae and Nycteribiidae. This grouping, however, was considered unnatural (Falcoz L, 1926, Dipterès Pupipares, Faune de France, 14, Lechevalier, Paris) as important differences were recognized between these taxa and affinity were observed between Hippoboscidae and Glossinidae (Zavattari E, 1928, Proc Soc it Sc nat, 67: 37-70), even assuming a common nidicole ancestor (Oldroyd H, 1964, The natural history of flies, Weidenfeld and Nicolson, London). The members of this family of Diptera Cyclorrhapha are bloodsucking obligate ectoparasites of mammals and birds. Some species have well developed wings, other vestigial, and in some taxa, as in the genus *Lipoptena*, the wings are cut at the base when the parasite reaches the host. In absence of their preferred hosts, Hippoboscidae can attack humans, some species even with a certain frequency. Their bite is rather painful. Some species may be vectors of pathogens including filarial *Dipetalonema dracunculoides* (Spirurida: Onchocercidae). In Italy, the species currently surveyed are 18, but the list can not be considered complete (Rivosecchi L, 1995, in Pape T et al, Diptera Hippoboscoidea, Oestroidea, Checklist of species of the Italian Fauna, 78. Calderini, Bologna). This work is aimed to give a contribution to the knowledge of Hippoboscidae in Sardinia, remarking on the environmental contexts of findings and on the hosts.

MATERIALS AND METHODS: The samples were detected in 7 different sites of the island, located in 4 Provinces. The specimens captured were suppressed in ethyl acetate and stored dry, or in 70% ethanol.

RESULTS: As summarized in the table below, 33 specimens were collected, belonging to four different species: *Crataerina pallida* (Olivier in Latreille, 1811), *Pseudolynchia canariensis* (Macquart in Webb & Berthelot, 1839), *Hippobosca equina* Linnaeus, 1758 and *Lipoptena cervi* (Linnaeus, 1758).

CONCLUSIONS: *C. pallida* was found in a cave entrance where *Apus apus*, *Ptyonoprogne rupestris*, and *Columba livia* usually nest; this species is reported to attack humans (Goebel B, 1960, Z Haut Geschlechtskr, 28: 302-304). *P. canariensis*, found exclusively in Cagliari, typically parasite *Columba livia domestica*, but given the promiscuity of this bird with anthropic environments, it can also attack humans and dogs. *L. cervi* seems strictly related to natural environments and wild mammals as deers, although it was observed that it may occasionally parasitize humans. *H. equina* is related to rural environments and parasitizes livestock, but also humans and dogs. This contribution shows that all the species of Hippoboscidae found in Sardinia seem not to be strictly host-specific, as when necessary, they can take advantage of the human presence as a trophic resource in urban, rural and natural areas.

Table 1. Species of Hippoboscidae collected in Sardinia.

Species	Locality	Provinces	Date	n°	Host / Environment
Crataerina pallida	Domusnovas	Carbonia Iglesias	20.VI.1981	1	cave
Pseudolynchia canariensis	Cagliari	Cagliari	12.X.1993	1	*Columba livia domestica*
Pseudolynchia canariensis	Cagliari	Cagliari	16.I.1998	1	*Canis familiaris*
Pseudolynchia canariensis	Cagliari	Cagliari	20.VIII.2005	1	*Columba livia domestica*
Pseudolynchia canariensis	Cagliari	Cagliari	8.III.2007	1	*Homo sapiens*
Lipoptena cervi	Seui	Ogliastra	09.XI.2007	3	*Dama dama*
Lipoptena cervi	Sinnai	Cagliari	15.VI.2008	10	*Homo sapiens* / light trap
Lipoptena cervi	Sinnai	Cagliari	10.XI.2008	6	*Cervus elaphus corsicanus*
Hippobosca equina	Domus de Maria	Cagliari	31.VIII.2009	3	*Capra hircus*
Hippobosca equina	Villacidro	Medio Campidano	3.VI.2011	3	*Homo sapiens*
Hippobosca equina	Cagliari	Cagliari	21.VI.2011	2	*Canis familiaris*
Hippobosca equina	Teulada	Cagliari	21.VIII.2011	1	*Homo sapiens*

New data on the presence of *Argas reflexus* (Ixodida: Argasidae) in Sardinia

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Argas reflexus (Fabricius, 1794), described for the first time on samples found in Italy, is a nidicolous, polyphasic and monothropic soft tick which originally parasitized several species of wild birds (Dusbábek F & Rosicky B, 1976, Acta Sci Nat Brno, 10: 1-43) and had as its preferred host the pigeon (*Columba livia livia* Gmelin). Consequently to its domestication, *A. reflexus* has associated with the domestic pigeon (*C. livia domestica* L.), colonizing rural and urban environments. *A. reflexus* is a Palaearctic species and in Italy its distribution is known in almost all regions, except for the southernmost regions (Sobrero L & Manilla G, 1988, Bonifica, 4: 111 pp). Its presence is signaled in Sardinia since the end of the '800 (Marcialis E, 1892, Saggio d'un catalogo metodico dei principali e più comuni animali invertebrati della Sardegna), notwithstanding some authors in the past have considered this report as unreliable. This record has been also reported later by Garneri G A (1902, Contribuzione alla fauna sarda, Aracnidi. Com. alla Soc. Zool. Italiana). More recently, *A. reflexus* was found in the town of Cagliari in the late 80 and several immature stages and adults were found inside some houses, whose attics housed colonies of pigeons (Pusceddu G, Ulteriori Studi sulle zecche (Ixodoidea) della provincia di Cagliari. Tesi di Laurea, Università di Cagliari. A.A. 1997-1998). The first official note on the presence of this species in Sardinia is nevertheless in the late 90: 21 adult specimens were sampled in a school in Cagliari (Contini C, 1998, Parassitologia, 40 (Suppl. 1), 37). The same paper reports additional findings relating to the years following the first discovery. Further unpublished records reported this species in the town of Cagliari in the same period (Figus V, personal communication). Subsequent scientific studies have reported *A. reflexus* in 2004 again in the town of Cagliari (Fois F et al, 2006, Parassitologia 48, 1-2: 349; Montarsi F et al, 2011, Proceedings TTp7, 213).

AIM: To confirm the presence of *A. reflexus* in Sardinia and make official its presence definitively in the light of more recent data acquired and in relation with 6 specimens found in the town of Cagliari between 2004 and 2011.

MATERIALS AND METHODS: The sampled specimens were fixed in 70% ethanol and morphologically determined by microscopic analysis according to Manilla's analytical keys (Manilla G., 1998, Fauna d'Italia, vol. XXXVI, Acari Ixodida. Ed. Calderini, Bologna. VIII + 280 pp).

RESULTS: All specimens were found in the town of Cagliari. One nymph was sampled in October 2004 on a windowsill of a building in the city center. In October 2007 another nymph was found in an apartment in another area of the town, and in June 2011 four other nymphs were found inside an apartment on whose balcony stopped domestic pigeons (*Columba livia domestica*). In the latter case, the ticks were particularly numerous and shown a particular attraction to humans.

CONCLUSIONS: These new data show that *Argas reflexus*, present in Sardinia already for some decades, is now firmly established. It is strictly associated with the domestic pigeon, which is very abundant and widespread, especially in major urban centers of the island. The potential of this tick as a vector of pathogens and its anthropofily, as demonstrated also by the recent reported cases, especially in case of serious infestations, makes it a species of health importance. *A. reflexus* can cause, through sensitization to salivary secretions, sometimes very severe anaphylactic reactions (Miadonna A et al, 1982, Ann Allergy, 49: 293-294; Khoury C & Maroli M, 2004, Ann Ist Super Sanità, 40: 427-432).

Preliminary notes on the presence of *Haematopinus apri* (Phthiraptera: Anoplura) in Sardinia

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AIM: *Haematopinus apri* Goureau, 1866 (=*Haematopinus aperis* Ferris, 1933) is an Eurasian Haematopinidae, reported in several European countries including Germany, Czechoslovakia, Poland, Bulgaria, Hungary and Switzerland (Durden L A, Musser G G, 1994, Bulletin of the American Museum of Natural History, 218, 90 pp; Piotrowski F, 1970, Parasit Hung, 3: 97-118). Its presence is known for northern Italy, but still had no data on its distribution in the rest of the Italian territory (Manilla, 1995, Phthiraptera, Checklist delle specie della Fauna Italiana, 39. Calderini, Bologna). *Haematopinus apri* is a sucking lice that typically parasitize wild boar (*Sus scrofa*), while *H. suis* (Linnaeus, 1758) is a cosmopolitan species typically associated with the domestic pig. Some problems related to the nomenclature of the species have created confusion between descriptions, synonyms and redescriptions; the currently accepted name is *Haematopinus apri*. In this note we report for the first time the presence of this species in Sardinia describing current knowledge on its distribution in the island.

MATERIALS AND METHODS: The samples analyzed were found during the diagnostic activities of Istituto Zooprofilattico Sperimentale della Sardegna. The sucking lice were collected directly from the carcasses of animals delivered to the investigation necropsy, fixed in 70% ethanol and identified by microscopic examination.

RESULTS: On 9 wild boars (*Sus scrofa meridionalis*) examined between 2007 and 2011, from 8 different locality and 3 different provinces of the region, 7 were parasitized by *H. apri* and 2 *H. suis*. A total 89 specimens (24 females, 28 males and 37 larvae) of *H. apri* and a female and a male of *H. suis* have been found. In two cases it was detected a co-infestation with ticks (Acarina: Ixodida) belonging to the species *Dermacentor marginatus* (Sulzer, 1776) and *Rhipicephalus turanicus* (Pomerantzev, Matikashvili et Lototsky, 1940). The 7 wild boars parasitized by *H. apri* came from Pula (Province of Cagliari), Santadi (Prov. of Carbonia Iglesias), Jerzu, Tortoli, Talana and Baunei (Prov. of Ogliastra); the 2 wild boars parasitized by *H. suis* came from Uta (Prov. of Cagliari) and Lanusei (Prov. of Ogliastra). The sucking lice were found from March to November, with a more substantial abundance of specimens found in the spring season.

CONCLUSIONS: By these data, at least relative on the distribution of this species in the central-southern Sardinia, it emerges that *Haematopinus apri* is present on the island for a long time and is fairly widespread. It is closely related to wild boar, although this Artiodactyla may also be parasitized from *H. suis*, typically associated with the domestic pig on which, until now, was not detected *H. apri*. Were not detected cases of co-infestation between the two Haematopinidae species, even if they are not to be excluded, at least in the wild boar. This important contribution provides preliminary data on *Haematopinus apri* extending the current knowledge on its distribution in Italy and the Mediterranean basin.

First record of *Linognathus africanus* (Phthiraptera: Anoplura) in Sardinia and current knowledge about its presence in Italy

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AIM: The Phthiraptera include the Anoplura (= Siphunculata) and the Mallophaga, respectively the so called sucking lice and chewing lice. They are obligatory permanent ectoparasities, characterized by a high host specificity. The species *Linognathus africanus* (Kellogg & Paine, 1911) was described on specimens collected from sheeps in southern Nigeria, West Africa (Kellogg VL & Paine JH, 1911, Bull Entomol, Res 2: 146). *L. africanus* is a hematophagous louse whose preferred hosts are goats and sheeps. In massive infestations it may cause pediculosis, exfoliative dermatitis with hair loss and anaemia. *L. africanus* is a species widely distributed and it has been reported in Africa, India, Mongolia, Philippines, Mexico, Australia, USA, Israel, Turkey, the former Soviet Union, China, Chile, Brazil. In Europe, the congeneric *L. stenopsis* (Burmeister, 1838) is the louse parasite of goats historically and widely disseminated, but also *L. africanus* has been sporadically reported as in Spain (Portus M et al, 1977, Rev Iber Parasitol, 37: 345-354) and Greece (Himonas CA & Liakos VD, 1989, Vet Rec 125: 420-421). In Italy, this species was recorded for the first time in Emilia Romagna (Calzolari M et al, 2006, Atti XVII Congr. SIPAOC, 17: 142); numerous specimens were found on a goat from Roncofreddo (Forlì-Cesena), which was delivered for autopsy to the Istituto Zooprofilattico della Lombardia ed Emilia Romagna. In this work, the presence of *L. africanus* is reported in Sardinia for the first time.

MATERIALS AND METHODS: The specimens sampled were found on goats (*Capra hircus*) from nine different localities in four different provinces of the island. The hosts were delivered for necropsy investigation to the Dip.to di Cagliari and to the Centro Territoriale di Tortolì of the Istituto Zooprofilattico della Sardegna from 2002 to 2011. The sucking lice were collected using entomological forceps, fixed in 70% ethanol and identified by microscopic analysis.

RESULTS: As summarized in Table 1, females, males and neanids of *L. africanus* were found. In four off the nine cases examined, it shared the infestation with *L. stenopsis*, and in two cases with the chewing lice *Bovicola caprae* (Gurlt, 1843) (Phthiraptera: Ischnocera), according to what reported in previous papers (Himonas C & Liakos VD, 1989, Vet Rec, 125: 420-421; dos Santos SB et al, 2006, Rev Bras Parasitol Vet 15: 41-43).

CONCLUSIONS: By the findings obtained and the data on its distribution, it is believed that *L. africanus* is widespread in Sardinia for a long time. Given the similarity of this species with other Haematopinidae, particularly with the congeneric *L. stenopsis* with which sometimes shares the infestation, it is supposed that its presence has not been properly recognized in the past. Current knowledge on the distribution of *L. africanus* in Italian peninsular territories are limited to Emilia Romagna, although there are elements on which we are still working, that suggest its wider distribution, at least in northern Italy.

Table 1. Specimens of *Linognathus africanus* collected in Sardinia (num. spec. = massive infestation by very high number of lice).

Locality	Provinces	Date	Female	Male	Neanid	Species in co-infestation
Talana	Ogliastra	04.II.2002	5	3	0	
Cardedu	Ogliastra	14.V.2005	3	2	1	
Villacidro	Medio Campidano	19.II.2007	1	0	0	*Bovicola caprae*
San Vito	Cagliari	15.V.2007	6	0	0	*Linognathus stenopsis*
Loceri	Ogliastra	02.VIII.2007	3	0	0	
Urzulei	Ogliastra	04.IV.2011	1	0	0	*L. stenopsis – B. caprae*
Arzana	Ogliastra	23.V.2011	4	4	0	*L. stenopsis*
Morgongiori	Oristano	09.IX.2011	1	0	0	*L. stenopsis*
Domus de Maria	Cagliari	03.XI.2011	num. spec.	num. spec.	num. spec.	

Distribution and abundance of questing ticks in three parks of Emilia-Romagna Region (Italy)

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AIM: The human risk of contracting tick born diseases is linked to the density of questing ticks; in particular for Lyme borreliosis, the risk is broadly linked to the nymphs density. Monitoring of ticks, in particular *Ixodes ricinus*, in areas used by people for leisure activities, make possible a valuation of the risk of ticks infestation and pathogen transmission (Kubiak K, Dziekonska-Rynko J, 2006, Wiad Parazytol, 52: 59-64).The following study was performed to quantify the presence and abundance of thick in three Emilia Romagna Regional parks, never checked before and usually haunted by family with children.

MATERIALS AND METHODS: Four sampling site were selected. In the "Gessi bolognesi and Calanchi dell'Abbadessa" Regional Park (Bologna province) two sampling site were selected: site A (locality "Ca' de Mandorli) and site B (locality Ciagnano); another site (C) was localized in the "Monteveglio Abbey Regional Park" (Bologna province) and lastly the site D was localized in the Carnè Park (Ravenna province). In these areas there are naturalistic pathway and picnic areas, interspersed with wood and meadow, that are usually frequented by many people. In these sites, areas or transects were selected and sampled every 15 days for questing ticks from April to October 2010 (from May to October in site D, for the annual closing of the park).Ticks were collected by flagging the upper trail edge line of the transects (KramerVL, Beesley C, 1993, J Med Entomol, 30: 549-554) or the whole areas with a 1m x 1m white cotton muslin cloth. The ticks were removed every 2 meters to reduce the effect of tick drop-off, according to Li X, DunleyJE (1998, Exp Appl Acarol, 22: 233-248). Environmental factor considered in each transect or area examined was the dominant vegetation (presence of grass or leaf litter) and the microclimate during each sampling, assessed by measuring the temperature and the percentage of humidity at 5 cm by the soil using a thermohygrometer (Oregon Scientific). Collected ticks were immediately put into alcohol 70% and afterward identified using identification keys (Manilla, 1998, Fauna d'Italia, XXXVI Acari Ixodida. Ed. Calderini, Bologna; Iori et al, 2005, parte III- Zecche d'Italia, In: Cringoli G (Ed) Mappe parassitologiche 6-Zecche, Rolando Editore, Napoli). Ticks species and stage of development were recorded for each transect or areas. Only for *I. ricinus*, the abundance index (AI) i.e. the number of ticks collected in 100 m^2, was calculated for larvae (AIL), nymph (AIN) and adult (AIA), according to Barandika et al, (2010,Vector-Borne Zoon Dis, 10: 1027-1035) and compared among sites, period of collection, temperature, humidity and dominant vegetation. Kruskall-Wallis test was used to compare the AI in the different sites; non parametric correlation (Spearman Rho)was used to quantify association between the AI andthe different temperature orhumidity value at the collection time.

RESULTS: A total of 8139 questing ticks, mainly larvae (6734), to a lesser degree nymph (1344) and only few adults (33 male and 28 female), were collected. The higher number of ticks were found (4187) in site B, followed by site A (2123), site D (967) and lastly site C (862). *I. ricinus* was predominant (8080 specimen) but also other few specimens were found such as *Dermacentor marginatus* (37), *Scaphixodes frontalis* (13), firstly described in Emilia-Romagna Region, *Hyalomma*spp. (6) and *Ixodes acuminatus* (3). In site B there was the major heterogeneity of ticks species. The average AI of *I. ricinus* was significantly higher in site B both for larvae (215.1 ticks/100m^2, $p<0.01$) and nymph (29.3 tick/100m^2; $p<0.05$). The higher AIL was found in July in sites A, B and C, while in August in site D; the higher AIN was found in May in all the sites. The AIL tend to increase, while the AIN and AIA tend to decrease significantly when the temperature increases, instead the AI is not correlated with humidity, even if the larvae were more collected with humidity lower than 35%.The overall AI was higher in transect or areas with leaf litter instead of grass vegetation.

CONCLUSIONS: It is highlighted the importance to monitoring the presence of ticks and to perform awareness campaign about prophylaxis measures.The attending of these areas can became hazardous for people because of potential transmission of vector borne diseases.

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Underwater survival of *Rhipicephalus sanguineus*

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Ticks (Acari: Ixodida) are blood-sucking ectoparasites of vertebrates, globally recognized as vectors of many pathogens (Sonenshine DE, Mather TN, 1994, Ecological dynamics of tick-borne zoonoses, Oxford University Press, Oxford). Their distribution is largely accounted for their off-host adaptability (Needham GR, Teel PD, 1991, Annu Rev Entomol, 36: 659–681). Among ticks of veterinary and medical concern, *Rhipicephalus sanguineus* is regarded as one of the most important arthropods since it is a vector of several pathogens (Dantas-Torres F, 2010, Paras Vectors, 3: 26) and extremely resistant to extreme environmental conditions (Dantas-Torres F et al, 2010, Vet Parasitol, 171: 327–330; Dantas-Torres F, Otranto D, 2011, Exp Appl Acarol, 54: 313–318;). Ticks are terrestrial arthropods, but they can survive for some period submerged underwater. This ability might be determinant for tick survival in certain areas exposed to environmental flooding.

AIM: To assess the ability of *R. sanguineus* females to survive and to lay eggs after water immersion.

MATERIALS AND METHODS: On April 2011, *R. sanguineus* engorged females were obtained from the environment of a private dog shelter in the province of Bari, southern Italy. Ticks were divided into three groups of 75 females; two were used as test groups (group T1 and T2), and one as control (group C). T1 and T2 females were placed in 100 ml plastic vials (5 females per vial) and filled with bi-distilled water for 1-15 days. Every 24 h, two vials were removed from water. Group C females (n=75) were put in individual tubes, closed with a cotton wool plug. Both control and test groups were maintained in an incubator at 26°C and RH > 80%, throughout the trial. The survival was evaluated daily, at 1-hour intervals, until the ticks had completely recovered, by placing them on a filter paper and by assessing their ability to move. To evaluate the effect of water immersion on egg viability, two replicates of 20 mg of eggs each were separated from groups T1, T2 and C, being the first egg batch flooded and the second used as control. Eggs were placed in glass tubes with water, which was removed every 24 h from 1 to 5 days.

RESULTS: All females survived water immersion for 48 h, some of them up to 72 h, producing egg masses equivalent to those of C group (Kruskal-Wallis test, $P = 0.88$), but with egg hatch rate negatively correlated with female submersion period. All females submerged for more than 72 h died displaying alterations in body shape and colour (i.e., blood leaking from the anal and genital openings, probably due to the inability to regulate osmotic balance for a long time). Indeed, no significant differences were found in relation to pre-oviposition and oviposition periods among test and control groups (Kruskal-Wallis test, $P = 0.31$ and $P = 0.18$, respectively). All eggs flooded for up to 120 h hatched successfully and no correlation was found between egg submersion period and egg hatch rate ($r = 0.37$, $P = 0.54$). Larvae hatched from flooded eggs behaved normally, like those of the control group.

CONCLUSIONS: These findings suggest that engorged females and eggs of *R. sanguineus* from southern Europe are able to survive for some period underwater without loosing biological activity. Whether water flooding impacts on the ability of ticks to transmit pathogens in endemic areas, deserves further investigation. More practically, the survival of *R. sanguineus* underwater could also suggest their resistance to washing. Consequently, people who live in close contact with their pets or returning home from places where ticks may occur (e.g., kennels, shelters, boarding for dogs), should pay attention to clothes, even after washing.

Preliminary investigation of *Nosema* spp infection in honeybee apiaries in Northern Italy

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Nosemosis is a microsporidial gut disease of adult honeybees, *Apis mellifera,* that until recently was thought to be caused only by *Nosema apis. N. ceranae*, originally found in *Apis cerana*, has now been recognized also in Europe, and considered a more common infection agent than *N. apis* (Fries I et al, 2010, J Invertebr Pathol,103, Suppl 1: S73-S79). *N. apis* and *N. ceranae* spores are not easily distinguishable from each other under microscopic examination, requiring molecular analysis for species identification.

AIM: The aim of this work was to investigate the presence of *N. ceranae* and *N. apis* in apiaries located in different regions of Northern Italy, and to determine which *Nosema* species is more prevalent.

MATERIALS AND METHODS: In the present study, 118 samples were collected during 2011 from honeybee colonies of 104 apiaries of the investigation area (14 from Friuli Venezia Giulia region: Udine, Gorizia, and Pordenone provinces; 6 from Trento and Bolzano provinces; 14 from Veneto region: Verona, Vicenza, Treviso; Padova, Rovigo, and Belluno provinces; 63 from Lombardia region: Milano, Monza-Brianza, Lodi, Como, Lecco, Varese, Brescia, Bergamo, Mantova, and Sondrio provinces; 4 from Piemonte region: Novara, Vercelli, Biella, and Torino provinces; 3 from Valle d'Aosta region). The diagnosis of *Nosema* spp. was performed on a sample of 25-30 adult honeybees as a balanced pool of individuals from each of 5-10 colonies of the apiary. In most cases, honeybee crushings underwent microscopic examination to assess the presence of *Nosema* spp. spores. For microscopically-positive samples and for samples not microscopically tested, DNA extraction and *Nosema* DNA amplification through species-specific PCR were performed using protocols already described in literature (Martin-Hernandez R et al, 2007, Appl Environ Microbiol, 73: 6331-6338;). To confirm the obtained results, 30 positive samples were also tested with two other protocols: PCR and sequencing (Higes M et al, 2006, J Inverteb Pathol, 92: 93-95) and a specific PCR for *N. apis* (Webster TC et al, 2004, Apidologie, 35: 49-54). PCR products obtained by Higes et al. PCR protocol were sequenced and similarity analysis was performed by using BLAST database search present in Genebank.

RESULTS: Of the 118 samples analyzed, 74 were positive for *N. ceranae* and it was detected in all the investigated regions, with an overall positivity rate of 62.7%. When PCR products were sequenced, all the sequences showed 100% identity to 16S rRNA sequence of *N. ceranae*. All the analyzed samples were negative for *N. apis* and *N.apis*/*N.ceranae* co-infection was never detected.

CONCLUSIONS: The results of this study suggest that *N. ceranae* is widespread also in Northern Italy, with detection ratio comparable to other European countries (e.g. 65.6% in samples collected in France from 2002 to 2005, (Chauzat MP et al, 2007, Apidologie 46: 127-128). In addition, *N. apis* seems to be undetected from the same geographic area. An increase of the number of apiaries under investigation and seasonal samplings are recommended in order to accurately determine the prevalence of *N. ceranae* infection, and to monitor the possible presence of *N. apis* in Northern Italy.

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The *Anopheles gambiae* salivary protein cE5 is a tight- and fast-binding thrombin inhibitorwhose tissue-restricted expression is regulated at the post-transcriptional level

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Mosquito saliva carries a large number of factors with anti-hemostatic, anti-inflammatory and immuno-modulatory activities. We have previously explored the salivary repertoire of the African malaria vector *Anopheles gambiae* and, among other components, we have identified a cDNA encoding the putative salivary protein cE5 (Arcà B et al, 1999, Proc Natl Acad Sci USA, 96: 1516). The cE5 protein was then shown to share sequence similarity with anophelin, a thrombin inhibitor from the saliva of the New World mosquito *Anopheles albimanus* (Valenzuela JG et al, 1999, Biochemistry, 38: 11209).

AIM: The aim of this work was to carry out a detailed molecular and biochemical characterization of the *An. gambiae* cE5 protein.

MATERIALS AND METHODS: The transcriptional profile of the *cE5* gene was determined by RT-PCR and 3'-RACE in salivary glands and several other tissues/organs of adult male and/or female mosquitoes (midgut, ovaries, heads, malpighian tubules, hemolymph, hemocytes). The *cE5* protein was expressed in recombinant form in *E. coli*, purified by affinity chromatography followed by ion-exchange chromatography and HPLC. An anti-cE5 polyclonal serum was obtained by immunization of BALB/c mice and employed for protein detection by Western blot analysis. The purified recombinant protein was used for biochemical assays to evaluate its anti-thrombin activity.

RESULTS: The *cE5* gene was found to encode mRNA isoforms carrying 3'UTRs of different length coexisting in several tissues of both male and female mosquitoes, a highly unusual profile for a gene involved in blood feeding and potentially encoding an anti-thrombin polypeptide. Interestingly, despite the widespread occurrence of *cE5* transcripts in different mosquito tissues, the corresponding protein was only found in female salivary glands, where it undergoes post-translational modification. Expression of recombinant cE5 protein and assessment of its activity and inhibitory properties showed that it is a highly specific and tight-binding thrombin inhibitor, which differs from the *An. albimanus* orthologue for the fast-binding kinetics.

CONCLUSIONS: Unusually, the tissue-specific restriction of *An. gambiae* cE5 is not achieved by transcriptional control, as common for mosquito salivary genes involved in hematophagy, but by post-trascriptional gene regulatory mechanism. Although *An. gambiae* and *An. albimanus* separated approximately 100 million years ago and the two proteins, anophelin and *cE5*, are quite divergent (43% identity, 57% similarity), their anti-thrombin function is fully preserved, since they were both found to be highly specific and tight-binding inhibitors of thrombin. On the contrary, they differ for the binding kinetics: the *An. albimanus* anophelin is a slow-binding thrombin inhibitor, whereas the *An. gambiae cE5* protein behaves like a fast-binding inhibitor, a property that may confer a significant advantage to a blood feeder like a mosquito. Our observations provide a paradigm of post-transcriptional regulation as key determinant of tissue specificity for a protein from an important disease vector and point out that transcriptomic data should be interpreted with caution in the absence of concomitant proteomic support.

Cutaneous myasis caused by *Dermatobia hominis* in an Italian traveller returning from Mexico: transmission by a cockroach?

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INTRODUCTION: The increasing travelers to the tropics lead physicians more frequently to deal with non-domestic diseases in Europe (et al, 2012, Der Hautarzt, 63: 325-328). Myiasis is a common travel associated skin disorder as a consequence of short visits to developing countries (Boruk M et al, 2006, Int J Pediatr Otorhinolarryngol, 82: 576–584; Hall M and Wall R, 1995, Adv Parasitol, 35: 257–334). It is the fourth most common travel associated disease. *Dermatobia hominis* is a bot fly which instar maggots are cause of myiasis in Central and South America and should be taken into account in furuncular skin eruptions of returning travelers. The tissues of humans can be infested by larvae hatching from eggs laid by non-biting flies glued to their underside on wet clothes, animal faeces and other humid objects. The eggs hatch and the first instar penetrates the skin and when mature, they wriggle out and fall to the ground to pupate (Messahel A et al, 2010, J Infect Public Health, 3: 43-45). The typical appearance of the skin eruption, with a central porus, seropurulent discharge and a whitish, tender moving mass within the nodule, is quite characteristic for myiasis. The patients often have intermittent sharp pain in the area of the affected skin, continuing growth of the nodules and a sensation of slight movement within the skin eruption (et al, 2011, Dtsch Med Wochenschr, 136: 309-312).

CASE REPORT: we describe an imported case of furuncular myasis caused by *D. hominis*. A 59-year-old man presented skin eruptions on dorsal area, due to the bite of an insect (cockroach) after returning from a trip in Mexico. The skin manifestation was characterized by two furuncles, one with a central red porus of about 2 cm in diameter and the other one of 1 cm. The patient referred after squeezing the exit of two whitish maggot from the first nodule and the only clinical sign present was fever (~ 39C°). The maggots were identified as *D. hominis* larvae also with the aid of transmission electron microscopy. The patient was treated with chinolones and ceftriaxone. After 2 months the patient has reported, after squeezing, again the exit of a new maggot, for this an accurate surgical toilet was indicated. The patient will be followed up to exclude further manifestations.

CONCLUSIONS: This case suggests with some others that with increased international travel physician should be aware of this parasitic infection in recent travelers to Central and South American countries. It is important to remember this parasitic disease in differential diagnosis in patients presenting boil-like inflammatory papules following travel to Latin America.

First report on natural infection of *Phlebotomus neglectus* (Diptera, Psychodidae) with *Leishmania infantum* in an endemic area of visceral leishmaniasis in Albania

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Zoonotic visceral leishmaniasis (VL) is a re-emerging disease in some Mediterranean countries. In Albania 1025 VL cases have been diagnosed in the 1998-2008 period, of which 88% were children. *Leishmania* infecting humans and dogs was typed as *L. infantum* zymodeme MON-1. Information on phlebotomine species distribution in the country is limited and their role as *Leishmania* vectors has not been elucidated yet. In 2006, nested-PCR was used to detect genomic *Leishmania* DNA in 425 *Phlebotomus* (*Larroussius*) females after species identification. Insects were pooled according to species, site and date of collection. Eight/54 pools (14.8%) were found positive for leishmanial DNA, of which 7/47 pools from *P. neglectus* and 1/1 (made of 3 specimens only) from *P. tobbi* (Bongiorno G et al, 2011, Proc ISOPS 7, 25-30 April 2011, Kusadasi, Turkey, p. 81). So far, the main criteria to incriminate definitively a sand fly species in Albania have not been met (i.e. complete development of promastigotes after blood digestion, and specific identification of the harboured *Leishmania*).

AIM: In 2011, an entomological survey aimed to assess the natural *Leishmania* infection rate in sand fly species was performed in Lezhë district, an endemic focus with a relevant cumulative incidence of VL morbidity.

MATERIALS AND METHODS: CDC miniature light traps were used to collect live sand flies during the first week of September (4-7) in rural and peri-urban areas of 4 villages of the Lezhë district (Koder Marlekay, Tresh, Gryke Manati, Manati). Before dissection the flies were anaesthetized for 5 min in a deep freezer and stored in sterile phosphate-buffered saline containing gentamicin (250 μg/ml), 5-fluorocytosine (500 μg/ml) and commercial baby shampoo (1 drop/30 ml). The specimens were identified by the morphology of pharyngeal armature and spermathecae according to Léger N et al (1983, Ann Parasitol Hum Comp, 58: 611-623). When flies were found to harbour promastigotes, some drops of Evans' Modified Tobie's Medium (EMTM) liquid phase were added to the dissected material and then the entire gut was aspirated and inoculated into screw-top vials containing EMTM solid phase (Maroli M et al, 1994, Acta Trop, 57: 333-335). *Leishmania* typing was performed by both ribosomal internal-transcribed spacer-1 (ITS-1) nested-PCR, followed by Restriction Fragment Polymorphism Length analysis (ITS-1 n-PCR-RFLP) (Schönian G et al, 2003, Diagn Microbiol Infect Dis, 47: 349-358; Alcover et al., 2012, Parasitol Res, in press) and Multilocus Enzyme Electrophoresis (MLEE) on 13 isoenzymes (Gramiccia M et al, 2003, Ann Trop Med Parasitol, 97: S65-S73).

RESULTS: A total of 387 sand fly females were dissected, identified and their guts microscopically examined for promastigotes. Among 361 specimens belonging to suspected vector species, *P. neglectus* was the most prevalent (59.8%) followed by *P. tobbi* (40.2%). Out of 64 dissected flies from suburbs of Lezhë town (Koder Marlekay), live promastigotes were detected in two *P. neglectus* specimens (3.1%), one of which found heavily infected with metacyclic forms in the foregut. Only these parasites have been successfully cultured. The strain IMJN/AL/2011/MJN2 was identified by both techniques as belonging to *L. infantum*. ITS-1 n-PCR-RFLP showed the specific pattern of this species (184-72-55 bp bands), and MLEE identified the strain as belonging to zymodeme MON-1, the most diffuse *L. infantum* zymodeme already reported as causing human and canine leishmaniasis in Albania.

CONCLUSIONS: The abundance of *P. neglectus* among the *Larroussius* phlebotomines recorded in the studied area and in all Albanian territories surveyed so far (63.7% prevalence among 17/34 districts; Bongiorno G et al, 2011, ibid.) as well as the *L. infantum* infection rates detected in this species, indicate that *P. neglectus* is indeed the main VL vector in Albania.

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Introduction and establishment in northern Italy of a new potential mosquito vector: *Aedes (Finlaya) koreicus*

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AIM: Since the discovery of *Aedes albopictus* (Skuse) in Italy in 1991, many local programs for its surveillance and control were established. During this routine surveillance activity in a tiger mosquito-free area (Belluno Province, May 2011) some larvae similar to *Ae. albopictus* were collected and were reared in laboratory. Ten newly emerged adults were clearly neither *Ae. albopictus* nor belonging to the Italian mosquito fauna. Further investigations based on morphological and molecular diagnosis identified them as *Aedes* (*Finlaya*) *koreicus* (Edwards, 1917) (Capelli G et al, 2011, Parasites & Vectors, 4: 188). After this finding, the surveillance was extended to surrounding areas to verify the distribution of this species and to attempt to trace back the possible route of entry. In this work we report the results of a year of surveillance and the implication for monitoring and control of a new species overlapping to another similar invasive species. In addition, the main features of Italian population of *Ae. koreicus* are described to help personnel involved in identification.

MATERIALS AND METHODS: The survey was performed from May to November 2011 in the Valbelluna valley (Belluno Province), Northern Italy. The area was characterized recording environmental and climatic data obtained from Land Use Map and from 18 meteorological local stations. All the potential breeding sites such as manholes, flower pots, man-made containers, tyres, vases in cemeteries were checked for larvae. Plant nurseries, garden centers and florists were also surveyed. Moreover, information obtained by ovitraps placed for the surveillance of *Ae. albopictus* were considered. A subset of eggs collected by this method were reared to obtain larvae IV stage. These were stored in 70% ethanol, clarified and identified. Adults were examined by means of a stereomicroscope or by microscope to record and describe useful morphological characters for identification.

RESULTS: A total of 22 out of 28 municipalities present in the valley were monitored and *Ae. koreicus* was found in 16 villages (73%) located at an altitude ranging from 244 to 1040 meters a.s.l. Garden centers and cemeteries were the most positive sites (13/17 and 10/14, respectively). The main breeding sites were artificial water containers and flowerpots and at a lesser extent also manholes and tires. The Larvae hatched from eggs in laboratory were identified as *Ae. koreicus*. The last positive ovitrap was found in October, 1st. The typical characters of larvae are represented by pecten teeth no spaced behind siphonal setae and shape of VIII segment teeth; the adults have an apical white band on all tarsomeri of the hind legs.

CONCLUSIONS: This is the first finding of *Ae. koreicus* in Italy and the second one in Europe, given that the species has been previously introduced in Belgium in 2008 (Versteirt et al, 2009, Brussels: Belgian Science Policy: 131 pp). This study demonstrated that *Ae. koreicus* is already established locally and further studies are in program to better define its range of distribution. The species seems to be well adapted to the urban environment and larvae develop in particular in artificial containers. Due to the predominant positivity of garden centers and cemeteries, introduction in Italy by trade of flower and plants was suspected, but not confirmed, as a possible route of entry. *Ae. koreicus* is involved in the transmission of the Japanese Encephalitis virus and *Dirofilaria immitis* (Myagi I, 1971, Trop Med, 13: 141-151). Studies on its vector competence for other arboviruses are now pivotal.

Emergence period of mature *Hypoderma lineatum* larvae in Northwestern Spain and its relation with early diagnosis and treatment

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AIM: After accomplishing a complex intraorganic migration, *Hypoderma* (Diptera: Oestridae) first instars finally reach the subcutaneous connective tissue of the back of the cattle where they penetrate the hide, molt to second instars and incite an inflammatory reaction which causes localized swellings called "warbles". The larvae grow and molt to the third-instars within these swellings. Finally, mature third instars exit the host through the breathing hole, drop to the ground and pupate within a short period of time. Climatic conditions have a direct influence on the development of free stages of the parasite, and therefore they influence the time of appearance of warbles in the back, the emergence of the mature grubs and pupation on the ground, just as adult emergence. Accurate diagnosis and treatment of this myiasis require the knowledge of the chronobiology of the parasite in a given area.
The main objective of this study was to monitor the occurrence of subcutaneous (SC) larvae of *Hypoderma* as a basis to establish the best time periods for early diagnosis and preventive treatment in this region.

MATERIALS AND METHODS: From January to April 2012, 541 cows were examined at weekly intervals in a slaughterhouse in Galicia (N.W., Spain). Sampled animals included dairy and beef cattle reared under semi-extensive or extensive management systems.The inspection for subcutaneous larvae (L2 and L3) of *Hypoderma* sp. was made by observation of the inner surface of the hides, after the skinning of the animals. All the warbles were opened and the larvae were collected and morphologically identified following the Zumpt's keys (Zumpt F, 1965, Myiasis in man and animals in the Old World, London, Butterworths, 267 pp).

RESULTS: At the beginning of the study, on 19th January, SC larvae were already present under the skin with a prevalence of 7.5% and a mean intensity of 3±4 larvae/infested animal. In next samplings, the frequency of SC larvae increased considerably with maximum levels on 23rd February (17.8%, 5.4±3.3). From this moment, the number of SC larvae decreased progressively until 12th April, when no larvae were recorded. All the larvae were identified as second and third instars of *Hypoderma lineatum*. It must be pointed out that from 1st March sampling, an increasing number of holes, scars and empty warbles were observed in the inner side of the skins.

CONCLUSIONS: In northwestern Spain *H. lineatum* larvae are present in the subcutaneous host tissues in winter months, with a peak in February. Maximum grub emergence was found in March so it can be assumed that in this area flies are active as soon as March or April. It has traditionally been recommended that early treatments against *Hypoderma* in NW Spain be applied from mid-September to October. However, given our results, we consider it is necessary to advance the application of treatment as soon as June-July. These results are supported by the observations of veterinary clinicians, who frequently detect adverse reactions when early treatments are applied at this time point (St-Oc). However, further studies to determine the presence and chronobiology of *H. bovis* in this region should be conducted because its presence could limit the efficacy of early treatments.

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Distribution and seasonal variation of *Aedes albopictus* in southwestern Sardinia

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AIM: The official appearance of the invasive exotic species, *Aedes albopictus,* in Sardinia dates back to 1994, where it was found in a used tire depot and soon eradicated (Romi R, 2001, Ann Ist Sup Sanità, 37: 241-247). After sporadic occurrence of the species in 1996-97 (Contini C, 2007, Parassitologia, 49: 33-35), *Ae. albopictus* was reported in 2006 in southern (Marchi A et al, 2007, Parassitologia 49: 71-72) and northern Sardinia (Cristo B et al, 2006, Bull Insectology, 59: 161-162) and again in 2009 (Culurgioni J et al, Parassitologia, 52: 163).
In 2010, after complaints of diurnal mosquito bites by local citizens in the town of Iglesias, a 16 months monitoring program (from 2010 to December 2012) was started, following request from local authorities. Aim of the project was to investigate 1) the presence of *Ae. albopictus* in the 23 municipalities of the Province of Carbonia-Iglesias in south western Sardinian and 2) the distribution and seasonal abundance of the species in Iglesias, one of the two major urban centres of the Province.

MATERIALS AND METHODS: Egg collections were obtained using standard ovitraps placed in shaded areas in public and private settings. In order to ascertain the presence of *Ae. albopictus*, at least 3 ovitraps were placed in the main (administrative) urban centre of each municipality and monitored for 6 weeks or more during the survey period. In case of negative results, ovitraps were operated up to 18 weeks. To achieve the second goal, 35 ovitraps were set in different districts of the town of Iglesias, from the centre to the periphery, and monitored for the entire survey period. Ovitraps were checked weekly, masonite pads were removed and replaced and the water changed. Pads were analyzed under a dissecting microscope and eggs counted, then air dried and subsequently immersed in dechlorinated tap water for hatching. The number of positive ovitraps against the total number of inspected traps and the mean number of eggs per positive ovitrap were used to measure the relative abundance of the species and the infestation rate in Iglesias. Percent of hatching was also estimated.

RESULTS: A total of 172 sites were monitored and 2234 inspections were carried on. Overall, 21.600 eggs were counted. Presence of *Ae. albopictus* was ascertained in 20 of the 23 municipalities. Negative results were obtained in Giba, Villaperuccio and Santadi on the southeast part of the Province, in spite of the extended survey period (18 weeks) and the increased number of monitored ovitraps (up to 9). However, the occurrence of *Ae. albopictus* at very low frequency or the chance of colonization in the near future cannot be ruled out considering the presence of the vector species in the surrounding municipalities and that easy spread of eggs and adults by passive transport. *Ae. albopictus* populations can be considered stable in about half of the positive municipalities, but still sporadic in others.

CONCLUSIONS: In Iglesias, colonization by the Asian tiger mosquito appears to be consolidated, with 80-100% positive ovitraps between the end of August and the first two weeks of October. Distribution was not uniform among sites and districts, being more concentrated in green areas with suitable microclimatic conditions (mean egg No. > 240 in September 2011); lowest abundance (mean egg no < 50 in September 2011) was detected in peripheral areas with scanty house density. Ovopositions were recorded from the beginning of May until the end of November. Eggs hatching was above 70% up to August, decreasing to 14% in October and November, as result of the increasing number of diapausing eggs. Although distribution and abundance of *Ae. albopictus* in 2010 and 2011 did not differ consistently, spatial and temporal changes were observed in the infestation rate between the two years, depending on climatic factors and partially on preventive control measures. In conclusion, *Ae. albopictus* appears to be widespread in the southwestern part of Sardinia (and likely in the rest of the island) with stable populations in many urban centres, especially the largest ones. Although nuisance by local people is still not very strongly perceived, effective and coordinated activities of prevention and control at the regional level are urgently needed.

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Comparative testing of the efficacy of two odour baits for sampling host-seeking *Aedes albopictus* in Rome, Italy

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AIM: The sampling of host-seeking mosquitoes is an important aspect for the understanding of mosquito-borne disease dynamics and for the development of vector control strategies. The capacity to collect this fraction of mosquito population by traps is strongly affected by the attractant used, which efficacy depends from trophic habits of the target species. *Aedes albopictus* is an aggressive daytime biter highly adapted to the urban environment with generalist host preferences (Valerio L et al, 2010, Vector Borne Zoonotic Dis. 10: 291-294). The capacity to be attracted by several host species made this mosquito an important vector for several human and animal arboviroses, such as Dengue or Chikungunya, as well as the filarial worm *Dirofilaria*. The goal of this research was to compare the efficacy in the attraction of *Ae. albopictus* of two baits conceived to capture host-seeking mosquitoes: the Biogents commercial bait "Sweetscent", already known to be effective for this species, and a novel synthetic odour blend recently developed for anthropophilic *Anopheles* species named "M'bita lure" (Mukabana WR et al, 2012, J Chem Ecol, 38: 235-244). These baits are also tested in combination with CO_2 as a potential enhancer of attraction.

MATERIALS AND METHODS: The study was conducted in Rome in October 2011, choosing two sites highly infested by *Ae. albopictus*: the garden of Anatomy building of "Sapienza" University, a small green area in a highly urbanized neighborhood, and the Verano cemetery. In the two sites six Biogents BG-Sentinel traps have been tested in a Latin square experimental design with the following attractants: no-bait (negative control), CO_2 only, Sweetscent lure, M'bita lure, Sweetscent+CO_2, M'bita+CO_2. The six BG-Sentinel traps were placed in the two study sites for 24 hours and then were rotated. In each of the two Latin squares the traps have been tested for 6 days, for a total of 12 replicates per trap. Specimens collected were identified by species and sex using a stereomicroscope. Log-transformed data were analyzed by Generalized Linear Model (GLM).

RESULTS: In total, 2039 mosquitoes have been collected, of which 94% were *Ae. albopictus* and 6% *Culex pipiens*. Among *Ae. albopictus* specimens, 55% were females. The results of GLM analysis of mean numbers of daily caught females of *Ae. albopictus* using the different baits in the two sites together showed a Sweetscent+CO_2 - Sweetscent - M'bita+CO_2 - M'bita - CO_2 - no-bait trend. In particular, t probabilities of pairwise differences showed that Sweetscent+CO_2 was significantly more effective than M'bita, CO_2 and no bait ($P<0.04$), whereas both M'bita and Sweetscent were significantly more effective than no-bait only ($P=0.006$ in both cases). However, the mean captures of Sweetscent+CO_2, Sweetscent, and M'bita+CO_2 were not significantly different.

CONCLUSIONS: The novel M'bita lure showed the same attractive effect for *Ae. albopictus* than the Sweetscent lure. On the other hand the supply of CO_2 had a supplementary positive effect in attracting *Ae. albopictus* in combination with both odour blends. These results could indicate that a lure specifically conceived to attract anthropophilic mosquitoes has not a higher effect than a less specific attractant when applied to collect generalist species.

New report of *Ixodes festai* Rondelli, 1926 (Acari: Ixodidae) in Italy (Apulia region)

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AIM: *Ixodes festai* is a poorly-known bird parasite tick. It was originally described by Maria Tonelli Rondelli in 1926 from a female specimen on *Alectoris barbara* (Phasianidae*)* in Derna (Libya). The species mainly occurs in the West Mediterranean (Tunisia, Morocco, Southern France, Corsica) (Pérez-Eid C, 2007, Les tiques. Identification, biologie, importance médicale et vétérinaire, TEC & DOC Lavoisier, Paris) but it has also been recorded in Switzerland (Papadopulos B et al, 2001, Acarologia, 42: 3-19), Germany (Horst H, 2003, Zeckenborreliose Lyme-Krankheit bei Mensch und Tier, Spitta Verlag GmbH & Co KG, Balingen, Germany) and Poland (Siuda K et al, 2006, Biological Letters, 43: 147-151). In Italy, its presence was reported for the first time in Sardinia in 1998 from female specimens collected on Turdidae in the winters of 1971 and 1997 (Contini C, 1998, Parassitologia, 40: 37) and on hunting dogs and feral cats (Garippa G et al, 1998, Parassitologia, 40: 70). Subsequently, its presence has been reported on the islands of Montecristo and Ventotene (Livorno, Tuscany and Latina, Latium respectively) on examining two unidentified engorged females collected by Manilla in April 1990 on *Turdus torquatus* and *T. philomelos* (Iori A et al, 2004, Parassitologia, 46: 134). More recently, a detailed redescription of the male of *I. festai* was performed using further specimens collected on Turdidae in Sardinia (Contini C et al, 2011, Parasite 18: 235-240). This note reports for the first time the collection of *I. festai* in continental Italy.

MATERIALS AND METHODS: The specimens were collected by dragging in Bosco Incoronata Regional Park (Foggia, Apulia region) in December 2011 and January 2012 respectively, as part of a survey aiming to improve our knowledge of tick-borne pathogens in protected natural areas of the region where human recreation (picnicking and hiking) is common.

RESULTS: The ectoparasites were identified as two female specimens of *I. festai* according to morphological keys by Manilla (Manilla G, 1998, Acari Ixodida, Fauna d'Italia, Calderini Edizioni, Bologna, Italy) and Iori (Iori A, et al, 2005, Zecche d'Italia, Mappe parassitologiche, Rolando Editore, Italy), respectively and on the basis of previously described morphological characters (Gilot B, Pérez C, 1978, Revue Suisse Zool, 85: 143-149; Manilla G, 1991, Parassitologia, 70: 197-206).

CONCLUSIONS: Bosco Incoronata Regional Natural Park consists of about 1,000 Ha, including 320 Ha of lowland forest and 115 Ha of pastures. Vegetation consisting of willow (*Salix alba*), poplar (*Populus alba, P. nigra*), pine (*Pinus* spp.), eucalyptus (*Eucalyptus* spp.) and oak (*Quercus pubescens*) where the undergrowth is sparse, consisting of a herbaceous evergreen shrub layer (Massarelli C, Tommaselli V, 2010, Interdipendenze, 1: 2-8). The mammalian fauna is made up of common predators such as the fox (*Vulpes vulpes*), polecat (*Mustela putorius*), badger (*Meles meles*), stone marten (*Martes foina*) and stray dogs (*Canis lupus familiaris*) as well as unconfirmed reports of otter (*Lutra lutra*), while the avian fauna is also numerous, consisting in both resident and migratory birds, including several species of Turdidae (*Turdus philomelos, T. pilaris, T. merula*) (Del Rosso G, 2007, Piano Comunale dei Tratturi, 6: 1-52). This Regional Park lies on migratory bird routes. Migratory birds have been suggested to aid the dispersal of several tick-borne pathogens as they are able to carry ticks over long distances. The finding of *I. festai* could be linked to the birds, including Turdidae, which are abundant in the study area and on which this tick was previously recorded in Italy. Interestingly, in addition to Turdidae, foxes (Gilot B, Pérez C, 1978, Revue Suisse Zool, 85: 143-149) and hunting dogs (Garippa G et al, 1998, Parassitologia, 40: 70) have also been recorded as hosts of *I. festai* in Southern France and in Italy (Sardinia), respectively. They might play a role in the dispersal of *I. festai* in this biotope. However, further research is needed to investigate whether the Park is a suitable habitat for this tick species.

Emerging Mosquito Borne Diseases in Italy: effectiveness of the available biocidal products, techniques and strategies for vector control

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AIM: The purpose of this presentation is to provide an update of the biocides available for use in public health and to present the "state of the art" of the laboratory and field trials carried out by a working group, recently implemented on voluntary basis, aimed to set up specific guidelines with standardized and detailed procedures for the correct execution of vector control operation in case of a Vector Borne Disease Outbreak.

MATERIALS AND METHODS: Among the human communicable diseases, the Vector Borne Diseases (VBD) represent a particular group, because of their peculiar epidemiology that involves, at least, 3 different organisms: the invertebrate vector, the pathogen and the human host. As most of the VBD are zoonosis (due to an infectious agent of livestock able to cause disease in humans and that is naturally transmitted between the two), one or more wild and/or domestic animals may also be involved as reservoirs of the pathogen). VBD are also considered the most susceptible to climatic and environmental changes, that may strongly influence the vectorial capacity of a competent arthropod. Moreover, the global increase in the average temperature recorded during the last decade, coupled with the great increase in human travel and of good trade (globalization), have resulted in a quick spread of both vectors and infectious agents into new areas.

RESULTS: In recent years, Italy experienced the introduction of 2 exotic infection transmitted by mosquitoes (MBD). The outbreak of Chikungunya fever occurred in 2007 (transmitted by *Aedes albopictus*) and the West Nile disease that is going to become endemic in our country (transmitted by *Culex pipiens*), highlighted the need to be prepared for facing a possible MBD outbreaks. The quick and drastic reduction of the vector density, represents the main way to control successfully an MBD outbreak and, at present, this is possible only with the use of the insecticides, by an effective strategy of intervention and by the correct execution of the larvicide and adulticide treatments. Nevertheless, since 2004, the European Parliament and the Council, adopting he Decision of the 6th Environment Action Programme, have recognised that the impact of pesticides on human health and the environment must be strongly reduced. Although the need to achieve a more sustainable use of pesticides is mainly targeted to the necessary crop protection, the recent introduction of the MBD in Europe, led to a renewed interest on the use of pesticides devoted to public health, given lost since malaria eradication. In 1998, the European Commission, releasing the DIRECTIVE 98/8/CE on BIOCIDES, has implemented a revision of the pesticides to be used in public health and progressively but dramatically reduced the number of active ingredients (a.i.) available for the control of pest arthropods, including those of medical interest.

CONCLUSIONS: Hence, detailed guidelines, for personnel belonging to both central and local structures of the National Health Service and to the Municipalities (first in charge of control activities) who should be able to plan, to direct and to evaluate a control intervention and operative protocols and courses for training the technical personnel devoted to work on the field should be made available. Starting from the result of our most recent trials, the characteristics and the efficacy of the available a.i., their formulations, strategy and techniques of intervention diversified by vector and disease, the correct use of the equipment, in particular those for outdoor ground space spraying, will be shortly but critically discussed, also in the light of the advancement reported in the recent literature.

Comparative performance of CO_2 traps and sticky traps to capture sand flies in northern Italy

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AIM: Several trapping methods have been developed and used to capture sandflies and to describe their ecological features. The choice of sampling method should take into account the advantages and disadvantages of each trap in relation to the aim of the study (Alexander B, 2000, Med Vet Entomol, 14: 109-122), environmental parameters (e.g. relative humidity) of the study area and the structure of each single site. The present study describes the comparative performance of two different traps, CO_2 trap and sticky trap, used in selected sites, during a 3-years entomological survey in Veneto Region, northern Italy.

MATERIALS AND METHODS: Sand flies were collected using CO_2 traps (1kg dry ice) for one night and sticky traps (20x20 cm papers coated with castor oil, generally 10 papers, min. 5, max. 20) for two consecutive nights in 14 different sites of Veneto Region, for a total of 44 captures. The collections were performed for 3 years (2009-2011) during sand flies activity season. Sand flies were stored in 70% ethanol and identified according to morphological features (Romi R et al, 1994, ISTISAN, 94/8: 33-42). Results of the performance were analysed using descriptive statistics and the correlation between the two methods was calculated using the Spearman's rank correlation coefficient (R software 2.14.1).

RESULTS: Overall, 2,862 sand flies were collected, 2,443 by CO_2 traps (85.4%) and 419 by sticky traps (14.6%). At present all specimens captured by sticky traps have been identified (82.3% *Phlebotomus perniciosus,* 6% *P. neglectus,* 0.7% *Sergentomyia minuta*). Out of the 44 sampling performed, 30 (68.2%) resulted positive by CO_2 traps and 19 (43.2%) by sticky traps. Considering only the positive captures, on average, the CO_2 traps captured 81.4 sand flies, whereas sticky traps 22.1. All the catches found to be negative by CO_2 traps, were negative also by sticky traps (n=14). Conversely, some sampling negative by sticky traps were positive by CO_2 traps (n=11). The seasonal trend of sand flies abundance was concordantly described by the two methods of capture, as evidenced in Fig.1. Considering the whole 44 sampling, the number of sand flies captured by sticky was positively correlated to the number of specimens collected by CO_2 traps (rho=0.77; p<0,001). Also considering only positive captures (n=30), correlation was significative (rho=0.67; p<0,001).

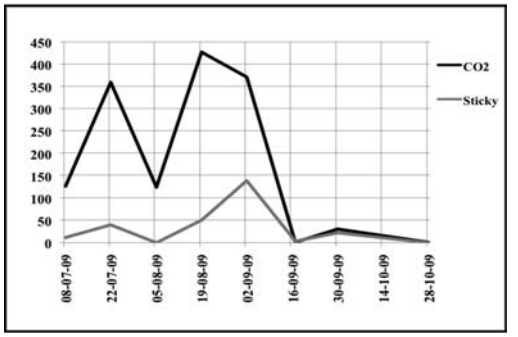

Fig. 1. Seasonal trend of sandflies collected in one sites by sticky and CO_2 traps in the year 2009

CONCLUSIONS: Sticky traps constitute an inexpensive and simple method for randomly determining species composition and seasonal trend of sand flies of a study area, and provide density values, in order to compare abundance of sand flies in different areas. Notwithstanding, they could be ineffective at low vector densities and may be highly influenced by correct positioning of the traps. Instead, CO_2 traps offer a more productive method for sampling sand flies than others attractive or not attractive traps (Veronesi E et al, 2007, J Vector Ecol, 32: 313-318; Kasap O E et al, 2009, Acta Vet Brno, 78: 327-335), but have rarely been used for describing the seasonal trends of sand flies.

The results support the idea that CO_2 trap could be a suitable method for describing the sand fly seasonal trend. The study shows a higher sensitivity of CO_2 traps compared to sticky traps, suggesting that they may be very effective to investigate unknown areas or to exclude with more certainty the presence of sand flies (e.g. in order to develop risk maps). The identification of caught sand flies has to be completed to verify which trap is more effective for the determination of the species composition.

Diversity of phlebotomine sand flies in different environments in southern Italy

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Phlebotomine sand flies (Diptera: Psychodidae) are vectors of several zoonotic pathogens including viruses, bacteria and protozoa (Tesh R, Guzman H, 1996, Sand flies and the agents they transmit, BJ Beaty& WC Marquardt (eds), University Press of Colorado, USA). They are the main vectors of *Leishmania* spp. the causative agents of the leishmaniases (Alexander B, Maroli M, 2003, Med Vet Entomol, 17: 1-18). Understanding the ecology of phlebotomine sand flies is pivotal to better control the pathogens their transmit, such as *Leishmania* spp. (Otranto D et al, 2009, Parasit. Vectors 2 (Suppl 1), S2).

AIM: To investigate the species composition of sand flies in a protected ecological reserve, in Basilicata, southern Italy.

MATERIALS AND METHODS: From March to November 2010, an entomological survey was conducted in the forest of Gallipoli Cognato, a protected reserve of historical, and ethno-anthropological value located in Basilicata region, southern Italy. Light traps were placed in six collection sites (CS), with the following characteristics: CS1, between a woodhouse (with some hares) and a stone wall, in an anthropized area surrounded by the forest; CS2, near some volcanic rocks, in a high-altitude forested area; CS3, on the trunk of a tree, at about 1 m high, in a forested area near a human house; CS4, near a sheep stable, in a small farm; CS5, near a chicken pen, in an urbanized area; CS6, nearby a house in an urbanized area. Collection sites were selected based on their characteristics, including presence/absence of animals, type of vegetation, and degree of urbanization. The light traps were installed at dawn and were operated for 12 consecutive hours and collected phlebotomine sand flies identified by classical morphological keys (Killick-Kendrick R et al, 1991, Parassitologia, 33: 335-34725; Corradetti A et al, 1960, Parassitologia, 3: 101-103).

RESULTS: Over 7,000 phlebotomine sand flies were collected and they belonged to six (*Phlebotomus papatasi*, *Phlebotomus perniciosus*, *Phlebotomus perfiliewi*, *Phlebotomus neglectus*, *Phlebotomus mascittii*, and *Sergentomyia minuta*) out of the eight species reported in Italy (Maroli M et al, 1994, Parassitologia, 36: 251-264). The most representative species were *P. perfiliewi* (n = 6,571; 90.23%) and *P. perniciosus* (n = 636; 8.73%) and the majority of them were collected in July (n=2,786, 38.26%) and August (n = 2,280; 31.31%), when the highest monthly mean temperature (21.4°C) and lowest monthly mean relative humidity (44.6%) rates were recorded. The highest number of specimens and the greatest species richness were recorded in sites located in urbanized areas (i.e., 6,102 specimens and up to 4 species in CS6; 864 specimens and 3 species in CS4 and 302 specimens and 5 species in CS5). On the other hand, no phlebotomine sand fly was trapped in CS3 or within the forested area (CS2), with the exception of the CS1, where few specimens (15 specimens and 3 species) were collected in July and August.

CONCLUSIONS: These findings confirm that phlebotomine sand flies are well adapted to environments in the studied area, where they find suitable conditions in terms of microclimate and host availability for their perpetuation. Moreover, the presence of vector species (e.g., *P. perniciosus*) near human houses indicates the potential risk for *Leishmania infantum* transmission in this region of southern Italy.

Acarologic investigation in the neighbourhood of Tarquinia, Latium region, Italy

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AIM: This study is aimed to deepen the knowledge of tick fauna composition and species distribution in Latium region and the possible presence of pathogens potentially circulating in such rural ecosystem. The research undertaken in May 2011 in collaboration with Università della Tuscia di Viterbo (VT) and Università Agraria di Tarquinia (VT), is still ongoing and the present abstract reports the data recorded until the early 2012.

MATERIALS AND METHODS: The study area consists of 4 sites situated at Ancarano, Roccaccia and Le Fornacette localities in the Northern outskirts of Tarquinia town. These sites were mainly selected on the base of the conditions considered suitable for the most common tick species occurrence, as cattle breeding pastures and wood areas alternate to fields. Moreover, sites were also chosen in the light of human activities there commonly carried out that could lead to suppose a high possibility of tick-human contact as open-air activities, hunting, timbering, camping and harvesting of various country products; this situation could favor a possible pathogens circulations. This research has been planned as an one-year tick surveillance of the study area. Until now monthly surveys were carried out from May 2011 to January 2012 in the selected sites, by flagging and dragging for 30 minutes/operator. During each sampling, temperature and relative humidity were recorded. The samples were transported in our laboratory and identified on the basis of morphological characters (Manilla G, 1998, Acari Ixodida, Fauna d'Italia, Calderini Bologna), and stored at –80°C for future molecular analysis.

RESULTS: The surveys allowed to collect 94 tick specimens belonging to 4 genera and 6 species: *Ixodes ricinus*, (n=63; 65%), *Dermacentor marginatus* (n=2; 2%), *Hyalomma marginatum marginatum* (n=3; 3%), *Rhipicephalus sanguineus* (n=3; 3%), *Rhipicephalus turanicus* (n=8; 8%), and *Rhipicephalus bursa* (n=18; 19%). *I. ricinus* constituted the main part of the sample while *D. marginatus* was the smaller. We compared the seasonal trend of the collection during the study period with the main climatic factors as falls, temperature and relative humidity and we noted that the most abundant collections were concentrated in May-July and in December-January, while in August-September collection was scarce or null.

CONCLUSIONS: The present inquiry pointed out a rather various ixodidic fauna composed by typical species of ecotonal environmets. Even if the number of specimens was not so abundant to completely define the seasonal trend of several species, tick composition seems to reflect the presence of wild animals and cattle in the study area. The scarce abundance of ticks in late summer and in early autumn was probably correlated to the prolonged absence of falls in that period. The results of this acarological research represent a significant contribution to the knowledge of the tick fauna of rural areas in Northern Latium region, as first step toward a future molecular investigation on pathogen circulation.

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SESSIONE 3

PARASSITI E FAUNA SELVATICA

Assessment of *Cephenemyia stimulator* infection in roe deer (*Capreolus capreolus*) from Asturias (North Spain) by ELISA

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AIM: In cervids, one of the most studied naso-pharyngeal myiasis caused by dipterous of the family Oestridae is *Cephenemyia.* Generally, all *Cephenemyia* species are very host specific and thereby also well adapted to their hosts. First time *Cephenemyia stimulator* was reported in Spain, in the mountains of Valdés Council (Asturias) by hunters in 2008. It might be the outcome of an irregular roe deer introduction from endemic areas. The mean drawback for oestrosis control is the difficult of its diagnostic. The aim of the present study was to estimate the seroprevalence of infection by *C. stimulator* in roe deer.

MATERIALS AND METHODS: During hunting seasons, 74 male roe deer were examined for myiasis. Animals were shot from March-June 2009 in Asturias (NW Spain). At the necropsy, the skin of the ventral side of the neck and head was removed and the esophagus and trachea opened. One blood sample of each animal was collected from the heart. Antibodies (IgG) were detected by ELISA using second instars (L2) *C. stimulator* excretory/secretory antigens (CsES) or *C. stimulator* somatic antigens (CsAS).

RESULTS: Sensitivity and specificity of this ELISA were 100% and 81%, respectively. The seroprevalence was 32.4% and 35.1% using CsES and CsAS, respectively. No significant differences were matched regarding the gender. We observed a high correlation between IgG values obtained using CsES and CsAS antigens (CC=0.760, P=0.000). We only detected a high correlation between IgG values and larvae total number (CC=0.516, P=0.004) using CsES. A significantly highest seroprevalence was detected according oestrids number (χ^2= 16.813, p= 0.032) (p< 0.05). Furthermore, we reported a high correlation between IgG values and animals weight (CC=0.477, P=0.005 and CC=0.461, P=0.007, using CsES and CsAS, respectively). As for sampling month, in March and June we did not detect seropositive animals; in April we reported the highest seroprevalence (40%), meanwhile in May only low values (28.6%) were registered.

CONCLUSIONS: We encourage the use of ELISA and L2 *C. stimulator* excretory/secretory antigens to detect antibody response in epidemiological enquiries, which are necessary for a real estimation of the distribution of oestrids in wild ruminants and like a viable alternative to the clinical or the post-mortem parasitological examination.

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Trichinella britovi spreads in Sardinia: from free-ranging pigs to red foxes (*Vulpes vulpes*)

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AIM: Up to 2004, the Italian Island of Sardinia has been considered *Trichinella*–free. In 2005, two trichinellosis outbreaks involving 19 people and a single case in 2007, occurred for the consumption of fresh homemade products from free ranging pigs infected by *Trichinella britovi* in the Orgosolo village (Nuoro province) (Cossu P et al, 2006, Parassitologia 48, 303; Pozio E et al, 2009, Vet Parasitol. 23, 159). In January 2011, a new human trichinellosis outbreak (6 hospitalised people) occurred in the same village of Orgosolo for the consumption of fresh sausages produced from free-ranging pigs. The aim of the present work was to investigate the spread of *T. britovi* in pig populations and hunted wild animals in the entire province of Nuoro and in the adjoining province of Ogliastra during the 2010-2011 and 2011-2012 winters.

MATERIALS AND METHODS: From October 2010 to February 2012 at the Istituto Zooprofilattico Sperimentale of Sardinia, muscle samples (5 g of diaphragm pillars from pigs and wild boars; 10 g of foreleg muscle from foxes and martens) from 5,541 animals were tested to detect *Trichinella* sp. larvae by artificial digestion according to Regulation (EC) No. 2075/2005. In particular: 1) 351 samples from free-ranging pigs illegally raised, 470 from legally breed backyards pigs slaughtered at the farms, 208 from wild boars (*Sus scrofa meridionalis*), 28 from foxes (*Vulpes vulpes*) and 1 from a marten (*Martes martes*), were collected from the Orgosolo municipality; 2) 308 samples from legally breed backyards pigs slaughtered at the farms, 866 from wild boars, 18 from red foxes and 1 from a marten were collected from the municipalities surrounding Orgosolo; 3) 863 from legally breed backyards pigs slaughtered at the farms, 2,390 from wild boars, 32 from red foxes and 5 from martens were collected from the other municipalities of the Nuoro and Ogliastra provinces. *Trichinella* sp. larvae were collected from each positive sample, stored in 90% ethyl alcohol and sent to the International Trichinella Reference Center of Rome for the identification at species level by multiplex PCR.

RESULTS: *Trichinella* larvae, were detected only in animals from the Orgosolo municipality. Specifically, larvae were collected from 9 (2.6%) free-ranging pigs, which had an average age of 5.3 years (range 1-10 years) and from 10 (35.7%) foxes. Infected pigs and foxes had an average larval burden of 127 larvae/g (range 0.4-543) and 79 larvae/g (range 3.4 - 565), respectively. All the other domestic (1,983) and wild (3,539) animals tested negative regardless their geographical origin. Multiplex PCR identified all the larvae as *Trichinella britovi.*

CONCLUSIONS: This is the first report of *T. britovi* in wild animals of Sardinia. The presence of this *Trichinella* species in the red fox is not surprising since this carnivore species plays the major role of reservoir of *T. britovi* in Europe (Pozio et al, 2009, Int. J. Parasitol. 39, 71) and consequently, it can be considered as a sentinel animal to assess the spreading of this zoonotic parasite in nature. At present, *T. britovi* infection is confined to the Orgosolo municipality even if its distribution area seems to be wider than that previously reported in free-ranging pigs (Pozio E et al, 2009, Vet Parasitol. 23, 159) and closer to neighbouring municipalities where it could rapidly spread. The further detection of *T. britovi* in uncontrolled and illegal free-ranging pigs and the existence of this pig breeding in many areas of Sardinia, could result in the spreading of this zoonotic pathogen in other areas of the island. We can speculate that the cannibalism among the illegal free-ranging pigs may occur as a consequence of the endemic presence of the African Swine Fever in this area, favouring the spreading of *T. britovi.* The discovery of *T. britovi* in foxes suggests that all the attempts to eradicate this infection from pigs of the Sardinia island could be frustrated by the presence of the sylvatic cycle.

Parasite load and hormonal immunosuppression in alpine chamois *Rupicapra rupicapra*

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AIM: Parasites indirectly affect life-history evolution of most species because of the costly immune defences required to control them. In vertebrate males, androgens, especially testosterone, affect mating success through the development of sexual signals, however they also can have immunosuppressive effects. This is also known as Immunocompetence Handicap-Hypothesis (ICHH) that was revealed controverted in different species. The aim of this work is to test the ICHH in an Alpine chamois (*Rupicapra rupicapra*) protected population.

MATERIALS AND METHODS: From January 2011 a faecal sample was monthly collected and analysed from 17 marked chamois from the Gran Paradiso National Park. For each sample, the parasitic load of different parasites (gastrointestinal nematodes, bronco pulmonary nematodes and coccidian protozoans) was studied with the zinc sulphate flotation method and the Mc Master counting procedure, whereas the testosterone metabolites level was analysed with an Enzyme Immunoassay (EIA). Parasite load and testosterone metabolites relationship was analysed using generalized linear mixed models (GLMM), considering other eco-ethologic covariates as individual age, mating behaviour and month.

RESULTS: From a preliminary analysis (January-May 2011), no correlation between testosterone and parasite appears. Parasite load doesn't show influence by mating behaviour and age, which is instead important for the production of testosterone in old animals. However the analyse of the whole year reveals a marked periodicity for parasites, showing high levels of lungworms during cold period and gastrointestinal nematodes in summer. Interesting, a pick of both nematodes happens during rut period. From statistical analysis, a positive correlation between testosterone and both lung (p_{value} = 0,049) and gastrointestinal (p_{value} = 0,000) nematodes is founded in autumn (October-December 2011) and also all long the year (p_{values} of 0,0001 and 0,0000 respectively).

CONCLUSIONS: Actually, sample collection and analysis are still in progress. Considering one year long, testosterone seems to be an important factor influencing the parasite infestation. In fact, during the rut season, where hormone levels are higher, nematodes from gut and from lung release the maximum number of eggs and larvae. These data seem to confirm ICHH, but testosterone pick happens together with a cortisol metabolites increasing. Much effort need to be done to understand the real role of testosterone in immunosuppression.

Demography and pathogens pressure on shaping variability at MHC DRB1 locus in chamois species

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AIM: Chamois (*Rupicapra* spp.) are mountain ungulates from Southern and Central Europe and the Near East. Alpine chamois (*R. rupicapra*) includes seven subspecies and Pyrenean chamois (*R. pyrenaica*) includes three subspecies. Population dynamics of *Rupicapra* spp. are influenced by environmental resources, climatic factors, anthropic impact and disease outbreaks. For instance, *R. p. parva* in Spain and *R. r. rupicapra* in the eastern Alps have been severely affected by sarcoptic mange due to *Sarcoptes scabiei* var. *rupicaprae* (Pence and Ueckermann, 2002, RevSci Tech Off Int Epiz, 21: 385-398; Rossi L et al, 2007, Eur J Wildl Res, 53: 131–141) and *R. p. pyrenaica* has been affected by Border Disease Virus (BDV) outbreaks that have led to a dramatic population decrease (Marco I et al, 2008, Vet Microbiol 127: 9–38). Epidemics can be even more important in mountain species inhabiting semi-isolated, fragile and fragmented habitats, due to low colonization rates and low gene flow between populations. Nowadays studies on immune system are growing in importance since infectious diseases are increasingly recognized for their crucial role in influencing host genetic variability, coevolution and altering species composition in ecological communities of natural systems. The Major Histocompatibility Complex (MHC) is a sensitive marker for genetic variation of populations: it is a multigene family and the variants at its loci influence many important biological traits. Moreover, its variability is affected by both pathogens and population dynamics and it is ecologically relevant, depending on host-pathogen relationships and life history. These features place MHC genes among the best candidates to study mechanisms and significance of molecular adaptation in vertebrates (Sommer S, 2005, Front Zool, 2:16; Mona S et al., 2008, Mol Ecol, 17:4053-4067).

MATERIALS AND METHODS: With the aim to investigate polymorphism at MHC class II DRB1 exon2 in relation to population structure and possible bottlenecks caused by exposure to parasitic infections, samples of 69 specimens of *R. r. rupicapra* in different epidemiological situations for sarcoptic mange and samples of 32 specimens of *R. r. tatrica* subspecies were studied. Tatra chamois is listed as critically endangered by IUCN since it has a very small population of less than 200 individuals. This is the first study on variability at immune system of *R. r. tatrica*.

RESULTS: Data obtained by haplotype inference, population genetics indexes and neutrality tests have revealed a high degree of nucleotide and aminoacid polymorphism, with most aminoacidic variation occurring within the PBR (Peptide Binding Region) in alpine species, but high level of homozygosity in Tatra samples.

CONCLUSIONS: The relative frequencies of alleles in the studied populations showed the presence of highly frequent haplotypes that are common in all samples and of several haplotypes showing low frequencies, related only to specific healthy categories (private alleles). These results have been also related to previous data on Pyrenean chamois (Cavallero S, 2012, Infect Genet Evol, doi: 10.1016/j.meegid.2012.02.017) to depict the action of balancing selection and the effect of bottlenecks on the amount of variation at DRB1 locus in different demographic, ecologic and environmental conditions.

Parasitic situation of the European mink (*Mustela lutreola*) in Navarra (Spain)

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AIM: At present, European mink (*Mustela lutreola*) population is restricted to two non-connected small sub-populations. One is located in the north-west of Russia and Rumania and the other in the south-west of France and north of Spain. The future of this specie is uncertain, consequence of several factors (Palazón et al, 2006 Proceedings of the International on the Preservation of European Mink, Logroño (Spain), p.143-150). Similar to the European situation the Spanish population of European mink is decreasing and the area in which is dispersed is just restricted to the regions of País Vasco, Navarra, La Rioja and the north-east of Castilla y León.

MATERIAL AND METHODS: The research was carried out in the Foral Community of Navarra, before and after the reproductive season, by means of a sample done in seven river sections using river trapping stations (Palazón et al, *Mammalia* 67:473-484). The survey was carried out during the pre-reproduction season (February-March) and post-reproduction season (September-December). Captured minks were tranquilized, tagged and biometric and biologic data and samples were collected for further health and genetics analysis.
The presence of ectoparasites was checked in 71 minks (26 and 45 from the pre-reproductive and post-reproductive season respectively), and 66 fecal samples (24 and 42 animals respectively). Furthermore parasites present in the feces, the small intestine, lungs, liver and muscles from eight carcasses from knocked down minks were also evaluated. Laboratory methods were the standard in the evaluation of ectoparasites (*MAFF*, 1986).

RESULTS: Ticks, from species *Ixodes hexagonus* and *I. acuminatus,* were present in 30.98 % of the minks checked with a mean of 2.8 tick/animal (min.1 and max.10). This data are according to those published by Torres J et al, 2006 (Veterinary Parasitology 137: 379-385). Season and sex variables had no effect in the results. 47.6 % of the parasited minks were from the river Argas. (Diez N et al, 2005, Acta Parasitologica Portuguesa 12: 335-336). 57.58 % of the minks had endoparasites (33.3 % and 71.4 % from the pre-reproductive and post-reproductive season respectively). Scarce number of intestinal nematode eggs (29% of Strongiloideos, 15% Ascaroideos) and 16.2 % of coccidia oocyst (4.1 % of *Eimeria spp.*, 2.7 % of *E. furoni*, 1.3 % of *E. mustelae,* 8.1% of *Isospora laidlawi*) (Hidalgo MR et al, 2005, *Acta Parasitológica Portuguesa* 12: 241-242). Larva from respiratory nematode were detected at 23.4 % of the animals with a mean of 8.1 larvae/mink (min. 1 max. 28). 3 % and 1.5 % of the minks presented eggs from Trematode and Cestode respectively.
62 % of the carcasses minks were parasite by a scarce number of larvae, eggs and oocyst of coccidia, microcysts of sarcosporidia and adults parasites of *Metorchis sp.* and *Mesocestoides spp.*

CONCLUSIONS: The results show that minks are frequently parasited, even when the amounts of parasites detected are low. Their presence has a negative effect on the immune system affecting their survival.

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Targeted selective treatments: The alpine ibex (*Capra ibex*) as study model for small ruminants to identify the most affected and infectious individuals

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AIM: Recent investigations are increasingly addressed to the development of targeted selective treatments (Cringoli G et al, 2009, Vet Parasitol, 164: 36-43) as approach to improve the efficacy parasite control and avoid the development of anthelmintic resistance. Indeed parasite infections are aggregated on a restricted number of host individuals and this pattern affect impact and transmission of infections, determining that a few hosts are responsible for the maintenance and transmission of the parasite population (Woolhouse MEJ et al, 1997 PNAS, 94: 338-342). However while the effects of some factors promoting these patterns are known (Wilson K et al, 2002 The Ecology of Wildlife Diseases, Oxford University Press) a broader comprehension of the mechanisms determining the most infected and infectious individuals is needed in order to predict their composition. Here we analyse the effect of animal life history on the determination of the most infected infectious individuals.

We analysed Alpine Ibex (*Capra ibex*), because its phylogenetic proximity with domestic small ruminants makes an appropriate study model. In particular ibex are characterized by a pronounced sexual dimorphism in several biological and life history traits. Ibex males carry large horns and weight till the double respect females. Differences in survival is observed with males showing high survival until the age of 11 years, followed by a drastic senescence while females, show a more gradual survival decline. This pattern is interpreted as a strategy where males allocate most of their energy to survival until reaching a definitive body size, which allows reproduction, while females optimize their fitness modulating reproduction instead of their survival. These pattern may affect parasite infection with sexual dimorphism determining male's higher parasite loads and infectivity. Moreover life-history should determine the control of parasite infection in males until the age of about 11 years when they invest more in survival and immune-response, afterwards parasite infection should increase. Females, on the other hand, should show lower parasite infections and no change during their life. In this study we tested two hypotheses: 1) males show higher infection intensities and infectivity; 2) in males infection increases with age while in females infection is constant. As final consequence, the most infected and infectious group in the population should be represented by the older males.

MATERIALS AND METHODS: We quantified the parasite Egg Per Gram (EPG) of 319 faecal samples collected during summer months from 52 individually marked ibex in the Gran Paradiso National Park in 2008-2009 and we estimated the direct parasite count in abomasa from 94 Swiss ibex hunted in 2007-2009. These dataset represents two complementary parasitological processes; EPG reflect the parasite transmission while parasite count represents actual infection.

RESULTS: Alpine ibex were infected by a parasite community composed by 10 *Trichostrongilidae* species with an overall mean abundance of 1891 (±125 S.E.) helminths/individual. The two dominant species *Teladorsagia circumcincta* and *Marshallagia marshalli*, represent together 91% of all found parasite species. Data confirmed their significant higher infection intensities and EPG in males. The generalized mixed effect models best describing the influence of age on parasite intensities and EPG evidenced in males an initial increase followed by constant parasite infection/EPG till the age of 11 yrs followed by a later progressive increase. Females did not show any change between ages.

CONCLUSIONS: These results showing that old males are the age/sex class most infected and possibly responsible in parasite transmission evidence as the parasite infection reflects the age survival profile and suggest the use of life-history as a predictive tool to identify the most infected/infectious host group that could be useful to identify animal groups to target selective treatments in livestock.

Health risk associated with the presence of coypus (*Myocastor coypus*) in Lombardy: an overview of *Toxoplasma gondii*, *Giardia* sp. and *Cryptosporidium* sp. infections

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AIM: The increasing presence of allochthonous species as the coypu (*Myocastor coypus*) underlines the necessity to state the possible risks associated to their presence in environment as concerning biodiversity, economic damages and health security. We focus the attention to important zoonotic, protozoan pathogens investigating prevalence in this species.

MATERIALS ND METHODS: From November 2008 to March 2011, 156 coypus were trapped and sacrificed following ISPRA (Istituto Superiore per la Protezione e Ricerca Ambientale) guidelines in Milano Province (Lombardy Region). Necropsies were performed; blood samples were collected, centrifuged and then sera stored at -20°C, lungs and kidneys were isolated and stored at -20°C. To evaluate the presence of intestinal protozoans *Giardia* sp. and *Cryptosporidium* sp., 75 fecal samples were tested using a commercial enzyme immunoassays (RIDASCREEN®R-Biopharm) based on antigen detection. For *Toxoplasma gondii*, sera of 128 coypus were tested using an indirect ELISA commercial kit (ID Screen® Toxoplasmosis). This was performed according to the manufacturer's instructions. Afterwards, to confirm *T. gondii* infection, from lungs and kidneys belonging to 11 positive animals DNA extraction was performed using QIAampDNA mini Kit (QIAGEN Inc, Valencia, US); purity and quantity of extract DNA were evaluated with NanoDrop 1000 Spectrophotometer. PCR protocol (Hurtado A et al, 2006, Vet Parasitol, 102: 17-27) used two pairs of primers: NN1 (5'-CCTTTGAATCCCAAGCAAAACATGAG-3') and NN2 (5'-GCGAGCCAAGACATCCATTGCTGA-3') that hybridize to a region of the ITS1 common to both *T. gondii* and *N. caninum*, and Tg-NP1 (5'-GTGATAGTATCGAAAGGTAT-3') and Tg-NP2 (5'-ACTCTCTCTCAAATGTTCCT- 3') that amplify a region of the same gene specific for *T. gondii*. PCR amplification of DNA was verified by electrophoresis in 2% agarose gel, and bands were visualized under UV light on a transilluminator. Once determined positivity for each PCR, specific bands were cut from gel, purified (Wizard® SV gel, Promega) and re-suspended in deionized water; finally DNA was quantified with spectrophotometer. Specimen were send to an external laboratory for sequencing; sequences comparison and alignment were conducted with GenBank database and using BioEdit v7.1.3, adding sequences of the same gene of *T. gondii* from other host-species (*Passer domesticus*, *Ovis aries*, *Felis silvestris catus*) and of *H. hammondi*, *N. caninum*, *C. parvum* and *P. falciparum*. Phylogenetic analysis were performed using Mega5 (Tamura K et al, 2011, Mol Biol Evol, 28(10): 2731-2739).

RESULTS: All samples tested for *Giardia* sp. and *Cryptosporidium* sp. resulted negative. On the contrary, serological positivity for *Toxoplasma gondii* was found in 37 coypus (P=28.29%); of these, in 19 cases the infection was acute (S/P_{value}>200), whereas 18 samples were considered only positive (S/P_{value} comprised between 50 and 200). No significant difference was noticed considering sex (Pearson's chi-squared test, p= 0.354), whereas considering age there was significant difference between positive young animals (P=5.55%) and adults (P=28.94%) (p=0.034). As regard PCR, all samples obtained from lungs were positive, whereas those from kidneys negative; serological positivity was confirmed in all the samples tested by PCR. BLAST analysis confirmed an homology of 99% with *T. gondii* founded in *Gallus gallus domesticus*. Phylogenetic analysis revealed that our samples clustered with those from cat, sheep, sparrow and chicken, indicating probably way of infection for coypus from definitive host. Sequences obtained revealed low genetic variability, being identical but a nucleotide substitution in one sample.

CONCLUSIONS: The absence of *Giardia* sp. and *Cryptosporidium* sp. is unattended, considering previous studies on coypus (Dunlap BG et al, 2002, J Parasitol, 88(6): 1254-1258) and considering the presence in rural environment and in cattle (Olson ME et al, 2004, Trends Parasitol, 20(4): 185-191). On the other hand, coypu resulted infected by *T. gondii* but, having not natural predators in the study area, the protozoan doesn't continue its biologic cycle. However, domestic carnivore animals as dogs or cats may feed on carcasses of coypus laying near urban areas, contracting the infection. Furthermore, considering the high prevalence obtained in our study, we can affirm coypu may have a role of sentinel in maintaining the infection in environment.

First record of *Capillaria hepatica* (syn. *Calodium hepaticum*) in a fox from north-west Italy

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AIM: *Capillaria hepatica* (syn. *Calodium hepaticum*) is a zoonotic cosmopolite nematode of the family Trichuridae, affecting mainly rodents but also a broad range of other mammals (Fuehrer et al, 2011, Parasitol Res, 109: 969-979). Adult worms live in the liver parenchyma, where they can cause hepatitis. The life cycle is direct and unusual. Females lay eggs in the liver, where they remain immature for the entire life of the host (Farhang-Azad A, 1977, J Parasitol, 63: 701-706). Eggs reach the external environment either through decay of the host's carcass, or through the shedding of eggs in the faeces of a predator animal with spurious infection. In the external environment eggs embryonate becoming infective for a new host.

Concerning canids, *C.hepatica* was found in wild canids (Ruas et al, 2003, Arq Inst Bio, 70, 2: 127-130; Wobeser G and Rock TW, 1973, J Wild Dis, 9: 225- 226) and in dogs (Lloyd et al, 2002, Vet Rec 151,419-420; Brander et al 1990). In Italy it has been detected in one dog in the urban area of Milan (Ceruti et al. 2001, J Vet Med B, 48, 235-240).

The aim of this work was to report an infection by *C. hepatica* in a red fox (*Vulpes vulpes*); this is, as far as we know, the first case reported in this species.

MATERIALS AND METHODS: From February 2010 to February 2012, in the framework of a larger on-going survey on canine endoparasites, 75 livers of foxes culled in north west Italy (Imperia district) were examined. Sex, age, weight and geographical origin of each animal were registered. The liver was first examined grossly to detect liver lesions. Gall bladders and bile ducts were examined separately. The gall bladders were transferred into a Petri plate, opened and observed under a stereomicroscope for the detection of adult parasites. The mucosa was washed in 50 ml of normal saline solution; the sediment obtained after 30 minutes in a becker was centrifuged (2500 rpm x 10 min) and observed under an optical microscope (magnification 40x). Liver lobules were sliced and smears were prepared. Then the entire liver was dissected along the bile ducts under a stereomicroscope. The liver was then washed in a conical becker and the the washing liquid was submitted to the same sedimentation procedure as above. Morphologic and morphometric analysis of eggs were performed with light and scanning electron microscopy (SEM). For SEM, eggs were isolated and washed, mounted on aluminium stubs, air dried, sputtered with gold and observed with JEOL JSM 5410.

RESULTS: Among the 75 fox livers examined, one presented an infection by *C. hepatica.* At light microscopy the eggs (54-65 x 22-33 µm) showed the typical barrel-shaped morphology with a double shell (outer and inner), bipolar plugs did not protrude beyond the outer shell. SEM showed in details the egg shell surface.

CONCLUSIONS: This case report is a contribution to the epidemiology of *C. hepatica* in Italy and shows that *Vulpes vulpes* is another potential host. Although this parasite affects mainly rodents, its presence in other wild and domestic animals should not be disregarded. The carcass of an infected fox may be a source of infection for animals with scavenging behaviour. The increase of foxes in urban and suburban areas may represent, through carcasses decomposition or spurious infections, a source of infestation for urban rodents and domestic animals, thus leading to a hypothetical higher risk for humans.

Capillaria plica (syn. *Pearsonema plica*) in red foxes (*Vulpes vulpes*) of Liguria, north-west Italy

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AIM: *Capillaria plica* (syn. *Pearsonema plica*) is a cosmopolitan parasite of the Trichuridae family, found in the urinary bladder and in the lower urinary tract of foxes, dogs and cats. The life cycle is indirect, with earthworms as intermediate hosts. *C. plica* infection can cause severe cystitis with haematuria and eventually pyelonephritis due to secondary bacterial infection, both in pets (Bork-Mimm S, Rinder H, 2011, Parasitol Res, 108: 1063-1067) and in foxes (Fernández-Aguilar X et al, 2010, Acta Vet Scand, 12: 52: 39). *C. plica* infection in red foxes has been reported in many European countries (Bork-Mimm S, Rinder H, 2011, Parasitol Res, 108: 1063-1067). To our knowledge, in Italy it was found in foxes more than 20 years ago (Iori A et al, 1990, Parassitologia, 32:153-154), then recently in a cat (Rossi M et al, 2011, J Feline Med Surg, 13: 793-795) and in a dog associated with glomerular amyloidosis (Callegari D et al, 2010, Vet Parasitol, 168: 338-341).
The aim of this study was to evaluate the presence of parasitic infections of the urinary bladder of foxes from Liguria, north west Italy.

MATERIALS AND METHODS: From January 2011 to February 2012, the urinary bladder of 51 foxes culled in N-W Italy (Imperia district) was examined. Foxes were collected by the Imperia section of IZS of Piedmont, Liguria and Aosta Valley. The bladder was opened, washed in a conic becker and the sediment was observed by stereomicroscopy in order to collect adult worms. The liquid was then centrifuged (700g, 5 min) to collect eggs. Adults and eggs were identified by optical microscopy on the basis of the characteristics described in the literature (Levine ND, 1968, Nematodes parasites of domesticated animals and man, Burgess Publishing, Minneapolis, USA). Furthermore, some eggs were isolated by flotation and sieving (modifying the technique of Al-Sabi MN et al, 2010, Parasitol Res, 107, 135–140), mounted on aluminum stubs, air dried, sputtered with gold and observed with a scanning electron microscope (JEOL JSM 5410). Prevalence with 95% confidence intervals (CI), abundancy, mean intensity and range were calculated.

RESULTS: *C. plica* was found in 30 urinary bladders (prevalence 59%, 95% CI 45-72%). A total of 198 adult worms were collected: the number of parasites in a single animal ranged from 0 to 20; the abundancy was 3.8 and the mean intensity was 6.6. Macroscopic pathologic alterations (i.e. bladder mucosal inflammation: reddish, thickened wall, haematuria) were detected in 70% of the positive foxes. Eggs at optical microscopy measured 50-68 x 22-32 µm; using SEM a net of anastomosed ridges on the egg shell and prominent polar plugs were observed.

CONCLUSIONS: Our results update the epidemiology of this parasite in foxes in Italy, showing a prevalence similar to other values in Europe (Bork-Mimm S, Rinder H, 2011, Parasitol Res, 108: 1063-1067). Foxes are considered a reservoir of the parasite, while its presence in pets is likely underestimated, since urine analysis is conducted only for symptomatic animals. Moreover, in faeces contaminated with urine, the eggs of *C. plica* may be confused with eggs of other Trichuridae. As a consequence, prevalence values in Europe are known for wildlife, while only case reports are available for pets; further studies would be needed for a better understanding of the epidemiology of this parasite.

Assessment of parasitic cardio-respiratory infections and decreased the populations of roe deer (*Capreolus capreolus*) in a mountainous area from NW Spain

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AIM: In recent years, roe deer (*Capreolus capreolus*)populations have increased greatly in Galicia (NW Spain), except in Ancares National Game Reserve (mountainous area) where roe deer numbers have decreased significantly. Wolves (*Canis lupus*) are their main predators in our study area. On the contrary, according to wolf census, their population has increased. Nowadays, according to Galician Ministry of the Environment, roe deer density in Ancares has decreased (1.30/km^2) and increased in other areas, respect to 90 s.
In this study, we determined the prevalence and/or intensity of parasitic cardio-respiratory infections (*Sarcocystis*, bronchopulmonary nematodes and *Cephenemyia*) during two different periods of time (1993-95 and 2007-09). Then we analysed if different prevalences found could be one of the reasons for the decline of roe deer in Ancares.

MATERIAL AND METHODS: In order to determine the prevalence and intensity of infection by *Sarcocystis* and bronchopulmonary nematodes, we used the compresorio method and the technique of larval migration, respectively. Seroprevalence of *Cephenemyia stimulator* infestation was determined by ELISA using a second instars (L2) *C. stimulator* excretory/secretory antigens.

RESULTS: The prevalence of *Sarcocystis* was very high in two decades in the roe deer hunted in Ancares (97.3 *vs* 100%) and those from other areas (81.1 *vs* 97.9%); however, the intensity of infection (cyst per sample, cps) with this protozoan Apicomplexa in roe deer from Ancares increased considerably in the last decade (507 cps *vs* 32 cps) relative to we observed in other areas (300 cps *vs* 9.2 cps). Also, in the last years, the prevalence of bronchopulmonary nematodes in Ancares (31.7 *vs.* 63%) has increased compared to other areas (8.4 *vs* 49%), although the average of larvae per gram of faeces (lpg) were low in the both areas and decades (12 *vs* 4 lpg in Ancares and 1.6 *vs* 10 lpg in other areas).
Pajares (2008) observed *Cephenemyia* by first time in roe deer hunted in NW Spain. subsequently, Arias et al. (2011, Congreso Ibérico de Parasitología. Zaragoza: 288) reported by ELISA an outstanding higher seroprevalence of antibodies against *C. stimulator* in wildlife animals from Ancares (60%) than in those roe deer from the rest of Galicia (<30%).

CONCLUSIONS: We can conclude that roe deer from Ancares National Game Reserve are heavily infested by parasites affecting cardio-respiratory system (*Sarcocystis* and especially *Cephenemyia)*. Our results suggest that these wild ungulates could be an easier prey for wolf than in other areas where roe deer have a less parasitic burden. These research findings could be related to the fact that roe deer numbers in this Reserve have decreased in recent decades. During this period their predator has increased according to Galician Ministry of the Environment. Surprisingly, roe deer census in the rest of Galician populations has increased considerably.

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The prevalence and distribution of *Alaria alata*, a potential zoonotic parasite, in foxes in Ireland

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AIM: The digenean trematode *Alaria alata*, an intestinal parasite of wild canids is widely distributed in Europe. The recent finding of the mesocercarial life cycle stage in the paratenic wild boar host suggests that it may potentially infect humans.

MATERIALS AND METHODS: Over 500 foxes were examined during a wildlife survey for the zoonotic diseases in 2009 and 2010. The location of foxes was geo-referenced and mapped using a geographic information system (GIS).
RESULTS: The prevalence of *A. alata* ranged from 21% to 26% in 2009 and 2010 and the intensity of infection varied, with the majority of foxes having between 1-10 trematodes, but a small number of animals had parasitic burdens greater than 500.

CONCLUSIONS: The results of the spatial analysis suggest that *A. alata* may have a limited distribution being confined mainly to areas of pasture especially in the central plain and north Munster. Hot-spot analysis indicated a clustering and that the level of parasitism was greatest in foxes from those areas where the prevalence of infection was highest.

Seroprevalence of *Toxoplasma gondii* in wild boars (*Sus scrofa*) in Tuscany

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AIM: The zoonotic protozoa *Toxoplasma gondii* infects domestic and wild food animals worldwide, including wild boars (Richomme C et al, 2010, Epidemiol Infect 138: 1257-1266; Opsteegh M, 2011, Plos One 20: 162-140). There are two major routes of postnatal transmission of *T. gondii* in animals and humans: a) by ingestion of food or water contaminated with oocysts excreted by infected cats or b) by ingesting uncooked or undercooked meat containing tissue cysts. Due to their omnivorous and voracious feeding habits, wild boars can be exposed to infection with *T. gondii* by both routes. Since they spend most of the time rooting and digging in search of fruits, seeds, mushrooms, nuts, tubers, roots, and bulbs, they can be infected by ingestion of sporulated oocysts present in soil. Moreover, since they are not adverse to feeding on small mammals, birds, and carrion, wild boars can also be infected by ingestion of cysts in tissues of infected prey.

In Italy, wild boars are widely spread throughout the country. Since several years, their number has increased in an uncontrolled way and they are generally considered agricultural pests. The wild boar is one of the most popular hunted species in this country and is commonly home slaughtered after hunting. Because of its tasty meat, the wild boar is part of the diet for humans and many regional specialties of the Italian cuisine are made of its meat, including sausages which are frequently eaten fresh. Tissue cysts can survive in food animals for years and virtually all edible portions of an animal can harbor viable *T. gondii* tissue cysts (Dubey JP et al., 1986, J Am Vet Med Assoc 188: 1035–1037). It has been shown that *T. gondii* is killed by the salting, curing, freezing, or heating procedures that are used in meat processing, and thus the major risk to consumers is believed to be from meat products eaten fresh and raw like sausages. Moreover, evisceration and handling carcasses of infected animals could pose further risk for transmission of *Toxoplasma* to humans.

With the goal of identifying potential sources for transmission of *T. gondii* to humans, we determined the seroprevalence of *T. gondii* infection in wild boars hunted in a geographical area of the Tuscany region.

MATERIALS AND METHODS: Between October and December 2011, 31 wild boars killed in the province of Pisa during the annual hunting season were examined. Blood samples were collected directly from the hearts during home slaughtering procedures and transported to our laboratory on the day of collection. Sera were collected from whole blood by centrifugation and stored at -20°C until tested. Pig sera were tested for *T. gondii* IgG antibody levels by indirect immunofluorescence technique (IIFT) (Toxo-Spot IF; Biomérieux, Marcy l'Etoile, France). Fluorescein isothiocyanate-conjugated goat anti-swine IgG (Kirkegaard & Perry Laboratories, Gaithersburg, MD, USA) was used. Prevalence values and corresponding 95% confidence intervals (95% CI) were calculated.

RESULTS – Overall, IgG antibodies to *T. gondii* were found in 6 (19.3%, 95% CI=5.4-33.3%) of the 31 wild boars examined with IIFT titers of 1:20 in 2 (6.4%, 0-15.1%), 1:40 in 3 (9.7%, 0-20.1), and 1:160 in 1 (3.2, 0-9.4%) of them.

CONCLUSIONS: Previous seroepidemiological data suggest that ingesting improperly cooked meat containing *T. gondii* cysts is a major source of infection for humans (Dubey JP, Jones JL, 2008, Int J Parasitol 38: 1257-1278). However, the contribution of foodborne toxoplasmosis to human infection needs to be further investigated. Wild boars killed and home slaughtered during hunting seasons are not tested for *T. gondii* infection in Italy. Therefore, the role *T. gondii*–infected wild boars have in the overall epidemiology of human toxoplasmosis in this country remains unknown. Acute toxoplasmosis, including blindness was reported in three patients who had consumed raw wild boar viscera (Choi WY et al., 1997, J Infect Dis 175: 1280-1282). As 19.3% of wild boars were found to be serologically positive for *T. gondii* antibodies, results of the present study suggest that these animals may harbor infective *T. gondii* cysts in their muscles and, thus, that wild boar meat products may be considered as a potential consumer concern when consumed raw, fresh or improperly cooked.

Prevalence and intensity of infection by gastrointestinal nematodes in roe deer (*Capreolus capreolus*): differences according to the sex

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AIM: Roe deer (*Capreolus capreolus*)is a wild ungulate with a seasonal strong territorial behavior in males, while females form small groups and live with their offspring. These behavioral differences may influence the rates of gastrointestinal nematode infection which is one of the most common parasitic infections in roe deer in Galicia (NW Spain).

MATERIAL AND METHODS: 218 roe deer (187 males and 31 females) culled during the 2007-2009 hunting seasons in different locations of Galicia, were studied in this work. The number of females was lower than those of males due to the particular conditions of hunting; since females are only hunted during the fall in order to keep a stable census. To obtain adults of gastrointestinal nematodes, the content of the digestive tract was washed, filtered and stored in 5% formalin. Adults were collected under stereomicroscope, counted and mounted in lactophenol-cotton blue 0.05% for microscopic identification.

RESULTS: All the examined roe deer were infected by gastrointestinal nematodes, presenting a mean intensity of 1070±1020 nematodes per animal.
When the prevalence of the different genera were related with the sex of the animals, it was observed that, in general, for most of the genera, males were more infected than females: *Ostertagia* (96.3 *vs* 93.5%), *Spiculopteragia* (95.7 *vs* 87.1%), *Nematodirus* (65.8 *vs* 45.2%), *Oesophagostomum* (52.4 *vs* 29.6%), *Trichuris* (59.4 *vs* 22.6%), *Trichostrongylus* (52.4 *vs* 29.6%) and in minor proportion, *Teladorsagia* (7.7 *vs* 0%), *Haemonchus* (1.6 *vs* 0%), *Chabertia* (2.2 *vs* 3.4%) and *Cooperia* (1.7 *vs* 3.3%); Chi-square test showed that those differences were only significant for *Nematodirus, Oesophagostomum* and *Trichuris*. In relation to the intensity of infection, it was slightly higher in females (1144±1835) than in males (1059±834). Those differences were more pronounced for *Nematodirus*, the genus with the highest intensity of infection for both sexes (123±192 in males and 863±2657 in females); ANOVA showed that the differences were statistically significant (F= 4.643; p= 0.032).

CONCLUSIONS: Our results revealed that the parasite burden of gastrointestinal nematodes in roe deer is moderate but it could cause serious pathological changes when coincide with other infectious or parasitic diseases.
Physiological and behavioral differences due to sex are reflected in the gastrointestinal nematode burdens. Both sexes have similar chances of infection, but probably due to the gregarious nature of females, the intensity of gastrointestinal nematode infection is greater than in males.

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Identification of Sarcocystis species infecting roe deer (*Capreolus capreolus*) in Galicia (NW Spain) by optical and transmission electron microscopy

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AIM: Galicia is a region which provides excellent orographic and environmental conditions for the presence of cervids. Roe deer hunting is an important economic activity for people inhabiting rural areas. For the maintenance of this natural resource is vital to keep their health status, which must be optimal for their survival in the habitat.

In previous studies conducted in the 90's, a high prevalence of *Sarcocystis* in roe deer from Galicia was observed, but the present species were not identified (López et al, 2003, Z Jagdwiss, 49: 211-218). In this work, the *Sarcocystis* species from roe deer were studies by means of optical (OM) and transmission electron microscopy (TEM)

MATERIAL AND METHODS: Muscular samples from 101 roe deer different localities of Galicia were analyzed during 2007 to 2009 hunting seasons.

Macrocysts were detected in the musculature of the heart, diaphragm and oesophagus by macroscopic examination, whereas microscopic cysts were detected and counted by using the compression method. Identification of *Sarcocystis* species was carried out by histopathology; tissue samples were fixed in 10% buffered formalin for 24 hours; processed and embedded into paraffin blocks. Sections were stained with Hematoxylin and Eosin and then examined by optical microscopy (OM). In order to study the ultrastructure of muscular cysts and to identify species, tissue samples were fixed in 2% glutaraldehyde, ultrathin sections were studied with a transmission electron microscope (TEM) model JEOL JEM 101.

RESULTS: 99% of the roe deer were infected by *Sarcocystis*, with a high number of cysts per sample (404 ± 812). The highest intensity of parasitation corresponded to the heart (830 ± 1281) and the lowest to the diaphragm (197 ± 190) and oesophagus (180 ± 205). Macrocysts (2055 µm in length) and microcysts (length <1500 µm) were detected in 48.5% of the roe deer in their oesophagi, while only microcysts were found in the heart and diaphragm.

By OM, we observed that all the animals and the 3 types of muscles examined presented thin-walled cysts, and 20% of the esophagus and diaphragm samples had also thick-walled cysts. Among the thin-walled cysts, only those in excellent histological preparations could be identified as *Sarcocystis gracilis* and the rest were considered as *Sarcocystis* spp. Thick-walled cysts can be distinguished more easily to each other by OM, because the features of the cyst wall are very different, being identified *S. hofmanni* and *S. capreolicanis*.

By TEM, we found three distinct groups of cysts in structure. We confirmed the presence of *S. capreolicanis* and *S. gracilis*, but in some cases, TEM did not permit an accurate specific identification, which shows the complexity of this identification within this protozoan Apicomplexa.

CONCLUSIONS: This is the first report of *S. gracilis, S. hofmanni* y *S. capreolicanis* identified in roe deer from Galicia. However, there are species that have not been identified by TEM yet, so further molecular biological studies are necessary to complete knowledge on the species of the *Sarcocystis* present in roe deer from this region of the NW of Spain.

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Ectoparasites of the red fox (*Vulpes vulpes*) in central Italy

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AIM: Available data on ectoparasitic infections of the red fox (*Vulpes vulpes*) in Italy (Berrilli F et al, 2002, Parasitol Res 88:772-777; Nardoni S et al, 2002, proceedings 6th FIMUA Italian meeting: 24-25) are limited. The aim of the present study was to investigate ectoparasitic species and prevalence of ectoparasitic infections in red foxes living in the province of Pisa (Tuscany, central Italy).

MATERIALS AND METHODS: From January 2010 to December 2010, fifty frozen red foxes of both genders (20 males and 30 females) and different age (27 < 1 year and 23 > 1 year) shot during the regular hunting season in the Province of Pisa (43°N, 10-11°E), were examined. Ticks and fleas were collected directly from the body of the animals during necropsy. Collected arthropods were fixed in 70% alcohol and identified after morphological examination under the stereo microscope and/or mounted with the Hoyer medium and examined under the light microscope. For the detection of *Malassezia* and ear mites, cytological and parasitological examination of earwax samples collected by means of cotton swabs was performed. Sarcoptic mange was diagnosed on the presence of clearly visible lesions in the skin of the carcasses with confirmatory demonstration of *Sarcoptes scabiei* at parasitological and histopathological analysis. Data were statistically analysed by using a χ^2 test with the Yates correction (significativity $P<0.01$; $P<0.05$).

RESULTS: An overall prevalence of 84% was found in examined red foxes. Concerning isolated ectoparasites, 8%, 82%, 38%, 6% and 6% of foxes resulted positive for *Otodectes cynotis, Malassezia* sp., *S. scabiei*, fleas (*Archaeopsylla erinacei erinacei* and *Ctenocephalides canis*) and ticks (*Ixodes ricinus*), respectively. A significant positive correlation was found between the age of foxes and *Malassezia* (P<0.01), with *Malassezia* infections resulting prevalent in animals older than 1 year of age.

CONCLUSIONS: Our results demonstrated a high prevalence of ectoparasitic infections in the examined red fox population and confirm previous observations about the diffusion of *Malassezia* infections in these animals. The prevalence of some ectoparasitic infections, such as those caused by fleas and ticks, might be underestimated mainly due to some limits of the method used for the collection of parasites. Based on the prevalence (38%), severity of lesions and poor body condition of most *Sarcoptes*-infected animals, sarcoptic mange should be considered as the most important ectoparasitic infection of red foxes in the examined area.

High burden infection by *Macracanthorhynchus hirudinaceus* (Archiacanthocephala: Oligacanthorhynchidae) in two Sardinian wild boars: a case report

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AIM: The aim of this paper is to report a case of high burden infection by *Macracanthorhynchus hirudinaceus* (Pallas, 1781) in two wild boars (*Sus scrofa meridionalis* Forsyth Major, 1882) hunted in Sardinia, and to discuss the risk related to zoonotic potential of this parasite (Leng et al, 1983, Ann Trop Med Parasitol, 77: 107-109; Radomyos et al, 1989, Trop Med Parasitol, 40: 476-477; de Estrada, 1997, Rev Med Exp INS, 14: 47-50). *Macracanthorhynchus irudinaceus*, known as "Giant Thorny-headed Worm" of swine, is a big acanthocephalan parasite which lives in the small intestines of domestic pigs and other suids like wild boars with a worldwide distribution in this latter (De la Muela et al, 2001, J Wildl Dis, 37: 840-843; Fernandez-de-Mera et al, 2003, Vet Parasitol, 115: 335-341; Foata et al, 2005, Acta Parasitol, 50: 168-170; Senlik et al, 2010, J Helminthol, 30: 1-5), but very occasionally also in humans (Pradatsundarasar A et al, 1965, Am J Trop Med Hyg 14(5): 774-6; Prociv, P et al, 1990, Medical Journal of Australia, Vol. 152: 215-216), and dogs (Dalimi et al, 2006, Vet Parasitol, 142: 129-133), especially hunting dogs, which can become infected and in turn increase the chance of transmission to humans. It is responsible of enteritis, peritonitis and gastritis. Its life cycle includes various beetles as intermediates hosts such as Coleoptera Scarabaeidae (Pavlovic I N et al, 2010, Matica Srpska Proceedings for Natural Sciences, 119: 89-95). The acanthocephalosis of suids is frequently in Italy (De Carneri I, 1997, Parassitologia generale e umana. Casa Editrice Ambrosiana, Milano).

MATERIALS AND METHODS: Two adults wild boars, one female four years old, and one male one year old hunted in territory of Pula (Province of Cagliari), were presented to IZS della Sardegna, Dip.to di Cagliari, to check the cause of death. In addition, both wild boars were examined externally for the detection of ectoparasites.

RESULTS: The two wild boars, were in good nutritional condition. Predominant pathological lesions were related to shots from firearm, of course, interesting head and thorax in the male and female boar respectively, but abdomen and its organs inside were in both boars intact. During necropsy were analyzed internal organs where, at the level of the small intestine wall, were found a massive infestation of *M. hirudinaceus*, about 20 specimens per wild boar, in different stages of development. In the serosal surface of small intestine wall, spread in several tracts, numerous hemispheric nodules, size up to 5 mm in diameter, with smooth and intact surface, were present. In the mucosal surface of small intestine, the nodules showed a slight relief, centrally umbilicated, where, in most of they, the specimen of *M. hirudinaceus* was attached by means of its proboscis enterely embedded in the nodule. None perforation of the intestinal wall due to this parasite was observed. Among the ectoparasites collected were identified: 2 females and 2 males of *Dermacentor marginatus* (Sulzer, 1776) (Acarina: Ixodida), a tick species typically associated to wild boar in Sardinia, as reported by other recent works (Fois F et al, 2006, Parassitologia, 48(1-2): 349; Piras P, Fois F, 2008, Book of Proceedings TTp-6, 255; Fois F et al, 2008, Book of Proceedings TTp-6, 310); 40 specimens (10 females, 16 males and 14 nymphs) of *Haematopinus apri* Goureau, 1866 (Phthiraptera: Anoplura), whose presence in Sardinia had not yet been documented.

CONCLUSIONS: It was a long time that in the Dip.to di Cagliari of IZS della Sardegna, during the diagnostic examination, not occurring cases of infestation by *M. hirudinaceus,* on wild boars, so relevant. It must also be taken into account that parasitism by *M. hirudinaceus* may not be diagnosed in vitam by common diagnostic methods since their eggs do not float well in saturated saline solution (Dangjin, L 1996, Vet Parasitol, 61: 113-117), therefore its presence can be misdiagnosed.

Parasites and biological invasions: do helminths play a role in facilitating grey squirrel (*Sciurus carolinensis*) settlement and in its competition with native red squirrel (*Sciurus vulgaris*)?

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AIM: Several studies have shown that parasites can play an important role in biological invasions (Dunn AM, 2009, Adv Parasitol, 68: 161-184), mainly via two different processes. First, during the invasion process, alien species often lose part of their parasite community with a positive impact on their population growth and consequently on their settlement and spread ("parasite release hypothesis", Torchin ME et al 2003, Nature, 421: 628-630). Second, parasites may mediate the impact of invasive species on native taxa ("parasite-mediated competition", Hudson P and Greenman J, 1998, Trends Ecol Evol, 13:387-390), introducing in the environment novel parasites to which native species are more susceptible or increasing the abundances of local parasite acting as additional reservoir (Tompkins DM, 2001 Parasitology, 1999: 187-193). Since 1948, the Eastern grey squirrel (*Sciurus carolinensis*), a North-American alien species, has been repeatedly introduced in Italy, causing local extinction of the native Eurasian red squirrel (*Sciurus vulgaris*), mainly through exploitation competition for food (Martinoli A et al, 2010, Hystrix It J Mamm, 21: 127-136). Our purpose is to explore gastro-intestinal helminth communities of grey and red squirrels in Italy in order to investigate the role of parasites in the settlement of the alien species and in its interaction with native one. In particular, we want to test two independent hypothesis: the parasite release and the parasite-mediated competition.

MATERIAL AND METHODS: We have sampled 8 populations (4 grey-only, 3 red-only and 1 red-grey area) in Piedmont and Lombardy by capturing both species with standard live-trapping techniques. Grey squirrels were euthanized immediately after capture, while red squirrels were marked and released after samples collection. Grey squirrels were then dissected and their intestinal content examined using standard parasitological techniques. For red squirrels, we performed coprological analysis and tape tests to obtain indirect information on their gastro-intestinal parasites. To provide a check-list of red squirrel parasites, we also dissected several roadkills collected from different sites in Northern Italy.

RESULTS: We dissected 142 grey squirrels in which we identified four different species of gastro-intestinal nematodes: *Strongyloides robustus*, *Trichostrongylus calcaratus*, *Trichuris muris* and *Aonchotheca annulosa* (prevalence: 74%, 13%, 6% and 2%, respectively). The species richness we observed is lower compared to what is reported for grey squirrel in their native range (Raush R and Tiner JD, 1948, Am Midl Nat, 39: 728-747). *S. robustus* is a parasite common and abundant in North-American squirrels (Bartlett CM, 1995, Folia Parasit, 42:102-114), but never recorded in Europe until now, thus likely brought here by grey squirrels during the invasion process. On the contrary, *T. calcaratus*, *T. muris* and *A. annulosa* should be considered as non-specific or accidental species, the latter two acquired by the grey squirrel here in Europe. As regards red squirrels, coprological analysis, tape tests and 26 roadkills collected in red-only areas have shown the presence of only one nematode, *Rodentoxyuris sciuri* (prevalence: 96%), typical of this species and already recorded in red squirrels in Europe (Hugot JP et al, 1996, Int J Parasitol, 26: 147-149). On the other hand, in red-grey areas, coprological analysis and roadkills examination have shown the presence of *S. robustus* in red squirrels, suggesting parasite transmission from the grey squirrel to the native species.

CONCLUSION: Our results lend support to the parasite release hypothesis, as grey squirrels in Italy are missing several helminths species usually present in North-America. Moreover, the lack of species-specific parasites is suggested by the presence of some accidental species, probably exploiting vacant niches. Concerning parasite-mediated competition, we found that *S. robustus* can be transmitted to the red squirrel, but so far we haven't found any evidence of helminths spillover from red to grey squirrel. To confirm that *S. robustus* actually plays a role in grey squirrel impact on the native species, further investigation on the pathogenic effect of this nematode on red squirrels is needed.

Infestation by *Mesocestoides* spp. (Cyclophyllidea: Mesocestoididae) and their pathological significance in reptiles of Southern Italy

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AIM: Life cycle of *Mesocestoides* spp. have not yet completely elucidated. A unique larval form, the tetrathyridium, is commonly found in mammalian, avian, amphibian and reptilian intermediate and/or paratenic hosts and is readily infective to predatory definitive hosts. When an infected arthropod is ingested by a second intermediate host, larva develops in a tetrathyridium, encapsulating on the serous cavities of the host. Because poor and/or scanty data are so far existing concerning the infection patterns and the ranges of their intermediate/paratenic hosts, aim of this study was to investigate the infestation by *Mesocestoides* spp. in several reptilian hosts from the Calabria region (southern Italy) and to describe the pathological consequences associated with those parasites.

MATERIALS AND METHODS: We examined 242 road-killed reptiles collected from Cosenza Province of Calabria region, between May and September 2008 to 2011. Reptiles included 70 Italian wall lizards (*Podarcis sicula*), 35 common wall lizards (*P. muralis*), 11 western green lizards (*Lacerta bilineata*), 32 Italian three-toed skink (*Chalchides chalcides*), 50 western whip snake (*Hierophis viridiflavus*), 22 grass snakes (*Natrix natrix*), 8 smooth snakes (*Coronella austriaca*), 6 asp vipers (*Vipera aspis*), 4 four-lined snake (*Elaphe quatuorlineata)* and 4 Aesculapian snake (*Zamenis longissimus*).

RESULTS: Tetrathyridium of *Mesocestoides* spp. were found in 7 out of the 10 species studied, including 3 lizards and 4 snakes species. The western whip snake showed the higher prevalence (P= 40%) and intensity (mean Intensity, Im= 224.1) infection values. Infected reptiles were generally in fair to good nutritional condition, except for a single adult western green lizard; it was lethargic, emaciated, and with distended abdomen: at the necroscopy, a total of 739 tetrathyridia were found free in the coelomic cavity and approximately other 300 were found encapsulated in the whole hepatic tissue. At the histological view, the larvae were found across the hepatic capsula and parenchyma and they were associated with infiltration of heterophils and macrophages and scattered peripheral foci of necrosis.

CONCLUSIONS: The western whip snake resulted the most important reptilian intermediate/paratenic host of *Mesocestoides* spp. in the study area. Pathological significance in lizards are associated to the heavy intensity of the infestation; functional capacity of host liver should be strongly reduced due to the extensive invasion of the hepatic tissue. The genetic/molecular identification of the parasites detected in the present study, allowing to elucidate also aspects related to the life cycle of these parasites, is in progress.

Helminths of owls (Strigiformes) in Calabria region of Southern Italy

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AIM: Here we examined and compared the helminth assemblage of 5 species of owls from the Calabria region of southern Italy. Moreover, we compared this data with that obtained on helminth communities of 6 species of birds of prey (Accipitriformes and Falconiformes) occurring in the same geographical region (Santoro et al., 2010, Vet J, 186: 119-122; 2012, J Parasitol, 98: 22-29).

MATERIALS AND METHODS: Owls naturally died between January 2005 and December 2011 at the Wildlife Rescue Centre in Rende, Cosenza (Calabria region), were examined for helminth parasites. We studied a total of 122 owls belonging to 5 species including 30 little owls (*Athene noctua*), 31 tawny owls (*Strix aluco*), 41 barn owls (*Tyto alba*), 10 long-eared owls (*Asio otus*), and 10 scops owls (*Otus scops*).

RESULTS: A total of 19 helminth taxa were identified including 3 cestodes *Choanotaenia littoriae, Passerilepis stylosa*, and *Paruterina candelabraria,* 3 acanthocephalans *Centrorhynchus aluconis, C. clitorideus and C. globocaudatus,* 10 nematodes *Capillaria falconis*, *Dispharynx nasuta, Hamatospiculum* sp.*, Heterakis gallinarum, Excisa excisiformis, Porrocaecum spirale, Synhimantus affinis*, *S. laticeps*, *Skrjabinura spiralis*, and *Subulura* sp.; and 3 digeneans *Brachylaima fuscatum*, *Neodiplostomum* sp., and *Zonorchis* sp.. Number of helminth taxa for host species ranged from 2 in long-eared owl to 12 in tawny owl. Twelve taxa were restricted to a single host species; 2 taxa were shared between 4 hosts; 3 taxa were shared between 3 hosts; and 2 taxa were shared between 2 hosts. In the Calabria region, owls and birds of prey share just 4 of the 50 helminth taxa found in total (19 in owls and 31 in birds of prey) including *C. globocaudatus* and *S. laticeps* (each shared with 5 birds of prey species) and *B. fuscatum* and *C. falconis* (each shared with 2 birds of prey species) (Santoro et al., 2010, Vet J, 186: 119-122; 2012, J Parasitol, 98: 22-29).

CONCLUSIONS: Data here obtained supports the hypotheses that each owl species host a specific helminth community; and their community are poor probably because of their high trophic specialization on a few prey species, and differ qualitatively and quantitatively by those of birds of prey from the same geographical area.

Muscle distribution and survival of *Trichinella pseudospiralis* larvae in a naturally infected wild boar (*Sus scrofa*)

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AIM: *Trichinella pseudospiralis* is a non-encapsulated species infecting both mammals and birds circulating worldwide (Pozio et al, 2009, Infect Genet Evol, 9:606-616). In Italy, this parasite has been reported in two night-birds of prey of Central Italy (Pozio E et al., 1999, J. Parasitol. 85, 759-761) and in three wild boars (*Sus scrofa*) of Northern Italy (Merialdi G et al, 2011, Vet Parasitol, 178:370-373). The wild boar is susceptible to *Trichinella* spp. infection and represents one of the most important source of infection for humans worldwide (Murrell KD and Pozio E, 2011, Emerg Inf Dis, 17:2194-2202). On February 28th, 2012, a regularly slaughtered wild boar was found positive for *Trichinella* sp. larvae. Parasites were identified as *T. pseudospiralis* by multiplex-PCR. The positive animal belonged to a wild boar family farm of the Udine province (Northern Italy), where two wild boars had been found positive for *T. pseudospiralis* in 2010. The aims of this study were to evaluate the larval burden in different muscle/s and the larva survival in refrigerated conditions.

METHODS: After slaughtering, eight muscle groups (neck, shoulder, foreleg, abdomen, loin, gluteus and ham) were collected and refrigerated at +4°C. Muscles from the head were not available for this study, except 8 g of tongue and 10 g of diaphragm, processed at slaughter time. Between 24 and 44 days post slaughtering (p.s.), 30-50 g of each muscle group were tested by enzymatic digestion (Regulation (EC) No. 2075/2005, European Commission, 2005). The recovered larvae stored in saline at 37°C, were counted and their viability, based on presence/absence of motility, assessed under a stereomicroscope.

RESULTS: The mean number of larvae of *T. pseudospiralis* per g of muscle or group of muscles ranged from 0.22 to 1.8, with the highest concentration in the diaphragm, followed by the shoulder and the neck (Table 1). At +4°C, *T. pseudospiralis* larvae survived in wild boar muscles at least up to 44 days p.s. Four weeks p.s., the survival rate ranged from 33% to 100% and, 6 weeks p.s., from 35% to 89% in gluteal muscles (Table 1).

CONCLUSIONS: To the best of our knowledge, this is the first description of muscle distribution and survival in refrigerated conditions of *T. pseudospiralis* larvae in a naturally infected wild boar. The diaphragm was confirmed as the target muscle for the inspection, while tongue was difficult to digest and showed a lower infestation level, even if these results can be biased by the low amount of digested muscle. This study highlights that the level of infestation of this nematode can be very low and variable in the carcass of a wild boar. The test of more than 10 g of muscles is recommended, especially if the diaphragm is not available. The non-encapsulated larvae of *T. pseudospiralis* survived at +4°C for at least 44 days, possibly allowing the transmission to a new scavenger host. However, the viability of larvae itself does not prove their infectivity and further experimental studies are needed to evaluate the survival and infectivity of this parasite in decaying muscle tissues.

Table 1. Mean number of *T. pseudospiralis* larvae and survival rate of larvae in wild boar muscles stored at +4°C at different time post slaughtering (p.s.).

Muscles	N. of digested g per time	Number of larvae/g	Days p.s.	Alive/total larvae	Days p.s.	Alive/total larvae
diaphragm	10	1.80	0	18/18	-	-
tongue	8	0.25	0	2/2	-	-
gluteus	100	0.22	24	13/13	38	7/9
loin	100	0.31	24	7/7	42	14/24
foreleg	100	0.35	30	11/11	43	9/15
abdomen	80	0.34	30	4/12	43	6/13
ham	80	0.27	36	3/5	43	3/13
neck	100	0.41	36	10/19	44	4/22
shoulder	100	0.50	37	28/28	44	3/22

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Description of the nematode communities of sympatric *Lepus europaeus* and *Sylvilagus floridanus* in Piedmont

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AIM: The cottontail rabbit, *Sylvilagus floridanus*, has rapidly spread in North Italy since its illegal introduction in 1966 (Silvano *et al.* 2000 Hystrix, Italian Journal of Mammalogy 11: 75-78). In parallel, a range of exotic parasite species have been unintentionally introduced, including four nematodes, *Obeliscoides cuniculi, Passalurus nonannulatus, Trichostrongylus affinis* and *Trichostrongylus calcaratus* (Meneguz and Tizzani 2002 Parassitologia Vol. 44: 111). Recently, specimens of the potentially pathogenic *O. cuniculi* have been observed for the first time in the stomach of an adult European brown hare (Tizzani et al 2011 Parasitol Res., 109: 963-966), raising concern on the possibility that "parasite mediated competition" (Price et al 1988. The American Naturalist, 131: 544-555) may occur in addition to other forms of ecological competition - between the fragile native hare and the invader cottontail. The aim of this survey was to study a larger number of sympatric hares and cottontails to test how frequent and intense is the cross infection of gastrointestinal nematodes under natural conditions.

MATERIALS AND METHODS: The study was carried out in an area of Alessandria province where *S. floridanus* and *L. europaeus* live in sympatry since approximately ten years. In this area, hares are managed as game, whereas cottontails are exclusively culled in the frame of pest control plans. We analyzed the stomach and the intestinal tract of 16 *S. floridanus* and 21 *L. europaeus* collected in fall. Standard laboratory techniques were used to recover and preserve the nematodes (MAFF, 1986, Manual of Veterinary Diagnostic Techniques, Ref Book 418). For identification, a morphometric approach and dichotomous keys were used (e.g. Skrjabin et al, 1954 Essential of Nematodology, Trichostrongylids of Animals and Man, 526 pp).

RESULTS: Six and three nematode species were found in *L. europaeus* and *S. floridanus*, respectively. Their prevalence, abundance and intensity rates are reported in Table 1. *T. calcaratus* and *T. affinis* are signaled for the first time in the European brown hare. Remarkably, evidence was found showing that *O. cuniculi* and two additional exotic nematodes, *T. calcaratus* and *T. affinis*, are well established in the native *L. europaeus*, representing all together the 61.9 % of the nematode specimens collected in hares. Instead, the nematode fauna of *S. floridanus* was not enriched by any hare-adapted species. Prevalence, abundance and intensity rates of all common nematodes were significantly lower in the hares than in cottontails. The one-way spillover from the invader to the native host, and not viceversa, is original intriguing phenomenon.

Sylvilagus floridanus			Nematode species	*Lepus europaeus*		
P	A	I		P	A	I
86.7%	96.8	149	*Obeliscoides cuniculi*	40.0%	10.5	26.2
68.8%	79.1	105.4	*Trichostrongylus affinis*	25.0%	19.3	77.2
85.7%	963	1,123.5	*Trichostrongylus calcaratus*	47.6%	17.0	35.7
0	0	0	*Trichostrongylus colubriformis*	5.0%	13.0	260
0	0	0	*Trichostrongylus retortaeformis*	19.0%	15.5	77.5
0	0	0	*Trichuris leporis*	20.0%	0.35	1.7

CONCLUSIONS: Results of this study suggest that a range of cottontail-adapted nematodes is transmissible to native hares under natural conditions. Accordingly, the concern of a "parasite mediated competition" to the detriment of native hares cannot be dismissed. Further investigation is warranted, under experimental conditions, to elucidate the pathogenic potential of the individual nematode species involved in the observed one-way parasitic spillover.

Alien parasites following the introduction of *Myocastor coypus* in northern Italy

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AIM: The coypu (*Myocastor coypus*), an exotic wild rodent introduced from South America, is widely diffuse in Lombardy and in the Milano province and it has been demonstrated that it is well adapted to the new environment in all respects (Prigioni C., Balestrieri A., Remonti L. 2003, Rapporto di Ricerca n°22, http://www.agricoltura.regione. lombardia.it). It is considered a pest species able to cause serious environmental changes so as to threaten the conservation of indigenous habitats and species or cause severe economic losses to human activities (*Life Focus*, Alien species and nature conservation in the EU. The role of the LIFE program, 2004). In this area coypus highly interact with species of zootechnical interest. The aim of the survey was to evaluate both the parasitological status and the possible role as reservoir host for parasite of veterinary interest or threat for indigenous species.

MATERIALS AND METHODS: From November 2008 to March 2011, 153 coypus were trapped in cage and sacrificed following ISPRA guidelines (Istituto Superiore per la Protezione e Ricerca Ambientale). Sampling was performed in 15 different municipality of Milano Province. Ocular and palpebral conjunctiva, heart chambers, pulmonary vessels, liver, gallbladder, urinary bladder and kidneys were inspected and/or dissected for adult parasite detection. Particularly, necropsies focused on detection of parasites such as *Fasciola hepatica*, *Thelazia* sp., *Dirofilaria immitis*, *Angiostrongylus* sp. and *Capillaria* sp. During necropsies, faecal samples were collected from each animal and were analyzed with FLOTAC double technique (flotation solution $NaNO_3$, 1200 s.g.) for parasite eggs and/or oocysts within 48 h. Further, the gastrointestinal tracts from coypus resulted positive at copromicroscopical analysis, have been processed as described in MAFF (1986) to isolate and collect adult nematodes for morphological identification.

RESULTS: Organs inspected and/or dissected resulted negative for helmintic infections. 146 fecal samples were adequate to perform copromicroscopic analysis; coypus were infected with *Strongyloides* sp. (P=63,70%), *Trichostrongylus* sp. (P=28,67%), *Eimeria seideli* (P=6,85%) and *Eimeria coypi* (P=93,15%). *Strongyloides* sp. resulted the most abundant taxon with an average of 174,30 epg (min-max= 2-2840 epg) respect to *Trichostrongylus* sp. (epg=5,80; min-max=2-30 epg). At the parasitological exam of the intestine were isolated adult nematodes. Females of genus *Strongyloides* sp. were found and were identified as *Strongyloides myopotami* (Rossin M. A. et al., 2009, Acta Parasitologica 54, 257-262). Further, several specimens of genus *Trichostrongylus* were recovered; the adult males were identified on the basis of morphology and measurements of spicules, gubernaculum and dorsal ray; all nematodes belonged to *Trichostrongylus durettae* (Rossin M. A. et al, 2006, Acta Parasitologica, 51, 286-289).

CONCLUSIONS: Parasites detected in *M. coypus* in this survey are typical for this species (*S. myopotami*, *E. seideli*, *E. coypi*) or for other species of Rodentia (*T. duretteae*). However, *S. myopotami* and *T. duretteae* should be considered exotic species and like the coypus seem well established in the environment of the study area. These parasites should be able to infect other host species related to coypus from zoological point of view and endangered. In fact, *T. duretteae* has been described in *Ctenomis talarum*, a Rodentia belonging to the same suborder of coypus (Hystricomorpha).
M. coypus in Milano Provinces doesn't seem involved in helmintic infection of great importance from veterinary point of view. It's partially unexpected the absence of *F. hepatica* in *M. coypus* in our study area: in fact coypus is well known for its susceptibility to this fluke (Issia L. et al., 2009, Vet Parasitol 165, 341-344; Menard A. et al., 2001, Vet Res 32, 499-508). Probably in the last few decades the necessary ecological conditions for the maintenance of fasciolosis in Milano Province have been heavily modified, in fact prevalence of fasciolosis are decreased also in farm animals. Finally, it should be emphasized that *S. myopotami* is a zoonotic parasite, the aetiological agent of lesions known as "marsh itches" or "nutria itches". Prevalence and intensity of *S. myopotami* observed in our study are particularly relevant and shouldn't be undervalued.

SESSIONE 4

BIOLOGIA MOLECOLARE

Molecular detection of *Neospora caninum* in fetal tissues from spontaneous buffalo abortions

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AIM: *Neospora caninum* is a recognized genus of pathogenic coccidian, closely related to *Toxoplasma gondii*, that can cause abortion or congenital disease in a variety of domestic animal hosts. The aim of this study was to describe the presence of *N. caninum* infection in fetuses of water buffalo *(Bubalus bubalis*) in the Campania region (Southern Italy) by PCR assay.The DNA of *N. caninum* was detected and identified in the heart of three buffalo fetuses. The amplified products were instead purified and sequenced, confirming the presence of *N. caninum* in the samples. The present study is the first to report evidence of *N. caninum* in aborted water buffalo fetuses in Southern Italy.

MATERIALS AND METHODS: Three aborted buffalo fetuses between the fourth and sixth months of gestation were sent to the IZSM in 2011 for general aetiological diagnosis. Different tissues were tested for each fetus, mainly brain and heart, target tissues for the diagnosis of *N. caninum*- related abortion. Fetal tissues were obtained and fixed in 10% buffered formalin embedded in paraffin, cut at 4µm and stained (H/E) for histopathological analysis. Samples of brain and heart were subjected to PCR for the detection of *N. caninum*. After DNA extraction with a commercial kit (QIAamp DNA mini kit, Qiagen), the amplification was carried out employing primers targeting a region of the small subunit (ssu)-rRNA gene (18S) as described by Magnino et al. (Magnino S et al, 2000, Sel Vet S15–S23). A restriction analysis was then performed on the amplified products by digestion with the BseDI enzyme (MBI Fermentas) for a simultaneous differentiation among *N. caninum* and *T. gondii*. To confirm the presence of *N. caninum*, the DNA isolated from fetal tissues was also tested by hemi-nested PCR of the Nc5 gene (Baszler TV et al, 1999, J Clin Microbiol 37: 4059–4064; Yamage M et al,1996, J Parasitol, 82: 272-9). Amplification products were analysed by automated electrophoresis (QIAxcel, QIAgen). The Nc5 amplicons were instead purified (Qiaquick purification kit,Qiagen) and bi-directionally sequenced using Big Dye Terminator cycle sequencing kit v.3.1 (Applied Biosystems). Sequences were analysed by multiple alignment using BioEdit software and the CLUSTAL W alignment method.

RESULTS: No macroscopic lesions were observed in aborted fetuses analysed. Histological lesions of tissue samples were observed in the three infected fetuses and were defined as "characteristic" of *Neospora* infection in according to previous descriptions (Gonzàlez L et al, 1999, Vet Rec 144: 145-150). In all analysed fetuses, samples of heart were positive for the small-subunit rRNA gene (18S). DNA amplification yielded a fragment of 294 bp, expected product length for *N. caninum* and *T. gondii*. (Fig.1). BseDI restriction patterns yielded three bands (size: 85, 92, 105 bp) that were very close and appeared as thick band in the gel, as expected for *N. caninum*. (Fig.2). Samples of heart were also positive to Nc5 gene, a band of 227 bp lenght were found in these samples. (Fig.3). Sequencing of *N.caninum* PCR product amplified, confirmed homology with the published Nc5 sequence deposited in GenBank by Chryssafidis et al. (DQ059068) (Chryssafidis AL et al, 2011, Parasitol Res 108: 741–743).(Fig.4).

CONCLUSIONS: The causes of reproductive failure in buffaloes due to *Neospora caninum* in Italy have not been characterized; nevertheless, a high proportion (34.6%) of *Neospora*-seropositive water buffalo have reported in Campania region (Guarino A et al, 2000, Vet Parasitol 91: 15–21). Although evidence of natural *Neospora* infections have been described (Rodrigues AAR et al, 2004, Vet Parasitol, 124: 139–150) but spontaneous abortion caused by *N.caninum* has not yet reported in water buffaloes. In this study we have reported the first molecular detection and identification of *N. caninum* in fetal tissues from spontaneous buffalo abortion in the absence of another pathogens associated with abortion and reproductive disorders in these species. We have demostrated that *N. caninum* plays a significant role in abortion of water buffalo and the PCR technique represents an excellent and pratical assay to neosporosis diagnosis in fetal tissues. It is likely that *N.caninum* infections are responsible for sporadics abortions in Southern Italy, rather than multiple cases, but more investigation is required for this assumption to be shown to be valid.

Molecular identification of *Entamoeba*, *Giardia* and *Trichuris* species in captive mammals from the zoological garden of Rome (Italy)

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Intestinal protozoa and helminths are frequently reported in a variety of wild mammal species kept in captivity. Among the others, *Entamoeba*, *Giardia*, and *Trichuris* sp. are commonly found in stool of zoo animals. These parasitic infections can represent a significant cause of diarrhoea and failure to thrive and pose a serious threat to endangered species, particularly in young animals. Moreover, from a public health point of view, some *Entamoeba*, *Giardia*, and *Trichuris* species can have zoonotic potential, being among the most common intestinal human parasites worldwide.

AIM: In this study, molecular identification of *Giardia duodenalis, Entamoeba* spp. and *Trichuris* spp. in several mammal species from an Italian zoological garden was carried out, to better understand the transmission patterns of these pathogens in and from zoo facilities and the role of zoo animal as potential reservoires for zoonotic transmission.

MATERIALS AND METHODS: A total of 210 samples including 184 stool specimens and 26 intestinal contents from 41 mammals species were collected from the Bioparco of Rome, one of the oldest zoological gardens in Europe, located in the city center. All samples were examined for intestinal parasites in the Laboratory of Parasitology of the Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana, using fresh smear and salt flotation. Samples positive at microscopy for *Giardia*, *Entamoeba* and *Trichuris* were sent to the Laboratory of Parasitology of the University of Tor Vergata in Rome for molecular characterization. Genomic DNA was extracted using QIAamp DNA Micro and Stool Mini Kit. Molecular identification was carried out by amplifying a region of the small subunit ribosomal RNA both for *G. duodenalis* and *Entamoeba* spp. and the ITS rDNA region for *Thichuris* spp. using conventional PCR and sequencing.

RESULTS: As regards protozoa, *G. duodenalis* was found only in *Lemur catta* (47.0%); molecular characterization assigned all isolates to the zoonotic assemblage B, sub assemblage BIV. Three *Entamoeba* species were identified: *E. hartmanni*, *E. coli* and *E. dispar*, detected in 4 non-human primates (NHP) species. The number of positive pools ranged from 5.9% in *L. catta* to 81.2% in *Mandrillus sphinx*; in *Pan troglodytes* the observed prevalence was 53.6%. A mixed *Entamoeba-Giardia* infection was recorded only in one sample of *L. catta*.
Trichuris spp. eggs were found in 7 stool samples from 4 different species (*Macaca fuscata*, *Chlorocebus aethiops, Lycaon pictus* and *Camelus bactrianus*). Adult worms were collected at necropsy in intestinal contents from one specimens of *M. fuscata*, two of *C. aethiops* and two of *Dolichotis patagonum.* The molecular analysis based on the comparison of *ITS1 5.8S* and *ITS2* ribosomal DNA sequences from GenBank showed clear differences among *Trichuris* populations from the different hosts. Of particular concern was the identification of zoonotic *T. trichiura* species from *C. aethiops*.

CONCLUSIONS: The presence of parasites with direct life cycles in zoos raises many management problems, linked to the difficulty of preventing cyst/eggs transport from one enclosure to the other, and to the zoonotic risk related to their arrival. Our results highlight the importance of regularly testing animals kept in zoos for the diagnosis of zoonotic parasites, and to limit their spread into the structure.

Molecular characterization of *Cryptosporidium* strains from foals: preliminary results

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Cryptosporidium spp., a common enteric pathogen of animals and humans, has been involved as a cause of diarrhea in foals both immunocompetent and immunocompromised (Sgorbini et al, 2003, Ippologia, 4: 5-9; Grinberg et al, 2009, New Zeal Vet J, 57: 284-289; Perrucci et al, 2011, Vet Paras 182: 333-336). Several aspect of this infection in horse, such as prevalence, clinical onset, risk factors, economic relevance and the species status are not completely defined (Veronesi et al, 2009 Zoon Pub Health, 57: 510-517). So far, the literature on equine cryptosporidiosis, describe the presence of *Cryptosporidium parvum* "cattle" genotype and *Cryptosporidium* "horse" genotype (Grinberg et al, 2003 Vet Rec, 153: 628-631; Grinberg et al, 2008 J Clin Microbiol, 46: 2396-2398; Chalmers et al, 2005, Vet Rec, 156: 49-50; Chalmers et al, 2009, Eurosurveillance, 14: 1-9; Xiao et al, 2009 J Clin Microbiol, 47: 3017-3020).

AIM: During the last horse breeding seasons at the Neonatal Intensive Care Unit of Department of Veterinary Medical Science Alma Mater Studiorum-University of Bologna during past several cases of cryptosporidiosis have been observed in foals and mare. With the aim of undertake a study of epidemiology of these outbreak and in order to set up adequate control measures, a molecular study on *Cryptosporidium* spp. isolates from foals hosted in this structure in 2007 and from diarrheic goat housed in 2006 in a separate experimental stable have been carried out. Furthermore a human isolate from a technician with gastro-intestinal symptoms working on goat during the outbreak was also analyzed.

MATERIALS AND METHODS: The following fecal samples, tested positive by Ziehl-Neelsen stain and stored frozen, were examined: 4 from foals with diarrheic syndrome, 1 from goat and 1 from man. All the samples were subjected to DNA extraction with QIAamp DNA Stool Mini Kit (Qiagen) and to nested PCR, amplifying the 18S rRNA (Miller et al, 2006, J Microbiol Meth, 65: 367-379) and the gene encoding the *Cryptosporidium* oocyst wall protein (COWP) (Traversa et al, 2008, Mol Cell Probes, 22: 122-128). The PCR products were sequenced in both direction by ABI 3730 DNA Analyzer at StarSEQ GmbH (Mainz, Germany).

RESULTS: Sequence analysis of both genes revealed that all the isolates (foal, goat and human) were identical to each other and showed 100% identity with the *Cryptosporidium parvum* "cattle" genotype.

CONCLUSIONS: The presence of *C. parvum* "cattle" genotype has already been recorded all around the world in a wide host range comprising human (Chalmers et al, 2005, Vet Rec, 156: 49-50; Grinberg et al, 2003, Vet Rec, 153: 628-630; Grinberg et al, 2008, J Clin Microbiol, 46: 2396-2398; Imhasly et al, 2009, Arch Tierheilkd, 151: 21-26; Veronesi et al, 2009, Zoon Pubbl Health, 57: 510-517; Traversa et al., 2010, Parassitologia, 52: 214; Perrucci et al, 2011, Vet Parasitol, 182: 333-336; Grinberg et al., 2012, Vet Rec, 153: 628-631) while only few researches reported the presence of *Cryptosporidium* "horse genotype" in foals (Ryan et al, 2003, Appl Environ Microbiol, 69: 4302-4307; Xiao and Fayer, 2008, Int J Parasitol, 38: 1239-1255; Xiao and Feng, 2008, FEMS Immunol Med Microbiol, 53: 309-323) with a single case of human infection (Xiao et al, 2009, J Clin Microbiol, 47: 3017-3020). Our results confirm the data obtained by Grinberg et al (2012) on the evidence that *C. parvum* "cattle" genotype could be a co-factor of foals diarrhea. The isolation of the same genotype also from goat and humans, suggest the possible cross-transmission between animals and man, pointing out the zoonotic professional risk linked to the presence of *C. parvum* "cattle" genotype as already described in previous studies reporting outbreak of cryptosporidiosis in vet students (Pohjola et al, 1986, Scand J Infect Dis, 18: 173-178; Levine et al, 1988, J Am Vet Med Ass, 193: 1413-1414; Reif et al, 1989, Am J Public Health, 79: 1528-1530; Preiser et al, 2003, J Am Coll Health, 51: 213-215; Gait et al, 2008, Vet Rec, 162: 843-845). Further investigations are necessary in order to verify the presence of sub-genotypes by studying other more suitable genes such as the GP60 and the Hsp70 in order to link the epidemiologically related isolates.

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Occurrence, molecular identification and evaluation of risk factors of Cryptococcus sp. in asymptomatic cats

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Cryptococcal organisms are yeasts causing a fungal disease in both animals and humans by inhalation of airborne fungi from an environmental source, followed by the colonization of the respiratory tract. In cats cryptococcosis is the most important deep fungal disease, however *Cryptococcus* species are isolated from the upper respiratory tract of healthy animals suggesting that they may act as asymptomatic carriers of fungal spores. Humans can be at risk of exposure to the pathogen (Ducan C., et al.2005).

AIM: The present study reports the occurrence of *Cryptococcus* spp from the nasal cavity of stray cats in relation to possible risk factors as age, breed, sex, geographical distribution and colony size in Verona area (Northeastern of Italy).

MATERIALS AND METHODS: From January 2009 to December 2011, 763 deep nasal swabs were collected from cats undergoing general anaesthesia for sterilization. Cats came from 133 cat colonies located in urban and suburban areas. Samples were cultured on Sabouraud dextrose agar (SDA) at 25 and 37°C. Yeast identification was achieved by PCR based molecular techniques with direct sequencing of ITS1-ITS2 rRNA amplicons. Nucleotide BLAST of the sequences obtained were aligned in the CBS Knaw database. Age, sex, season, anamnestic data and digital geographic information of colony cats were recorded. Epidemiological data and colony size were offered to binary logistic regression models in order to find possible predisposing factors for infection.

RESULTS: 86 out of 763 cats (11.3%) from 43 out of 133 colonies cats (32.3%) showed positive cultures for *Cryptococcus* species as reported in table1. Size of positive cat colonies ranged from 3 to 100 animals aged from 4 mouths to 10 years. Tabby cats was the only breed described for the 50 females and 83 males. Generally, cats appeared in good health without clinical signs of cryptococcosis and corneal ulcer (2 cats), otitis externa (2 cats) and traumatic lesions to legs (5 cats) were only reported. Among three years of sampling yeasts were isolated in all seasons from both urban and suburban areas. Despite the presence of univariate correlations among data and prevalence, the multivariate analysis failed to find significant risk factors for cat infection.

Table 1. Prevalence (P) of Cryptococcus species identified by sequencing of ITS1-ITS2 rRNA amplicons.

Cryptococcus species	Pos/tot	P (%)
Filobasidiella neof ormans	9/86	10,50
Cryptococcus magnus	32/86	37,20
C. albidus	14/86	16,30
C. carnescens	9/86	10,50
C. adeliensis	3/86	3,50
Cryptococcus spp.	3/86	3,50
C. laurentii	2/86	2,30
C. oeirensis	2/86	2,30
C. victoriae	2/86	2,30
C. watticus	1/86	2,30
C. luteolus	1/86	1,20
C. anemochorus	1/86	1,20
C. aerius	1/86	1,20
C. diffluens	1/86	1,20
C. dimnnae	1/86	1,20
C. flavescens	1/86	1,20
F. floriforme	1/86	1,20
F. globisporum	1/86	1,20

CONCLUSIONS: *Filobasidiella neoformans* and other *Cryptococcus* organisms were isolated from nasal cavity of apparently healthy cats suggesting that potential sources of infection are present indifferently in urban and suburban areas. As regards to risk factors and occurrence of *Cryptococcus* yeasts any positive correlation seems to exist among isolates and the cat colony size or the season of sampling. In addition our findings strongly support the data published by Malik et al (2011), who concluded that sex, breed and age don't represent factors that predispose cats to develop cryptococcosis.

Molecular detection of *Eucoleus aerophilus* in naturally infected dogs and cats

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Eucoleus aerophilus (syn. *Capillaria aerophila*) is a trichuroid parasitic nematode affecting domestic (i.e. dogs and cats) and wild carnivores (e.g. foxes, mustelids) and, occasionally, humans. The lungworms live embedded in the epithelium of bronchioles, bronchi and trachea. After mating, the females lay eggs that are coughed, swallowed and released *via* faeces into the environment. The host infection relies on the ingestion of environment embryonated eggs or earthworms. Pulmonary capillariosis in pets is characterized by respiratory signs of different degrees and when complicated by bacterial infections, bronchopneumonia and life-threatening respiratory failure may occur (Traversa D et al, 2010, Parasit Vectors, 3: 62). The infection is cosmopolitan but knowledge on its distribution is fragmentary and several questions on its biological cycle remain unanswered. Such gaps are due to major hindrances inherent to conventional diagnostic methodologies and to the lack of molecular researches.

AIM: Given the merit in enhancing knowledge on this parasite, the present study has assessed the in field diagnostic efficiency of a PCR specific for the mitochondrial *cox*1 gene of *E. aerophilus*.

MATERIALS AND METHODS: Adult stages of *E. aerophilus* were collected from different animals in Serbia, Portugal, Romania and UK. Samples were molecularly characterized by a PCR carried out on partial (~344bp) *cox*1 gene using a degenerated set of primers designed in the present work. Amplicons were sequenced and sequences were used to validate the diagnostic PCR described on the follows. Faecal samples from 44 animals (34 dogs and 10 cats) microscopically positive for eggs of *E. aerophilus* and other parasites (i.e., lungworms, whipworms, roundworms, hookworms, tapeworms and/or coccidia), and from 44 animals negative for *C. aerophila* but positive for the aforementioned different endoparasites, were molecularly examined by a semi-nested PCR protocol using an internal primer specific for the *cox*1 gene (~299bp) of *E. aerophilus*. Sequences were compared with each other and with those of the Capillariinae *cox*1 available in GenBank. Pairwise comparisons of sequence differences were made and the open reading frames (ORFs) were confirmed by conceptual translation of all nucleotide into amino acid sequences using the invertebrate mitochondrial code.

RESULTS: Thirty-three dogs and 10 cats scored PCR-positive for *E. aerophilus*, showing a sensitivity of 97-100% and a specificity of 100%, confirmed by sequence analysis. None of the samples copromicroscopically negative for *E. aerophilus* eggs was PCR-positive and no aspecific amplicons were generated for other endoparasites. Eight sequence types (designated haplotypes I–VIII) were detected, with a nucleotide sequence variation from 0.4 to 5.5%. The most prevalent haplotypes were HI, HII and HIII, with HI detected in 27 dogs and 5 cats. The eight haplotypes showed a maximum identity of 87-88%% with the *cox*1 gene of *Capillaria* spp. from Australian marsupials, while HI-HIV showed 100% homology with *E. aerophilus* isolates affecting wildlife in different European countries.

CONCLUSIONS: The present findings open new avenues for the diagnosis of lung capillariosis by *E. aerophilus.* The molecular assay here described contributes to the diagnosis of the infection, which cannot be achieved by clinical examination due to the many other conditions with overlapping clinical pictures in pets. The definitive diagnosis of canine and feline lung capillariosis relies on the detection of the typical trichuroid eggs through standard fecal floatation but it may present major constraints in the identification of *E. aerophilus* eggs due to their similarity with those of other pet trichuroids, e.g. *Trichuris vulpis*, *Capillaria bohemi*, or with pseudo-parasitic findings, e.g. *Capillaria annulata* or *Capillaria hepatica* from preys (Conboy G, 2009, Vet Clin North Am Small Anim Pract, 39: 1109-1126; Traversa D et al, 2010, Parasit Vectors, 3: 62). The distribution of the infection in animals cohabiting the same geographic areas and the phylo-geography of different *E. aerophilus* populations should be better investigated. In conclusion, this PCR-based method is a powerful tool for holistic studies on lung capillariosis and provides a basis for a better understanding of poorly known aspects of biology, epidemiology, pathogenesis and taxonomy of *E. aerophilus*.

Genetic characterization of *Eucoleus aerophilus* from different hosts and Countries

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The parasitic nematode *Eucoleus aerophilus* (syn. *Capillaria aerophila*) infects the respiratory tract of various carnivore species. Occasionally, *E. aerophilus* may infect and cause damages in the lungs of humans and can even mimic carcinoma-like masses (Lalosevic D et al, 2008, Am J Trop Med Hyg, 78: 14-16). Despite the importance of *E. aerophilus* and the recent evidence of an increasing trend in the prevalence of infection in companion animals (Traversa D et al, 2010, Parasit Vectors, 3: 62), several aspects of lung capillariosis remains to be elucidated, particularly in the area of genetic studies.

AIM: To gain insights into the population structure, geographical distribution and host affiliation, a target region of mitochondrial DNA of *E. aerophilus* isolates from wild and domestic animals has been here characterized.

MATERIALS AND METHODS: Single adult nematodes from 14 red foxes (4 from Romania, 3 from Portugal, 3 from Serbia, 3 from UK and 1 from Canada), from 3 beech marten from Portugal, and 44 egg batches from 33 dogs, 10 cats and 1 fox from Italy, were subjected to a PCR-coupled sequencing protocol to amplify a ~344 bp informative region within mitochondrial cytochrome *c* oxidase subunit 1 gene of *E. aerophila*. The open reading frames were confirmed by conceptual translation using the invertebrate mitochondrial code by MEGA5 (Tamura K et al, 2011, Mol Biol Evol, 28: 2731-2739). The evolutionary relationships of taxa belonging to Capillarinae available in GenBank™ with the sequences herein generated were inferred using the Neighbor-Joining method (Saitou N, Nei M, 1987, Mol Biol Evol 4: 406-425).

RESULTS: Sixty-one amplicons of all *E. aerophilus* isolates were sequenced: fifteen sequence types (i.e. haplotypes I–XV) were found. The haplotypes I (n = 36.59%), II and III (n = 8, 13.1%) represented the prevalent sequence types, followed by the other twelve haplotypes (n = 17, 27.8%). Haplotype I was represented by sequences of *E. aerophilus* from dogs, cats and fox from Italy, as well in foxes from Serbia and Romania. All 15 haplotypes were aligned over 299 positions. Sequences included 274 conserved and 25 variable sites, of which 21 were singleton and 4 parsimony-informative. The majority of the variable sites (n = 18; 72%) was at the third codon position, whereas the remainder (n = 7; 28%) at the first and second codon positions. Almost all the intraspecific nucleotide variations were synonymous. Amino acid sequences had an open reading frame in first position and did not contain a further stop codon. The mean difference in the intraspecific nucleotides among the haplotypes was 1.5%, ranging from 0.4 to 5.1% in haplotype VIII (e.g., dogs, Italy) *vs* haplotype XIV (e.g., foxes, UK). All 15 haplotypes clustered together, with strong nodal support (≥49%), and were separated from sequences representing other Capillarinae, showing that all haplotypes, irrespective of their geographical and host origin, represented *E. aerophilus*.

CONCLUSIONS: This study identified significant genetic variation among sequences of *E. aerophilus* found in domestic and wild animals from different countries. Although 15 distinct haplotypes were identified, the sequence variation found in the *cox*1 region examined was lower than that among nematodes for which homologous sequence data are available in public databases. The existence of these population variants might be due to a higher mutation rate in the *cox*1 region, to inbreeding within particular host populations and geographical regions. Some haplotypes were shared between domestic and wild animals in different areas, thus indicating that *E. aerophilus* populations co-infect pets and wildlife. In summary, these results represent a foundation for addressing investigations on the epidemiology, phylo-geography and population-genetic make-up of this neglected lungworm in different hosts and countries.

Genetically-engineered *Aedes aegypti* strain for population control: a case study

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AIM: A genetically-engineered strain of the dengue mosquito vector *Aedes aegypti*, designated OX3604C (Fu G et al, 2010, Proc Natl Acad Sci, 107: 4550-4554), was evaluated in large outdoor cage trials for its potential ability to improve dengue prevention efforts by inducing population suppression. OX3604C is engineered with a repressible genetic construct that causes a female-specific flightless phenotype. Wild-type females that mate with homozygous OX3604C males will consequently not produce reproductive female offspring. Natural populations could potentially be reduced or eliminated by repeated releases of OX3604C males. In previous laboratory cage experiments, weekly introductions of OX3604C males eliminated all three targeted *Ae. aegypti* populations after 10-20 weeks (Wise de Valdez MR et al, 2011, Proc Natl Acad Sci 22;108: 4772-5). Comparison of transgenic mosquito performance in laboratory versus semi-field conditions is expected to provide valuable data for planning subsequent experimental assessments and refine strategies for disease prevention (Knols BGJ, Louis C, 2006 Bridging laboratory and field research for genetic control of disease vectors. Wageningen Frontis Series, The Netherlands).

MATERIALS AND METHODS: As part of the phased, progressive evaluation of this technology, we carried out a follow-up assessment in large, more environmentally natural, outdoor field enclosures (Facchinelli L et al, 2012, Am J Trop Med Hyg 85: 248–256), in *Ae. aegypti* and dengue endemic southern Mexico. OX3604C males were introduced weekly into field cages containing stable target populations, initially at a 10:1 ratio.

RESULTS: While after 17 weeks density reduction was significant in four of five target populations, we did not observe population elimination in any of the cages within the expected timeframe.
A series of mating competitiveness experiments, carried out to explore the discrepancy between lab and field cage results, revealed a maximum 59.1% mating disadvantage for OX3604C males, which accounted for a large part of the 97% fitness cost that a mathematical model predicted would be necessary to obtain the field cage results.

CONCLUSIONS: Implications of laboratory cage experiment, field cage experiments and short-term mating competition experiments in large field enclosures are discussed in view of the release of a genetically-engineered mosquito strain for control of vector borne diseases.

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Clinical and molecular findings of *Alaria alata* infection in dog from Aosta Valley, Italy

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AIM: *Alaria alata* is an intestinal fluke of canids that develops in a three-host life cycle, having as first intermediate host fresh-water snails (*Planorbis*-, *Heliosoma*-, *Lymnea*- and *Anisus* species) (Wójcik AR et al., 2001, Wiad Parazytol 47: 423–426). The second intermediate hosts are different species of frog and toad, in whose skeletal muscles, as well as in a wide variety of paratenic hosts, the infective mesocercariae are found. *Alaria alata* is currently the only member of its genus known to be endemic to Europe (Riehn K et al, 2011, Parasitol Res, 108: 1327-1332). Due to the lack of reports in Italy, we point out the clinical findings and the later molecular analysis, that led to the diagnosis of *A. alata* in a dog, living in Northwestern Italy.

MATERIAL AND METHODS: The owners of a 1 year old, female Border Collie, living in Aosta, noticed a somewhat-like *Taenia* parasite in the feces of their dog, which was treated with Fenbendazole at recommended dosage for 5 days. The parasites recurred every 15 days from the end of treatment, which was administered again twice. The parasites where first noticed in dog's feces, 16 days after it was taken to a mountain lake (2066 mt a.s.l.) in Aosta Valley. The dog has never displayed any kind of distress nor symptoms during the above parasite infection. In order to have a confirmatory diagnosis, several adult flukes as well as fecal samples were taken to the University of Turin for morphological-morphometrical analysis (Mohl K et al., 2009, Parasitol Res,105:1–15) , and for PCR-molecular identification (Riehn K et al, 2011, Parasitol Res, 108: 1327-1332).

RESULTS: Morphological-morphometrical analysis of adult flukes, the recovery of characteristics eggs in feces, as well as PCR, identified the recurrent parasites as *Alaria alata*. Sequencing will confirm conclusively, the specificity of the PCR assay and of other diagnostic procedures.

CONCLUSIONS: This is to our knowledge the first recorded case of alariosis in a dog from Italy. To date the identification of *A. alata* adult flukes has always been based on external characteristics and comparative morphology, here we confirmed the validity of the biomolecular assay. Epidemiological surveys are ongoing, in order to evaluate presence and distribution of the parasite in wild and domestic animals from the same area.

Molecular survey on tick-borne pathogens from ticks removed from wild animals in 2010

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AIM: Various tick-borne pathogens occur in Emilia-Romagna region, where Lyme disease, transmitted by *Ixodes ricinus*, is frequently diagnosed in humans (Ciceroni L., and Ciarrocchi S, 1998, New Microbiol. 21 407-18). In this work we investigated the occurrence of *Borrelia burgdoferi s.l., Rickettsia spp.* and *Anaplasma phagocytophilum* in ticks collected from wildlife during 2010.

MATERIALS AND METHODS: Ixodid ticks were collected from wild animals hunt-killed or found dead in 2010. Several pathogens were investigated by PCR. The detection of Rickettsia was performed using two sets of primers: *gltA* and *ompA* genes (Labruna et al., 2004, J Clin Microb, 42(1), 90-98; Roux et al., 1996, J Clin Microb 34, 2058-65.). For *Anaplasma* DNA detection the primers described by De la Fuente et al. (2005, J Clin Microb, 43, 1309-1317) for the msp4 (major surface proteins ,MSPs) gene coding regions of *A. phagocytophilum*, *A. marginale, A. centrale,* and *A. ovis* were used. *Borrelia burgorferi* senso latu complex DNA was detected by the use of primers described by Marconi and Garon (1992, J Clin Microbiol, 30, 2830-2834) that amplify a 357 bp fragment of 16S gene. To identify infective species, positive amplicons produced by the primers were further sequenced and compared with sequences in the GenBank database.

RESULTS: A total of 379 (24 nymphs, 141 males and 214 females) ticks collected from 58 wild animals from all nine provinces of the region were examined by PCR. Ticks were collected from roe deer (*Capreolus capreolus*; number of ticks collected=147), wild boar *(Sus scrofa* ; n=113), wolf (*Canis lupus*; n= 34), red fox (*Vulpes vulpes*; n=28), european brown hare (*Lepus europaeus;* n=26), hedgehog (*Erinaceus europaeus;* n=18) and red deer (*Cervus elaphus;* n=13). Exemplars were identified as belonging to six tick species: *Ixodes ricinus* (*n*=203), *Rhipicephalus sanguineus* (*n*=108), *Dermacentor marginatus* (*n*=40), *I. hexagonus* (*n*=18), *I. canisuga* (*n*=8), *Hyalomma marginatum* (*n*=2).
A total of 70 samples (18.4%) yielded positive results for *Rickettsia sp*. By DNA sequencing o f 61 samples they were identified as belonging to six *Rickettsia* species: *R. monacensis* (29 samples), *R. massiliae* (16), *R. slovaca* (12), *R. aeschlimannii* (2), *R. conorii* (1), *R. raoultii* (1).
Five *I. ricinus* samples results positive for *A. phagocytophilum* (1.3%). One of these was also positive for *R. monacensis.* None of the 379 samples analysed results positive for *B. burgdoferi s.l.*

CONCLUSIONS: The results obtained in this study shows that *Rickettsia* species and *A. phagocytophilum* occurred in northern Italy in ticks collected on wild animals, whilst detection of *B. burgdoferi* is difficult in these type of samples. Therefore the epidemiological role of wildlife should be considered and the risk of infection in humans and domestic animals should be better assessed.

Use of microsatellite markers for typing of Microsporum canis isolates causing pseudomycetoma in cats

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AIM: Pseudomycetoma (PM) is a rare subcutaneous form of dermatophytosis caused by *Microsporum canis*. A genetically transmitted immune defect is supposed to be responsible for this clinical entity, since it is mostly described in Persian cats. However, the question may arise as to whether animal hosts harbor mixed genotypes of *M. canis* that differ in their potential to cause pseudomycetoma. Genotyping methods are expected to be useful tools to address this question. In this work we applied a multi locus microsatellite typing (MLMT) method to study strains of *M. canis* cause of PM in cats.

MATERIALS AND METHODS: We used eight microsatellite markers in order to genotype 20 *M. canis* isolates that caused pseudomycetoma and we compared them with 103 isolates involved in classical ringworm episodes. Fungal DNA was extracted using a commercially available kit. Concerning pseudomycetoma samples, they consisted of paraffin embedded histological specimens. For these, DNA extraction was performed according to Lau et al. (2007) (J Clin Microbiol, 45:380-385) and Munoz-Cadavid et al. (2010) (J Clin Microbiol, 6: 2147-2153). PCR amplification of microsatellite markers was performed using specific fluorescence labeled primers. PCR products were screened for length polymorphisms using ABI 300 genetic analyzer.

RESULTS: A Bayesian and a distance approach were followed to structure the *M. canis* samples. The isolates responsible for pseudomycetoma episodes did not cluster. Moreover, most of them were genetically very close – if not equal - to other isolates responsible for classical ringworm.

CONCLUSIONS: Multi locus microsatellite typing (MLMT) represents a random strategy that gained in popularity owing to the highly variable nature and rapid mutability of microsatellite markers. MLMT has the potential to correlate specific genotypes with "phenotypical" features of interest in fungal strains. Actually, the loci under study are unlikely to be based on genes involved in virulence or other features of interest, but, due to the clonal mode of reproduction of dermatophyte fungi, genomes are transmitted to the next generation in unaltered condition and thus associated genes – such as virulence genes and microsatellite markers – may be linked. Results obtained with this technique support the conclusion that pseudomycetoma in cats is due to host factors rather than to the aptitude of particular genotypes of *M. canis*, and that this clinical form can be caused by any strain of *M. canis*.

Polymorphism analysis of Intron-1 of the sodium channel gene in species and molecular forms of the *Anopheles gambiae* complex

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AIM: In a previous geographical survey of genetic variation at Intron-1 of the voltage-gated sodium channel gene in M and S molecular forms within the major malaria vector *Anopheles gambiae* s.s. (Gentile et al., 2004, Ins Mol Biol, 13: 371-377), we found two major haplotypes separated by a single mutational step (a C/T substitution at position 702), which co-segregate almost completely with the IGS rDNA sites identifying M and S. We also reported 10 additional haplotypes stemming from the two major haplotypes, mostly present in single localities. We here present the results of a novel survey of the same intron region mostly carried out on M and S populations from areas of putative secondary contact between the two forms (Caputo et al., 2011, *PLoS One*, 6:e16415) and on other 4 members of the *A. gambiae* complex.

MATERIALS AND METHODS: Eighty-seven *A. gambiae* s.s. females from 12 African countries (65 of which from The Gambia and Guinea Bissau) were processed for DNA extraction, identified based on a PCR-RFLP approach (Fanello *et al*., 2002, Med Vet Entomol 16: 461–464) and on the *SINE200* method (Santolamazza *et al*., 2008, Malaria J, 7:163), and a 531 bp fragment of Intron I was sequenced (Gentile *et al*., 2004). Furthermore, 23 *A. arabiensis*, 4 *A. melas*, 2 *A. merus* and 1 *A. quadriannulatus*-A were identified and sequenced. Estimates of DNA polymorphism at Intron-1 were obtained using DnaSP v. 5

RESULTS: A total of 56 polymorphic sites and of 37 intron-1 haplotypes were detected. Fixed differences were found among the 5 members of the *A. gambiae* complex analysed. Twenty-six haplotypes (out of 172 alleles) were observed in *A. gambiae* s.s. (Hd=0.830): alleles in populations from The Gambia and Guinea Bissau were grouped in 20 haplotypes (Hd=0.827), while most alleles from populations of other African regions were grouped in only 10 haplotypes (Hd=0.748). Average nucleotide diversity in *A. gambiae* s.s. was π=0.51%, but this was higher in populations from Guinea Bissau (π=0.54%) and The Gambia (π=0.56%), than that from the rest of Africa (π=0.24%). Statistics applied to detect departures from neutral expectations were negative in *A. arabiensis* (with a significant D value=-1.93) and in *A. gambiae* s.s. sample (F*=-2.51), and, in particular, in the S form (D*=-2.54; F*=-2.66), thus indicating an excess of rare or recent mutations that could be due to a sudden demographic expansion or to positive selection. Differently, positive values for neutrality statistics were scored in Guinea Bissau and The Gambia M and S populations, where significant positive values of D and F* statistics were observed, indicating population structure, possibly due to a decrease in population size and/or to balancing selection. All M-form individuals were C/C homozygotes at position 702 (with a single exception), but a C/T polymorphism was observed in S-form samples from The Gambia, Guinea Bissau and Rwanda.

CONCLUSIONS: The above results, the relationships among haplotypes and their geographical distribution will be discussed with particular reference to their contribution in shedding light to the unusual situation of putative secondary contact between M and S forms in the western extreme of *A. gambiae* s.s. range.

Genetic characterization of macroscopic sarcocysts found in sardinian sheep

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Four species of Sarcocystis are reported in domestic sheep: *Sarcocystis ovicanis, S. arieticanis, S. ovifelis,* and *S. medusiformis*. During 2011 macroscopic cysts characterized by diaphragmatic and skeletal muscles location and small, elongated shape were found in of Sarda breed at slaughterhouse inspection. Those small, fusiform shaped sarcocysts (SFS) differ from the large, oval shaped (LOS), macroscopic cysts, which are found prevalently in the oesophagus of sheep during the slaughter process, largely recognized as *S. ovifelis*.

AIM: The aim of this study was to determine genetic differences, based on partial sequences of the nuclear ribosomal DNA among the macroscopic sarcocysts of sardinian sheep.

MATERIALS AND METHODS: Several cysts of each morphotype were isolated from the tissue of each animal at slaughterhouse and stored frozen at −20 °C. Individual isolated sarcocysts were placed in 1.5 ml Eppendorf tubes with 20 l distilled water and DNA extracted.
The amplification of the 28S rRNA gene fragment was performed using the primer pair KL4 and KL6b , whereas of the 18S rRNA gene fragment using the primer pair 3L and 3H . Partial gene sequences were analysed with an ABI Prism 377 automatic DNA sequencer using the same primers as in PCR. The specificity of the end sequences was analysed with the BLAST programme comparing the identified sequences with those stored in the GenBank and having the highest homology for the identified sequences. Comparison of gene sequences with the identified homological sequences of Sarcosystis species, estimation of genetic distances and reconstruction of phylogenetic relationships in the Sarcocystidae family were performed using the MEGA4 program. Phylogenetic relationships were reconstructed from 1000 bootstrap replicates under the criterion of minimum evolution, using Kimura 2-parameter distances.

RESULTS: The DNA extracted from infected tissues was used as a template for genetic characterization of the 2 different macroscopic sarcocyst types: primers targeting the lsu rRNA gene and the ssu rRNA gene yelded an 800bp and 500bp fragment, respectively, for all the samples. Type LOS sarcocysts were identified as *S. ovifelis*, showing a 99% nucleotide sequence similarity with *S. ovifelis* sequences deposited in Genbank for both genes. Type SFS sarcocysts showed a 95% nucleotide sequence similarity for both genes. A phylogenetic tree was constructed using the criterion of minimum evolution method based on comparison of partial 18S and 28S rRNA gene sequences and SFS sarcocyst types resolved as discrete group within the sarcosporides having felids as definitive host.

CONCLUSIONS: The ssu and lsu rRNA gene have been used frequently in phylogenetic analyses to infer relationships among species and higher taxa within the Apicomplexa. Both genes are abundant in the genome of many apicomplexans, including the Sarcocystidae, and its double feature of hypervariable regions interspersed within highly conserved DNA sequences, makes the genes suitable for differentiation between many eukaryotic species. Moreover, the fairly large number of rRNA genes sequences from different Sarcocystis species available in GenBank provides data for construction of informative phylogenetic trees of the Sarcocystidae. The phylogenetic tree constructed in this study, based on comparison of partial 18S and 28S rRNA gene sequences of macroscopic sarcocysts from naturally infected Sardinian sheep and sequences of other taxa belonging to the Sarcocystidae family deposited in GenBank, showed that there is the possibility of the presence of a distinct subgroup among Sarcocystis having felids as definitive host. The genetic difference between ruminant Sarcocystis species having canids and felids as definitive host has been well established . The SFS sarcocyst types resolve as a distinct, highly homologous group within the “felid group”. Diagnostic ultrastructural features and complete genome characterization should aid in future studies and communications regarding this possibly new taxon, which lends itself to experimentation because its sarcocysts are macroscopic and easily excised from infected animals.

Detection of genomic regions affecting gastro-intestinal nematode resistance in dairy sheep

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AIM: On a global scale, sheep diseases caused by gastrointestinal nematode parasite infections have a great impact upon animal health and sustainability (Nieuwhof GJ, Bishop SC, 2005, Anim Sci, 81: 23-29). The evidence of a genetic variation for resistance at gastro-intestinal nematodes has been documented (Sechi S et al, 2009, IJAS, 8: 156-158). The main aim of this work was to detect genomic regions (QTL) affecting gastro–intestinal nematode resistance in sheep by using innovative molecular tools. The results are expected to produce a set of genetic markers to be used for selecting resistant animals.

MATERIALS AND METHODS: From 2000 to 2010 faecal eggs count (FEC) were recorded on average two times per year mainly in September and July on an experimental population. The naturally infected population consisted, at the beginning of the experiment, of 915 Sarda x Lacaune backcross ewes (BC). Successively, 1,479 descendants were generated using Sarda purebred rams (SA). Faeces were processed by flotation in saturated salt solution in a McMaster camera and the eggs counted according to Raynaud (Raynaud JP, 1970, Ann Parasitol Hum Comp, 45, 321-342). FEC measurements were log-transformed prior to further analysis. Genotypes of the ewes at 54,241 single nucleotide polymorphism (SNP) spread all over the 26 ovine autosomes were determined. The molecular tool which permits this kind of analysis was the Illumina OvineSNP50 BeadChip. Linkage analysis was performed for BC and SA families separately in order to detect QTLs specific of the Sarda breed.

RESULTS: The analysis was highly successful since 25 genomic regions affecting resistance against gastro-intestinal nematodes across different chromosomes were found. Several QTLs were located in 11 chromosomes (chromosomes 1, 2, 9, 11, 12, 14, 15, 16, 20, 24 and 25). The most important genomic regions were found on chromosomes 20 and 12. On chromosome 20 is located the Major Histocompatibility Complex (MHC) that is implied in the regulation of immune responses. Some of the genes of the MHC (e.g DRB1) have been previously identified as associated with resistance against gastro-intestinal nematodes (Hassan M, 2011, Vet Res, 42: 46; Stear M, 2009, Paras Immun, 31 : 274-282). The QTLs detected on chromosome 12 confirmed previous results on the same population (Sechi S et al, 2010, Large Animal Review, 5(suppl): 104).

CONCLUSIONS: The QTLs detection analysis using the Illumina OvineSNP50 Beadchip detected many significant regions affecting gastro-intestinal nematode resistance. Some genes located in the detected regions will be further investigated to identify causal mutations. The precise localization of these regions and a more detailed knowledge of these genes will permit selecting resistant animals.

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Development of a Real Time PCR based on OmpB gene for *Rickettsia* spp. detection and quantization

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The genus *Rickettsia* belongs to the family of Rickettsiaceae in the order of Rickettsiales and constitutes a group of obligate intracellular endosymbionts of eukaryotic cells. *Rickettsia* is an important cause of emerging infectious disease in people and animals and rickettsiosis is one of the oldest known vector-borne diseases. Currently used diagnostic tests have limitations. Serological tests are the easiest methods for the diagnosis of tick-borne rickettsioses but the interpretation of serological data can be complicated by the cross-reactivity among the spotted fever group rickettsiae. Molecular methods based on PCR utilize different primer sets targeting various rickettsial genes and constitute sensitive and rapid tools for the detection of rickettsiae (Brouqui P et al, 2004, Clin Microbiol Infect; 10: 1108–1132). Recently quantitative methods based on Real Time PCR were developed (Kidd L et al, 2008, Vet Microbiol. 129: 294-303) for diagnosis of Spotted Fever Group *Rickettsia*. One of the *Rickettsia* gene better characterized is the OmpB gene codifying an outer-membrane protein widely studied due to its exposed location and to the presence of conserved epitopes. OmpB gene is commonly employed for phylogenetic analysis.

AIM: This study was aimed to develop a quantitative PCR assay targeting the OmpB gene and involving the use of SYBR Green method for the diagnosis of *Rickettsia* spp. infection in order to simultaneously detect and quantify the presence of the parasite from blood samples. Analyses were conducted in order to test sensitivity and specificity of the proposed assay.

MATERIALS AND METHODS: Genomic DNA was extracted from 200 l of blood samples and screened by traditional PCR (Tzianabos T et al, 1989, Journal of Cinical Microbilogy, Dec: 2866-2868) to detect the presence of *Rickettsia* spp. DNA. OmpB gene sequences coming from many different species of *Rickettsia* (*R.helvetica*, *R.monacensis*, *R.australis*, *R.prowazekii*, *R.heilongjiangensis*, *R.felis*, *Candidatus R.hoogstraalii*, *R.japonica*, *R.slovaca*, *R.parkeri*, *R.rickettsii*, *R.honei*, *R.sibirica*, *R.mongolotimonae*, *R.rhipicephali*, *R.aeschlimannii*, *R.raoultii*, *R.africae*, *R.massiliae*, *R.conorii Israeli tick typhus*, *R.conorii subsp. Caspia*, *R.conorii Indian tick typhus*, *R.conorii conorii*, *R.montanensis*, *R.endosymbiont of Ixodes scapularis*, *R. conorii Malish7*) were selected from GenBank and aligned using Clustal W in order to identify the appropriate region for primer design using the Primer Express 3.0 software. For the assay optimization many annealing temperatures and primers concentrations were tested. Each reaction was performed in duplicate and in presence of at least a no-template control. The Real Time PCR was set up in a CFX96 Biorad Termocycler using the iTaq SYBR Green Supermix with ROX (Biorad) with the optimized thermal protocol. The fluorescence increase was detected during the polymerization phase. At the end of each reaction, a melting curve analysis was performed to test PCR products specificity. The assay was utilized to test the presence of pathogen DNA belonging to different species of *Rickettsia* (*R.conorii, R.massiliae, R.aeschlimannii, R.raoultii, R.monacensis R.slovaca* and *R.felis*) and also in presence of DNA from pathogens others then Rickettsie such as *Anaplasma*, *Ehrlichia*, *Babesia* and *Theileria.*

RESULTS: The customary values obtained (using CFX Manager 1.6. software) for the standard curve (r = 0.999; slope = - 3.349) indicate that the reaction was well optimised. The reaction resulted positive for all the analyzed *Rickettsia* species. The assay was very specific, since the parasites DNA was amplified only in those samples previously selected as positive with other techniques. Furthermore, all the samples positive for the other related pathogens resulted negative. The sensitivity of the Real Time PCR was also calculated and the reaction resulted to be very sensitive. The limit of detection was of 0.1 pg of pathogen DNA per reaction. This study allowed developing a new and powerful diagnostic method able to detect and quantify pathogen DNA of species of *Rickettsia* genus and also to evaluate the diagnostic utility of this assay. The assay is rapid and easy to perform and it is also sensitive e specific. The analysis has provided some information about OmpB gene, suggesting it can be used for diagnostic analysis. The use of a quantitative test with high sensitivity is extremely useful since it is able to detect, for example, possible changes in the parasitemia in the different stages of infection.The research was supported by the Italian Ministry of Health (IZSSI 07/08). The authors thank to Rosa Filippi and Franco Ferraro for their technical support.

Cryptospordium parvum sporozoites have three rhomboid proteins localized in different cell-compartments

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AIM: The rhomboid proteins constitute a large family of intramembrane serine proteases that are present in all branches of life (Urban S and Dickey SW, 2011, Genome Biol, 12: 231). A distinct subclass of rhomboids (PARL) are specifically associated with mitochondrial functions and are present in Eukarya (Hill RB and Pellegrini L, 2010, Semin Cell Dev Biol, 21: 582-92). In Apicomplexa, rhomboids are, in most of cases, involved in shedding adhesins from the surface of the zoites during motility and host cell entry (Santos JM and Soldati-Favre D, 2011, Cell Microbiol 13: 787-96). We previously described the first rhomboid in *Cryptosporidium parvum* (Trasarti E et al, 2007, Mol Biochem Parasitol 152: 159-169). The aim of this study is the characterization of all *C. parvum* rhomboids to define the minimal repertoire of rhomboids for an invasive apicomplexan. To this end, we expressed the predicted rhomboid proteases encoded by the *C. parvum* genome and verified their expression and localization using specific sera.

MATERIALS AND METHODS: Putative genes for rhomboid proteins were identified in CryptoDB (http://cryptodb.org/cryptodb/) screening by BLAST the database with the sequence of CpRom1 and with other rhomboid domains. Phylogenetic analysis was conducted at http://www.ebi.ac.uk/Tools/msa/clustalw2/ by neighbor joining method. Rhomboid coding sequences were amplified by RT-PCR from *C. parvum* sporozoites and cloned for the expression as 6His-tagged peptides. Fusion proteins were purified and used to immunize Balb-C mice to obtain specific sera then used in Western blots and in immunofluorescence on sporozoites.

RESULTS: The homology search revealed that *C. parvum* as well as the strictly related *Cryptosporidium hominis* have three genes encoding for rhomboids, thus the two novel rhomboids were named CpRom2 and CpRom3. The phylogenetic analysis showed that CpRom1 and CpRom2, which are strictly related, clustered with apicomplexan rhomboids responsible for the cleavage of the adhesins (i. e. TgRom4, TgRom5 and PfRom4) during the invasive process. Differently, CpRom3 resulted related to the TgRom2 that has been localized in the Golgi complex of *Toxoplasma gondii*. Noteworthy, none of these rhomboids of *C. parvum* showed homology with mitochondrial rhomboids whereas the related *Cryptosporidium muris* has a typical mitochondrial PARL in its predicted. The western blot analysis demonstrated that all the three rhomboids are expressed at the oocyst-sporozoite stage, even if there were remarkable differences among them. CpRom1 was present as 110 kDa form both in quiescient oocysts and sporozoites, but in sporozoites compared also a higher form (approximately 130 kDa). On the contrary CpRom2 was present as unique form of 51 kDa in sporozoites whereas in oocysts we observed also two smaller forms. CpRom3 was present in sporozoites as an approximately 80 kDa band but was completely absent in oocysts indicating that this protein is newly synthesized during the excystation process. The immuno-localization on sporozoites showed that the three rhomboids were located in different cell compartments. CpRom1 was mainly accumulated at the posterior pole behind the nucleus. CpRom2 was equally distributed along the anterior pole of the sporozoites apparently on the external surface. CpRom3 resulted accumulated in proximity of the nucleus in a zone compatible with the endoplasmic reticulum or an internal vesicular organelle (*C. parvum* Golgi apparatus is not yet identified).

CONCLUSIONS: *Cryptosporidium* genus has a limited repertoire of rhomboid proteins if compared with other apicomplexan parasites such as *T. gondii* or *Plasmodium falciparum* (both parasites have 6 rhomboids). It is remarkable that *C. parvum* as well as the strictly related *C. hominis* lack of mitochondrial rhomboids and this fact is consistent with the absence of a typical mitochondrion in these species (Putignani L, 2005, Parassitologia, 47: 217-225). The homologies with the other apicomplexan rhomboids and the sub-cellular localizations indicate that two of these rhomboids (CpRom1 and CpaRom2) are probably involved in processing the adhesins during the invasion of the host cell. Differently, the third rhomboid (CpRom3) is probably associated with an internal membranous organelle that has a role in protein trafficking. *C. parvum* has three rhomboids expressed at same stage but restricted in different areas of the sporozoite cell and this allocation probably contributes to the specificity in cleaving the proper substrates.

First Molecular Identification and Phylogeny of a *Babesia* spp. from a Symptomatic Sow (Sus scrofa Linnaeus 1758)

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AIM: Babesiosis, caused by intraerythrocytic parasites of the genus *Babesia*, is one of the most frequently reported infections of free-living and domestic animals. Interest in babesiosis is rising sharply due to its worldwide distribution and public health concerns; indeed, babesiosis is considered an emerging zoonosis of humans. Although porcine babesiosis can be responsible for serious economic loss outbreaks of babesiosis in pigs are seldom reported, and the etiological agents still remain genetically uninvestigated. Here we report the first molecular identification and phylogeny of a *Babesia* sp. isolated from a symptomatic pig.

MATERIALS AND METHODS: DNA was extracted from blood samples obtained from a 2.5-year-old pregnant sow reared in a family farm located the region of Anglona (North Sardinia, Italy). The animal showed signs indicative of babesiosis, such as anorexia, depression, lameness, reluctance to move, and high fever, with consequent abortion. Diff-Quick-stained blood smears revealed the presence of babesial inclusions in erythrocytes, with approximately 10% of erythrocytes being parasitized. DNA samples were tested by PCR as in Zobba et al., 2011, J. Clin. Microbiol. 49: 2321-2324. PCR products were cloned, sequenced, and a 1,650 bp consensus sequence corresponding to the 18S rRNA gene was generated. Phylogenetic analyses were conducted by by use of MEGA upon alignment of the consensus sequence to 34 sequences of other members of the Piroplasmida, representative of the 5 groups identified within this order.

RESULTS: The partial 18S rRNA nucleotide sequence of *Babesia* sp. Suis was deposited in GenBank under accession number HQ437690. Phylogenetic analyses using both neighbour joining and maximum parsimony yielded coinciding trees where *Babesia* sp. Suis falls into a distinct branch of the Ungulibabesids group. In this study we report the first molecular characterization of a piroplasm in pig. This species, tentatively named *Babesia* sp. Suis, was detected in a sow from a family farm located in North Sardinia showing symptoms typical of porcine babesiosis, including abortion. Interestingly, an outbreak of porcine babesiosis characterized by a high mortality rate was previously reported in the same area in 1993 (Ligios C., Scala A.. 1993, Soc. Ital. Sci. Vet. 47:1379–1383). However, the lack of molecular data in that previous study and the impossibility of obtaining suitable samples for molecular comparisons render it impossible to verify the homology of *Babesia* sp. Suis with that responsible for the 1993 outbreak.

CONCLUSIONS: phylogenetic analysis allowed us to place Babesia sp. Suis in a distinct ancestral branch of the Ungulibabesids group. The host tropism of its most close relatives (*Babesia* sp. *Kashi*, B. occultans, B. sp. Sable Antelope, and B. orientalis), and their geographical distribution indicate that *Babesia* sp. Suis represents a porcine-specific pathogen. This first molecular characterization paves the way for investigating a possible role of porcine piroplasms as zoonotic agents and establishes a milestone for future molecular epidemiology studies. More data are needed to assess the clinical relevance, the geographical distribution, and the tick vector associated with this Babesia sp.

SESSIONE 5

PARASSITI E FAUNA ACQUATICA

Anisakid nematodes in some fishes collected in the middle Adriatic sea: epidemiological analysis and molecular characterization of recombinant genotypes

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AIM: epidemiological study to assess anisakids infection in anchovies (*Engraulis encrasicolus*), blackspot seabreams (*Pagellus bogaraveo*) and horse mackerels (*Trachurus trachurus*) coming from the middle Adriatic sea and molecular identification of collected larvae.

MATERIALS AND METHODS: from February 2009 to May 2010 we collected 1198 anchovies and between March and June 2010 we collected 26 blackspot seabreams and 10 horse mackerels, in the Adriatic sea, northeastern Mediterranean (44°6 -42°30 N; 12°41 -14°50 E). After measurement of total length, fishes were dissected and visceral cavities and muscles were carefully examined for the presence of anisakids larvae. After larval DNA extraction, PCR-RFLP technique on ITS region and DNA sequencing were conducted for species identification (D'Amelio S et al, 2000, Int J Parasitol, 30: 223–226; Pontes T et al, 2005, J Parasitol, 91(6): 1430-4).

RESULTS: 1167 larvae were recovered from anchovies. The prevalence of infected fishes was higher during the spring than during the winter (table 1). Infestation also increases with the increase of the length of anchovies (table 2). 1492 anisakids were recovered from blackspot seabreams (the prevalence of infected fishes was 96,15%). A little increase of larvae in fishes of the biggest class was registered. 756 larvae were recovered from horse mackerels (the prevalence of infected hosts was 80%): here, no difference in the amount of parasites was registered in relation to the length. PCR-RFLP technique, conducted on 227 larvae, revealed that 214 corresponded to *Anisakis pegreffii*, displaying the typical restriction profiles (D'Amelio S et al, 2000, Int J Parasitol, 30: 223–226), 1 specimen (coming from anchovies) showed the profile corresponding to *Hysterothylacium auctum* (Szostakowska B et al, 2002, Mol Cell Probes, 16: 111-118) and 12 specimens showed the profile corresponding to recombinant genotypes between *A. simplex* s.s. and *A. pegreffii* (Abollo E et al, 2003, Infect Genet Evol, 3(3): 175-81). Up to now, 10 recombinant worms were also studied by ITS sequence analysis: one individual showed heterozygosis C/T in the first position and a T in the second position, 2 individuals showed both heterozygote positions (fig.1) whereas 7 specimens showed C in both positions.

Table 1. No. of analyzed and infected anchovies and relative prevalences in reference to the months of sampling

Month of sampling	Fishes examined	Fishes infected	Prevalence (%)
Feb '09	276	106	38,4
March '09	604	190	31,5
April '09	232	118	50,9
Dec '09	28	7	25,0
Jan '10	15	6	40,0
March '10	15	5	33,3
May '10	28	14	50,0

Table 2. No. of examined anchovies and no. of recovered parasites relative to host length during the sampling

Length class	No. anchovies examined	Parasite
10 cm - 11 cm	184	33
12 cm - 13 cm	709	283
14 cm - 16 cm	305	851

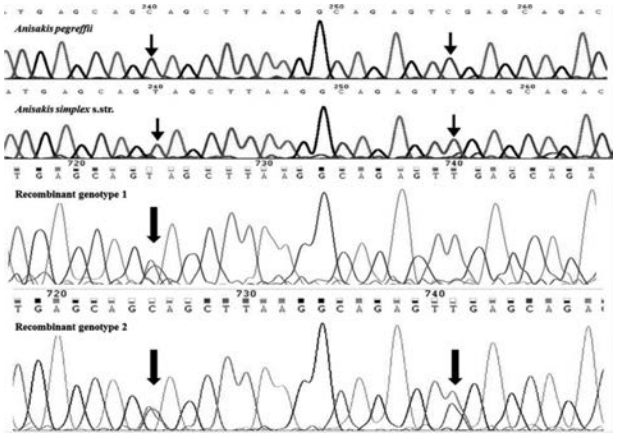

Fig. 1. Electropherograms related to 2 recombinant states compared with the reference sequence of A. *pegreffii* and *A. simplex* s.s.

CONCLUSIONS: the increasing of anisakids infection in anchovies during the spring can be due to the rise of the zooplankton which represents the intermediate host for larvae and food for anchovies; the increasing in biggest fishes can be probably due to larvae accumulation into the coelomatic cavity. PCR-RFLP analysis revealed that *A. pegreffii* is the main anisakid worm in the Adriatic sea: the predominance can be due to the occurrence of various dolphin species, like *Tursiops truncatus*, one of the principal definitive host of *A. pegreffii* in the Mediterranean (Mattiucci S et al, 2004, J Fish Biol, 65: 495-510). Molecular analysis also detected some recombinant states: up to now, DNA sequencing revealed both heterozygote positions C/T only in 2 individuals, confirming their putative hybrid identity. The specimen with heterozygosis only in the first position can be the result of a gene flow or incomplete concerted evolution. The 7 heterozygote patterns showing C in both positions (the typical polymorphism of *A. pegreffii*) can be probably due to incomplete DNA digestions. The occurrence of recombinant forms may be due to the passive transport of eggs of heterozygote genotypes by plankton and drifts from sympatric zones where the distributional areas of *A. pegreffii* and *A. simplex* s.s. overlap (Abollo E et al, 2003, Infect Genet Evol, 3(3):175-81; Meloni M et al, 2011, J Parasitol, 97(5): 908-14).

Occurrence of *Clinostomum complanatum* (Trematoda: Digenea) metacercariae in European Newts *Triturus carnifex* and *Lissotriton vulgaris* (Caudata: Salamandridae) from Tuscany, Central Italy.

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AIM: *Clinostomum* is a Digenetic Trematode living at the adult stage in the oral cavity, pharynx or oesophagus of fish-eating birds, reptiles and occasionally mammals, including man and recognize as second intermediate host many fish species. Metacercariae of the genus *Clinostomum* have been described from a variety of American anurans and urodeles.
We report the first record of *Clinostomum complanatum* in European Amphibians, with the finding of metacercariae in Italian crested newt (*Triturus carnifex*) and smooth newt (*Lissotriton vulgaris*).

MATERIALS AND METHODS: From October 2010 to March 2012, eighteen Italian crested newts (4 metamorphic males, 4 metamorphic females, 2 paedomorphic females and 8 larvae) and four smooth newts (1 paedomorphic male, 3 paedomorphic females) with evident yellow grub cysts were observed in an artificial pond in the protected area A.N.P.I.L. "Podere la Querciola" (Sesto Fiorentino, Tuscany, 43.824703N, 11.173299E). The infected newts were anesthetized with tricaine methanesulphonate (MS222). The cysts were incised with a scalpel and the metacercariae removed and fixed in 90% ethanol. The newts were rinsed with freshwater until recovery and then a small amount of ciprofloxacin ophthalmic ointment was placed on the wounds. The metacercariae were subjected to morphological and molecular identification amplifying the ITS rDNA region and the COI mtDNA genes (Caffara M et al., 2011, J Parasitol, 97: 884-891)

RESULTS: A total of 35 cysts were collected, 30 from the Italian crested newts and 5 from smooth newts. In *L. vulgaris* all the cyst (5) were in the head, while in *T. carnifex* the cysts were recovered from different part of the body as head (14), snout (6), throat (4), mouth (5) and tail (1).
The morphological observations carried out on 11 metacercariae allowed to refer them to *Clinostomum complanatum* (Digenea: Clinostomidae). The analysis of the sequences of both genes ITS rDNA and COI mtDNA showed 99.9-100% identity with this species.

CONCLUSIONS: The taxonomy of the genus *Clinostomum* is still confused and should be addressed, in order to avoid misidentification, by combining the morphological and the molecular approach. The presence of *Clinostomum* spp. in Amphibia has been recorded in North America and Mexico since many years (McAllister CT et al, 1990, J Helminth Soc Wash, 57: 69-71; Miller DL et al, 2004, J Helmith, 78: 373-376; McAllister CT et al, 2007, Texas J Sci, 59: 321-326; McAllister CT et al, 2010, Comp Parasitol, 77: 25-30; Cabrera-Guzman E et al, 2010, J Parasitol, 96: 736-739) where the species described so far are *C. marginatum, C. attenuatum* and *C. complanatum*, even if most of the works do not include detailed morphological and/or molecular descriptions. Concerning our specimens the morphological characters clearly allowed to refer the parasites to the species *C. complanatum*, data supported by the molecular analysis. This is the first record of *C. complanatum* in European newts and, more in general, in amphibians from Europe. Further analyses are required in order to establish the impact of this parasite on wild populations of European newts.

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Occurrence of anisakid nematodes in commercially important fishes from markets in central Italy

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AIM: Nematodes of the genus *Anisakis* are parasites of fishes and marine mammals; their accurate identification at any life cycle stage is important both to deepen the knowledge on their taxonomy, ecology, epidemiology and for diagnosis and control, as larval stages cause a clinical disease in humans known as anisakidosis. With the aim to monitor the presence of anisakid larvae in fishes usually intended for human consumption and identify them at species level, specimens of commercially important fishes were collected from markets in central Italy.

MATERIALS AND METHODS: Specimens of Atlantic mackerel *Scomber scombrus* (L), European hake *Merluccius merluccius* (L), European anchovy *Engraulis encrasicolus* (L), European pilchard *Sardina pilchardus* (Walbaum, 1792), red mullet *Mullus barbatus* (L), blue whiting *Micromesistius poutassou* (Risso, 1827) and Mediterranean horse mackerel *Trachurus mediterraneus* (Steindachner, 1868) were collected. Abdominal cavity and muscles were accurately checked for the presence of anisakids. Nematodes were counted and stored in ethanol for molecular characterization. A total of 2890 larval nematodes were found in 446 fish specimens. Epizootiological parameters such as prevalence, abundance and mean intensity were estimated; genetic identification was performed on representative nematodes from fish species using a PCR-RFLP diagnostic key based on nuclear ribosomal ITS region (D'Amelio S et al, 2000, Int J Parasitol, 30: 223 – 226).

RESULTS: Data obtained about epizootiological values and species-specific identification were summarized in the Table.

CONCLUSIONS: *A. pegreffii* is confirmed as the prevalent species in fishes from the Mediterranean basin; moreover, new records of the presence of the heterozygote genotype (*A. pegreffii -A. simplex* s.s.) at rDNA marker are reported, confirming the importance to deepen their identity and evolutive meaning. The high epizootiological values here reported for Atlantic mackerel, blue whiting and Mediterranean horse mackerel, confirm the widespread occurrence of anisakids in fish generally intended for human consumption. The epidemiological survey of zoonotic anisakids species in fish is of particular interest, since previously reported cases of human anisakiasis were supposed to be caused by infected typical Italian preparations based on marinated anchovies (Fumarola L et al, 2009, Foodborne Pathog Dis, 6: 1157–1159; Mattiucci S et al, 2011, BMC Infect Dis, 31: 11–82). This raw dish is highly popular also in other Mediterranean countries and it is supposed to be the cause of most cases of anisakiasis in Spain, too (Rello et al, 2009, Int J Food Microbiol, 129: 277–281). Finally, the accurate identification of anisakid nematodes at any life cycle stage is crucial to deepen the knowledge on several aspects of their biology, but also to screen the safety of fish products, since their occurrence in fishery products can cause both public health and economic problems.

Table 1. N°h (number of fish examined); Go(Geographical origin); N°L (number of larvae recovered); P (prevalence); A (abundance); Im (mean intensity); Molecular N-S (number of individuals identified and species); NAO: North-Atlantic Ocean.

Host species	N° h	Go	N° L	P (%)	A	Im	Molecular N-S
Scomber scombrus	17	NAO	65	35.29	3.82	10.83	2 - *Anisakis simplex s.s.*
Scomber scombrus	15	fao37	31	6.67	2.07	31,0	3 - *A. pegreffii*
Merluccius merluccius	47	fao37	427	19.15	9.08	47.44	5 - *A. pegreffii*
Engraulis encrasicolus	216	fao37	70	16.20	0.32	2,0	63 - *A. pegreffii* 7 - heterozygote
Sardina pilchardus	93	fao37	3	1.08	0.03	3,0	2 - *A. pegreffii* 1 - heterozygote
Mullus barbatus	39	fao37	4	7.69	0.10	1.33	4 - *Hysterothylacium aduncum*
Micromesistius poutassou	9	fao37	965	88.89	10722	120.62	5 - *A. pegreffii* 1 - heterozygote
Trachurus mediterraneus	10	fao37	1325	100	132.50	132.50	9 - *A. pegreffii* 1 - heterozygote

Molecular and morphological evidence for a cryptic species of the *Rhabdias bufonis* (Hartwich, 1972) *s.l.* species complex (Nematoda: Rhabdiasidae) from the green frogs of *Rana esculenta* species complex in Italy, and genetic differentiation from its congeners in frogs and toads

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AIM: The nematodes of the genus *Rhabdias* Stiles & Hassal, 1905 are common lung parasites of amphibian and reptile species throughout the world. Distinguishing the species belonging to *Rhabdias* is complicated due to their high morphological uniformity, parallelism and convergence (Kuzmin Y et al, 2007, J Parasitol, 93: 159-165). The molecular/genetic approach applied recently to species of this genus has revealed that several parasite species are indeed complexes of morphologically similar species (Tkach V et al, 2006, J Parasitol, 92: 631-636). The resolution of some of the taxonomic issues relating to *Rhabdias*, has been achieved by a parallel analysis of morphological traits and genetic characterisation. The aim of this study was to investigate the morphological and the genetic variation of these nematodes from frogs and toads simpatrically collected in several localities of Italy in order to estimate their genetic differentiation, provide a differential morphological analysis and define the host specificity.

MATERIALS AND METHODS: Several specimens of *Rhabdias* collected from the green frogs of the *Rana esculenta* species complex in Italy (i.e. *R. lessonae* Camerano, and *R. esculenta* Linnaeus, identified genetically by diagnostic allozyme loci) and common toad *Bufo bufo* Linnaeus, were analysed, based on DNA sequence analysis at multiple loci (i.e. mtDNA *cox-1*, 12S rRNA, ITS-1 and partial ITS-2 regions of the nuclear rDNA) and by morphometrical analysis.

RESULTS: Three different taxa were identified in the survey: a new cryptic species, *Rhabdias* n. sp., differentiated genetically, at both mitochondrial and nuclear level, from *Rh. bufonis* (*sensu* Hartwich, 1972) and *Rh. sphaerocephala* Goodey, 1924. The new taxon resulted to be different from the other species of *Rhabdias* previously sequenced and deposited in GeneBank. Phylogenetic analyses (MP and ML) were congruent in depicting *Rh. esculentarum* n. sp. as forming a distinct and highly supported clade from the sympatric species *Rh. bufonis* and *Rh. sphaerocephala*. A concatenated phylogenetic analysis (combined mtDNA *cox-1* and 12S rRNA) was used in order to maximize the power of the phylogenetic inference. The differential diagnosis of specimens of *Rhabdias* n. sp. have revealed differences in several characters in comparison with the type-species, *Rh. bufonis.*

CONCLUSIONS: The results achieved in the present study suggest that *Rh. bufonis* could be a complex of cryptic species. *Rhabdias* n. sp. is genetically closely related to *Rh. bufonis* in all of the phylogenetic trees, even if it is distinct from the lineage formed by specimens of *Rh. bufonis*. This seems to indicate that *Rhabdias* n. sp. represents a sister species of *Rh. bufonis*. This results add to our knowledge the occurrence of *Rhabdias* spp. in amphibians in Italy, and indeed Europe, and represent the first genetic/molecular characterization of *Rh. bufonis* (*sensu* Hartwich 1972). The data so far collected appear to indicate a remarkable host-preference of *Rh. esculentarum* for *R. lessonae* and *R. esculenta*: it is the only lung parasite to have been recovered from all 30 green frogs examined.

Preliminary studies on gill parasites and pathologies of cage reared *Thunnus thynnus* (Osteichthyes: Scombridae) from the western Mediterranean Sea

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AIM: Atlantic bluefin tuna (BFT) ongrowing is a promising activity in the Mediterranean aquaculture and BFT intensive culture is an important challenge for the next years. One of the aspects that must be considered and evaluated is the pathology that could compromise the correct development of this activity. The aim of study is to describe the parasites and gill pathologies affecting tunas from a large production batch. Based on preliminary studies (Mladineo I, Tudor M, 2004, Bull Eur Ass Fish Pathol, 24: 144-152), gills are one of the main targets for many important diseases affecting this species.

MATERIALS AND METHODS: Gills of 15 farmed tunas were analysed for metazoan parasites within 4 hours after landing. Selected filaments of the first left holobranch (10% formalin fixed *in toto*) were processed for routine histopathological studies. The rest of the gills were immediately frozen. Thawed holobranchs were excised and individually examined by naked eye and under a stereomicroscope. Two filaments (from inner and outer hemibranch) from 5 areas of each holobranch were excised and washed in order to look for blood fluke (Trematoda: Aporocotylidae) eggs. The location of the parasites was recorded according to Mele S et al (2010, Dis Aquat Org, 97: 219-225).

RESULTS: Most of the fish (73%) harboured at least one gill parasite. Nine parasite species were found: 7 trematodes (eggs of Aporocotylidae gen. sp., *Didymocystis* sp. 3 *sensu* Rodríguez-Marín et al, 2008, *Didymosulcus wedli*, *Didymosulcus* sp. 2 *sensu* Rodríguez-Marín et al, 2008, *Didymosulcus* sp. 3, *Didymozoon pretiosus*, *Wedlia bipartita*) and 2 copepods (*Euryphorus brachypterus*, *Pseudocycnus appendiculatus*), with a mean total intensity of 11 (1-35) for didymozoids and copepods. Didymozoid trematodes were the most abundant and prevalent parasites, mainly finding *D. wedli* [mean intensity, 14 (2-35); prevalence, 53%]. Blood fluke eggs were detected in the gill filaments of 60% of the fish. No adult aporocotylids were observed. Regarding the copepods, only 1 *E. brachypterus* and 7 *P. appendiculatus* were found in 3 fish. The histological study revealed the presence of blood fluke eggs and adult didymozoids. Eggs were spherical to elongate, about 20 in diameter, located within capillaries of gill lamellae, surrounded by a low to moderate inflammatory response of the host. Different degrees of miracidial development were observed. Adult didymozoids corresponding to *D. wedli* were mainly found in the outer margin of the gill filaments as whitish nodules with hard consistency. Histological sections of these nodules revealed encysted parasites covered by a thin layer of connective tissue and without an apparent inflammatory reaction surrounding them. Moreover, unspecific gill pathologies were also observed, including hypertrophy of lamellar epithelium with extensive inflammation or granulomatous inflammatory responses.

CONCLUSIONS: These preliminary results show that very few ectoparasites seemed to survive in culture conditions. Endoparasite, and particularly didymozoid, richness of farmed BFT is similar to that of wild BFT (Rodríguez-Marín E et al, 2008, Aquat Living Resour, 21: 365-371). *D. wedli* is the most prevalent, as occurs in BFT from the Adriatic Sea (Mladineo and Tudor, 2004). Interestingly, this species seems not able to cause significant pathological effects, apart from local mechanical disturbances due to its encapsulation. On the opposite, eggs of aporocotylids were found mainly within the gill lamellae, functionally sensitive structures, obstructing gill capillaries, triggering inflammatory response and possibly reducing the respiratory exchange area of the gills. These findings are similar to those described in cultured BFT from Spain (Ruiz de Ybáñez R et al, 2011, Fish Pathol, 46: 87-90).

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Molecular characterization of *Contracaecum rudolphii* (Nematoda: Anisakidae) from *Phalacrocorax carbo sinensis* from Sicily

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AIM: Several studies have demonstrated that the ITS and the mitochondrial *rrn*S gene are valuable genetic markers for the accurate identification of cryptic species and morphospecies within the genus *Contracaecum* (Li A et al, 2005, Parasitol Res, 96: 361-366; D'Amelio S et al, 2007, Parasitology, 134: 1041-1051; D'Amelio S et al, 2012, Syst Parasitol, 81: 1-16). Specimens of *C. rudolphii* Hartwich, 1964 (Nematoda: Anisakidae) from *Phalacrocorax carbo sinensis* (Linnaeus 1758) from Sicily were collected and characterised genetically using PCR-RFLP analysis of the rDNA internal transcribed spacers (ITS-1, 5.8S and ITS-2) and of the small subunit of the mitochondrial rRNA (*rrn*S).

MATERIALS AND METHODS: An individual of *Phalacrocorax carbo sinensis*, the Eurasian subspecies of the Great Cormorant which is usually observed in the winter season also in Sicily, was found and collected from the staff of Wildlife rescue Center of Cattolica Eraclea in province of Agrigento, probably coming from the near Platani river. A total of 92 nematodes, at larval and adult stage, were collected from the stomach at necropsy and analyzed in the present study. Nematodes were repeatedly washed in physiological saline, stored in 70% ethanol and cleared in glycerine for morphological studies on the anterior and posterior ends by light microscopy (morphology of lips and interlabial tips, length of spicule and morphology of the spicule tip) (Abollo et al, 2001, J Helminthol 75: 209-214). A subsample of nematodes (n=30) was characterized using genetic markers defined previously in the internal transcribed spacers (ITS) of nuclear ribosomal DNA and in the small subunit of the mitochondrial ribosomal RNA gene (*rrn*S).

RESULTS: The adult nematodes recovered from *P. carbo sinensis* from Sicily were morphologically identified as *C. rudolphii* (s.l.). The molecular characterization using the PCR-RFLP analysis of the ITS and *rrn*S allowed the identification of the specimens as *C. rudolphii* B. These results have been also confirmed by sequences analysis of representative specimens, BLAST search and alignment with already characterized individuals.

CONCLUSION: Several studies describe *C. rudolphii* Hartwich, 1964 (s.l.) as a common anisakid of fish-eating birds, with a worldwide distribution: these nematodes have been so far reported in the definitive hosts, mainly cormorants, also in Europe and in Italy, where the two cryptic species A and B have been recorded (Mattiucci S et al, 2002, Parassitologia 44: 105; Farjallah S et al, 2008, Parasitol Int, 57(4): 437-440). From an ecological viewpoint, Mattiucci et al (2002 Parassitologia 44: 105) considered *C. rudolphii* A as a species occurring in brackish waters, as is *C. rudolphii* C (D'Amelio S et al, 2007, Parasitology, 134: 1041-1051), in contrast to *C. rudolphii* B which occurs mostly in freshwater habitats. The possible origin of the definitive host from inland waters in Sicily seems to support such hypothesis. The preliminary results reported provide information regarding the species of *Contracaecum rudolphii* complex parasites of cormorants in this area.

Parasites of the grass goby *Zosterisessor ophiocephalus* (Pallas, 1814) from Porto Pino lagoon (Sardinia, Western Mediterranean)

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AIM: The grass goby *Zosterisessor ophiocephalus* (Pallas, 1814) lives in shallow brackish waters of the Mediterranean basin, including the Black Sea and the Sea of Azov, preferring soft-bottom substrates on seagrass meadows (*Zostera* spp.). It is a very appreciated and marketed fish in the Northern Adriatic coast, especially in the Venetian Lagoon. In Sardinia, this species is quite rare but a consistent population lives in Porto Pino lagoon (Southern Sardinia, Western Mediterranean), where it is regularly exploited by the local fishery. Various aspects of the biology, behavior and ecology of *Z. ophiocephalus* are studied in the Adriatic area. This preliminary study is aimed to improve the knowledge about the parasitic fauna of this species, since to date this subject has been mainly treated in the eastern Mediterranean basin than in the central-western one (Kvach Y, 2002, Vestn Zool, 36(3): 71-76; Kvach Y, 2005, Acta Ichthyol Piscat, 35(2): 103-110; Marcer F et al, 2010, Parassitologia, 52(1-2): 355), and no data are reported on populations from Sardinian waters.

MATERIALS AND METHODS: A total of 41 *Z. ophiocephalus* from Porto Pino lagoon (length 22.0-26.0, weight 159-241 g) were provided by local fishermen between February and May 2011. In the laboratory, the samples were examined fresh or after freezing at -20°C. The parasites were detected by dissecting microscope, or by artificial digestion of muscle portions (pepsine + HCl), then examined and identified by morphology under light microscope. Epidemiological indices were calculated according to Bush et al (1997, J Parasitol, 83 (4): 575-583).

RESULTS: All the gobies examined (P = 100%) harboured from 2 to 8 parasite species. Overall, 11 taxa of parasites were observed: one Microsporidia species, one Nematoda, one Acanthocephala, eight Trematoda Digenea. The data are summarized in the Table below.

CONCLUSIONS: All the parasites reported in *Z. ophiocephalus* from P. Pino are generalist species, already observed in other fish from brackish waters of Southern Sardinia (Culurgioni et al, Parassitologia 52(1-2): 350), except the unidentified "diplostomuli" (Digenea, Diplostomidae). On the other hand, it is interesting to point out that the acanthocephalan *T. exiguus* is the only metazoan parasite species shared by the grass gobies object of this study and the specimens living in the Venice lagoon examined by Marcer et al (2010, Parassitologia, 52(1-2): 355). The digeneans *C. longicollis* and *C. labracis* dominated the parasitic fauna, due to the high values of prevalence and intensity. It is also remarkable that the highest prevalence (78.0%) was showed by the zoonotic anisakid *C. rudolphii*. This considerable occurrence of parasitic larval stages (8 off the 10 metazoan species detected) emphasizes the important role of the grass goby in the food web of the lagoon, particularly as prey for the sea bass, *Dicentrarchus labrax* (which is the definitive host for *C. labracis*, *B. labracis*, *B. minimus*, *T. imbutiforme*), and for fish-eating birds (definitive hosts for *C. longicollis*, *C. rudolphii*, and Diplostomidae).

Table 1. Parasites of *Zosterisessor ophiocephalus* from Porto Pino lagoon. Total Prevalence (P%), mean intensity (MI) and intensity range (IR) of infections are reported.

Parasite	Stage	Site of infection	P% - MI	IR
Microsporidia sp.	Spores	intestinal mucosa	9.8	-
Bacciger bacciger (Digenea)	Adult	middle intestine	19.5 - 6.8	1 - 22
Cainocreadium labracis (Digenea)	Metacercaria	muscles, fins	75.6 - 17.10	1 - 125
Bucephalus labracis (Digenea)	Metacercaria	muscles, fins	22.0 - 6.3	1 - 12
B. minimus (Digenea)	Metacercaria	muscles, fins	14.6 - 5.0	1 - 12
Timoniella imbutiforme (Digenea)	Metacercaria	cranial muscles	34.1 - 2.6	1 - 12
Cardiocephalus longicollis (Digenea)	Tetracotyle	brain	75.6 - 27.2	3 - 64
Diplostomidae sp. (Digenea)	Diplostomulum	muscles	4.9 - 1.5	1 - 2
unidentified digenean	Metacercaria	cranial muscles	26.8 - 2.8	1 - 2
Contracaecum rudolphii (Nematoda)	3rd stage Larva	Intestinal serosa	78.0 - 5.9	1- 16
Telosentis exiguus (Acanthocephala)	Adult	posterior intestine	12.2 - 1.8	1 - 5

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Parasitofauna of *Histioteuthis bonnellii* (Férussac, 1835) and *H. reversa* (Verril, 1880) (Cephalopoda: Teuthoidea) from Sardinian waters (western Mediterranean)

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AIM: The umbrella squid *Histioteuthis bonnellii* (Férussac, 1835) and the reverse jewel squid *H. reversa* (Verril, 1880) are the only two species of Histioteuthidae (Cephalopoda: Teuthoidea) living in the Mediterranean Sea (Roper CFE, Jereb P, 2010, Family Histioteuthidae. In Jereb P, Roper CFE, Cephalopods of the world. An annotated and illustrated catalogue of species known to date. Vol 2. Myopsid and Oegopsid Squids. FAO Spec Cat Fish Purp, Rome, 4(2): 223-236; Cuccu D et al, 2007, Biol Mar Medit 14(2): 262-263). These mesopelagic squids are inedible for humans, but they have a key role in the marine food chain both as predators and preys (Hochberg FG, 1983, Mem Nat Mus Vict, 44: 109-145; Xavier J et al, 2007, Arquipélago, Life Mar Sci, 24: 41-48). At the same time they serve as intermediate, paratenic or reservoir hosts in various parasite life cycles (Pascual S et al, 1996, Aquaculture 142: 1-10), including zoonotic species as *Anisakis* spp. This study is aimed to extend the amount of data concerning the parasite fauna of these two Histioteuthidae species in Mediterranean, since to date reports on their parasites are restricted to nematodes (Dollfus RP, 1958, Faune Mar Pyren Orient, 1: 61-72; Culurgioni J et al, 2010, Bull Eur Ass Fish Pathol, 30(6): 220-228).

MATERIALS AND METHODS: Thirty-seven *H. bonnellii* (mantle length 2.1-21.6 cm, weight 5.1-2603 g) and 24 *H. reversa* (m.l. 3.4-12.0 cm, w. 16.8-263.4 g) were caught by trawl fishing in waters surrounding Sardinia at a depth of 550 to 700 m between February 2009 and March 2012. The samples were examined fresh or after freezing at -20°C. The parasites were detected by dissection of the organs under stereomicroscope, and by observation of tissue portions in fresh mounts at light microscope. Their identification was mainly done by morphology. Sequence analysis of the mitochondrial gene cytochrome oxidase II (mtDNA *cox 2*) was carried out on several specimens of *Anisakis* spp. larvae, according to the procedure given in Mattiucci et al (2009, Syst Parasitol, 74: 199-217). Epidemiological indices were calculated according to Bush et al (1997, J Parasitol, 83 (4): 575-583).

RESULTS: As summarized in the Table below, 6 taxa of parasites were detected: three species of anisakid third-stage larvae (Nematoda) in the mantle and outer surface of organs, didymozoid metacercariae (Trematoda Digenea), tetraphyllidean plerocercoids (Cestoda), and different stages of *Aggregata* spp. (Apicomplexa) in the digestive tract. Molecular analysis showed that the *Anisakis* larvae extracted from these squids belonged to the species *Anisakis pegreffii* and *A. physeteris*. The parasitic infections occurred in *H. bonnellii* and in *H. reversa* with total prevalence of 37.8% and 70.8%, respectively.

CONCLUSIONS: Overall, the highest prevalence was observed in both hosts for the protozoans of the genus *Aggregata*. The morphologic and morphometric features of these oocysts, sporocysts and sporozoites differ from those recorded from other cephalopods in Mediterranean and Atlantic (Gestal C et al, Syst Parasitol, 47(3): 203-206), suggesting the possible presence of at least one new species of *Aggregata* in Histioteuthidae from Mediterranean. The most abundant metazoan parasite, particularly in *H. reversa*, was *Lappetascaris* sp. at larval stage, whose presence, until recently, was known only in Pacific waters (Nagasawa K, Moravec F, 2002, J Nat His, 36: 883-891). Differently, *A. physeteris* showed a complete host preference for *H. bonnellii*. These results extend the knowledge on the hosts and distribution of most of the parasites observed in *H. bonnellii* and *H. reversa*, representing also a potential additional tool in the study of biology and ecology of these species.

Table 1. Parasites of *Histioteuthis bonnellii* and *H. reversa* from Sardinian Channel. Prevalence% and mean intensity of infections are reported.

host	parasite					
	A. pegreffii	*A. physeteris*	*Lappetascaris* sp.	Didymozoidae metacercariae	Tetraphyllidea larvae	*Aggregata* spp.
H. bonnellii	2.7% - 1.0	13.5% - 1.6	16.2% - 4.5	2.7% - 2.0	2.7% - 4.0	29.7%
H. reversa	4.2% - 1.0	0.0%	41.7% - 13.2	8.3% - 2.5	8.3% - 1.0	66.7%

Cryptosporidium sp. and *Giardia* sp. in edible bivalves: results of a one-year monitoring on three sites in Sardinia

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Protozoan parasites of the genera *Cryptosporidium* and *Giardia* are worldwide responsible for enteritis in several animal species. Some of them are etiological agents of zoonotic infections, that may be severe, particularly in immunocompromised patients (Hunter PR, Nichols G, 2002, Clin Microbiol Rev 15: 145-154). The transmission of infective stages of these parasites, i.e. oocysts of *Cryptosporidium* sp. and cysts of *Giardia* sp., occurs mainly by fecal contamination of food/water. These resistant forms have long time of survival in the aquatic environment, and they can be accumulated by organisms such as bivalves, which feed by filtering large volumes of water. It has been shown that in these organisms oocysts and cysts are retained a longer time than that of treatment needed to remove other contaminants, such as fecal bacteria (Tamburrini A, Pozio E, 1999, Int J Parasitol 29: 711-715).

AIM: The aim of this work is to evaluate for the first time the presence of oocysts of *Cryptosporidium* sp. and cysts of *Giardia* sp. in edible bivalves from three farming localities of the western and northeastern coasts of Sardinia (western Mediterranean Sea).

MATERIALS AND METHODS: From April 2011 to February 2012, 72 samples of *Mytilus galloprovincialis* from the Gulf of Olbia (51 samples) and Arborea (21), 16 of *Crassostrea gigas* and 1 of *Tapes decussatus* from the Stagno of San Teodoro (OT) were collected. Depending on the size of specimens, each sample was composed of a pool of 12-15 specimens of mussels or clams, and 4-5 oysters. The organs examined were the hepatopancreas and the gills (including labial palps). Samples were homogenised in 0.04 M phosphate-buffered saline (PBS), sieved through a 40 m mesh, suspended in PBS/diethyl ether (2:1) and concentrated by centrifugation (3x5 min at 1000 g). To search oocysts/cysts three different diagnostic techniques were used: 1) staining, Ziehl-Neelsen modified by Angus KW (1987, In Pract, 9: 47-49) and Auramine O for *Cryptosporidium* sp., Kohn's Chlorazol Black and Lugol's iodine for *Giardia* sp.; 2) direct immunofluorescence (IF) test Merifluor® for *Cryptosporidium*/*Giardia* (Meridian Diagnostics, Inc., Cincinnati, Ohio); 3) molecular analysis, genomic DNA was extracted from all samples and then the GDH target gene for *Giardia* and the 18S rRNA for *Cryptosporidium* species were amplified by PCR.

RESULTS: After the application of the three diagnostic techniques, none of the 89 samples examined was positive for oocysts of *Cryptosporidium* sp. and/or cysts of *Giardia* sp. For each session, positive controls of oocysts and cysts confirmed the proper execution of the procedures: staining, IF and PCR.

CONCLUSIONS: The results of this one-year study represent the first data from Sardinia, and consistent to their preliminary character they allow to estimate as very low the zoonotic risk related to *Cryptosporidium* sp. and *Giardia* sp. in commercial bivalves, especially *M. galloprovincialis* and *C. gigas*. This fact also gives an optimistic view in terms of fecal contamination (of both human and livestock origin) in the investigated areas, especially if compared with previous studies conducted on bivalves from the Italian peninsular coasts (Giangaspero A et al, 2004, Parassitologia, 46, 153; Berrilli F et al, 2008, System Copy s.a.s., Ozzano Emilia).

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Geographic and host-dependent morphological variability of representatives of the genus *Ligophorus* Euzet et Suriano, 1977 (Platyhelminthes: Monogenea)

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The genus *Ligophorus* Euzet et Suriano, 1977 comprises 41 species, whose differentiation is based on the morphometry of the hard parts of haptor, male copulatory organ (MCO) and vagina. So far, the intraspecific variability of these characters has not been studied enough and only data from the Mediterranean Sea have been early analysed.

AIM: In the present study the geographic and host-dependent variability of representatives of the genus *Ligophorus* spp. from the Mediterranean and Black seas is analysed.

MATERIALS AND METHODS: Four species of *Ligophorus*: *L. vanbenedenii*, 130 specimens *ex Liza aurata* from two regions of the Black Sea (44°28'N, 33°31'E and 45°17'N, 36°29'E), 17 spm *ex L. aurata* from the Mediterranean Sea (39°54'N, 8°28'E) and 18 spm *ex Oedalechilus labeo* from the Adriatic Sea (42°8'N, 18°52'E); *L. szidati*, 130 spm *ex L. aurata* from the Black Sea (44°28'N, 33°31'E and 45°17'N, 36°29'E), 15 spm *ex L. aurata* from the Mediterranean Sea (39°54'N, 8°28'E) and 15 spm *ex L. saliens* from the Black Sea (44°28'N, 33°31'E); *L. cephali*, 25 spm *ex Mugil cephalus* from two regions of the Black Sea (46°8'N, 30°41'E and 44°28'N, 33°31'E) and 12 spm from the Mediterranean Sea (39°15'N, 0°7'W); *L. mediterraneus*, 15 spm *ex M. cephalus* from the Black Sea (44°28'N, 33°31'E) and 14 spm from the Mediterranean Sea (39°54'N, 8°28'E), were measured according to Dmitrieva E et al (2007, Syst Parasitol, 67: 51-64). A total of 35 characters relative to attachment structures, MCO and vagina were included. Data analysis was carried out using independent t-test, coefficient of variation (CV), calculated as a percentage of the standard deviation of the mean, and Principal Component Analysis (PCA).

RESULTS: The comparison of the four species of *Ligophorus* from the type host species from the Mediterranean and Black seas revealed significant differences in: 22 characters for *L. vanbenedenii*, 26 for *L. szidati*, 18 for *L. cephali* and 11 for *L. mediterraneus*. Among them, the length of vagina differed in all species. However, a general regularity in their variability was not found, and only *L. vanbenedenii* and *L. szidati* from the Black Sea had larger dimensions of all significantly different characters of attachment structures. Moreover, the data on *L. vanbenedenii* and *L. szidati ex Liza aurata* and on *L. cephali ex Mugil cephalus* were analysed from two different regions of the Black Sea, and also between these groups significant differences were found: in 12 and 14 characters for the first two species, respectively, and in total length of both anchors and width of the two bars for *L. cephali*. Comparison of specimens of *L. vanbenedenii* from two host species (*L. aurata* and *O. labeo*) from the Mediterranean region and of specimens of *L. szidati* also from two host species (*L. aurata* and *L. saliens*) from the Black Sea showed that specimens from the type host (i.e. *L. aurata*) have significantly smaller haptoral structures (differing in 24 characters in the former and in 20 in the latter). The analysis of the total variability of the 35 investigated characters was carried out for each species including all specimens, i.e. collected in different regions, seasons and from different hosts. Despite the geographic and host-dependent variability, the CV of most of the analysed characters are consistently low (<10%) in each of the four examined species, except lengths of roots and of base of anchors. Moreover, the 394 measured specimens were clearly divided into four groups (according to each species) on the PCA plots based on their scores in the first plane of the Principal Component Analysis (PCA) (explaining 68 % of the overall variance), ran on metrical data for the 18 more stable characters relative to haptor and lengths of the accessory piece and of the tube of MCO.

CONCLUSIONS: Geographic and host-dependent variability were shown for investigated species. However, the characters proposed for the differentiation of *Ligophorus* spp. (Dmitrieva et al, 2007) are rather stable and good to discriminate representatives of this genus.

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A survey aimed at mapping the "*Anisakis* risk" in anchovies (*Engraulis encrasicolus*) and sardines (*Sardina pilchardus*) caught off the Ligurian and north-western Adriatic coasts

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AIM: According to EFSA Scientific Opinion on Risk Assessment of Parasites in Fishery Products (2010), all wild fish should be considered at risk of containing any viable zoonotic parasites if these products are to be eaten raw or almost raw, pointing out the need to carry out epidemiological surveys on presence/diffusion of zoonotic parasites in all fishery grounds. Therefore surveys aimed at mapping the presence of zoonotic anisakids, i.e. *Anisakis* spp., in commercially important fish are strongly encouraged. Since most of the cases of human Anisakiasis reported till now in Italy have been referred mainly to consumption of marinated/pickled anchovies and/or sardines, a quali-quantitative parasitological survey has been carried out on these two species from different Italian fishing areas.

MATERIALS AND METHODS: From October 2010 to February 2012 a total of 3808 anchovies (*Engraulis encrasicolus*) and 2636 sardines (*Sardina pilchardus*) caught off the Ligurian and north-western Adriatic coasts were examined for the presence of anisakid larvae (see Table 1). Fishing areas were identified by geographic coordinates. All the anisakids recovered during the survey were fixed in 70% ethanol, cleared and identified at genus level by light microscope. A representative pool of larvae was subjected to molecular identification by PCR-RFLP of ITS rRNA using restriction enzymes *Hinf*I e *Hae*III. Data on localization, viability and number of anisakid larvae were recorded. Prevalence and mean intensity MI) were calculated as suggested by Bush AO et al. (1997, J Parasitol, 83: 575-583).

RESULTS: Anisakid larvae have been detected in 791 (20.8%) anchovies and 531 (20.1%) sardines. Among anisakids, *Anisakis* sp. larvae were detected in 111 (2.9%) anchovies and 5 (0.2%) sardines, while *Hysterothylacium* sp. larvae, considered nonpathogenic to humans, were found in 703 (18.5%) anchovies and 526 (20%) sardines. Striking differences in prevalence values were observed among fishing areas and fish species, as detailed in Table 1. Sardines showed very low infection rates by *Anisakis* larvae (0-0.3%) from all fishing areas, while anchovies from Ligurian sea showed *Anisakis* prevalence values higher than those from northern Adriatic sea (0.9-9.8 *vs*. 0-0.8%). *Hysterothylacium* larvae were found in all the batches examined, with prevalence ranging from 2.3 to 46.3%. MI of *Anisakis* larvae was generally around 1, except for anchovies from Imperia (2.5), and MI of *Hysterothylacium* between 1 and 2.9, with highest values in Adriatic fish. Larvae were always viable and located in body cavity. Molecular

Table 1. Fishing areas, fish species (A: anchovy; S: sardine), No. and % of fish positive for anisakid larvae/examined and No. and % of fish positive for Anisakis sp. and *Hysterothylacium* sp. larvae.

Fishing area	Imperia IM	Savona SV		Arenzano GE	La Spezia SP		Piombino LI		Caorle VE		Cesenatico FC		Rimini RN	
Fish Species	A	A	S	A	A	S	A	S	A	S	A	S	A	S
No. positive/examined	89/441	39/450	13/520	34/164	192/715	18/212	36/342	15/313	235/507	210/550	104/819	172/681	62/370	103/360
%	20.2%	8.7%	2.5%	20.7%	26.9%	8.5%	10.5%	4.8%	46.3%	38.2%	12.7%	25.3%	16,7%	28.6%
No. positive *Anisakis*	19	39/450	1	16	46	0	21	1	0	0	0	2	3	1
%	4.3%	8.7%	0.2%	9.8%	6.4%		6.1%	0.3%				0.3%	0.8%	0.3%
No. positive *Hysterothylacium*	70	35	12	18	165	18	17	14	235	210	104	170	59	102
%	15.9%	7.8%	2.3%	11%	23.1%	8.5%	5%	4.5%	46.3%	38.2%	12.7%	25%	15.9%	28.3%

analyses allowed to identify all the *Anisakis* larvae as owing to the species *A. pegreffii*, except for a specimen identified as an *A. pegreffii*/*A. simplex* hybrid, and all the *Hysterothylacium* larvae as *H. aduncum*.

CONCLUSIONS: The results of this survey showed a very low *Anisakis* risk in anchovies caught off north-western Adriatic coast and in sardines from all the fishing areas under study. However, it should be stressed that prevalence values observed in anchovies from Ligurian sea (0.9-9.8%) were strongly lower than those reported by Rello FJ et al. (2009, Int J Food Microbiol, 129: 277-281) who found *Anisakis* sp. larvae in 21.88% of anchovies from Ligurian sea, but examining just 64 fish "landed at La Spezia and Piombino". Influence of fish population structuring and dynamics, host ecological/trophic attitude and hydrogeographical factors are discussed in relation to the parasitological findings.

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Survey of parasite fauna in *Rana* kl. *esculenta* in Ravenna Province: preliminary results

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AIM: In literature there are only few record about parasites infecting amphibians in Italy (Galli P et al, 2001, Parassitologia, 43: 147-149). In the latest year, the interest about these animals are increasing, because of the diffusion of *Batrachochytrium dendrobatidis* (Ficetola GF et al, 2011, Acta Herpetol, 6: 297-302), but other parasites are scarcely considered. The aim of this note is to contribute to the knowledge on the parasite of *Rana* kl. *esculenta* in Italy.

MATERIALS AND METHODS: In the framework of a project carried out in collaboration with the provincial authorities on the assessment of the status of Anura population in Ravenna province (Italy) twenty-three specimen of *R. esculenta* (12 male and 11 female) apparently healthy, were captured in the Regional Park of "Vena del gesso romagnola" (44°15'2"N and 11°40'55"E), having a special permission from the Ravenna Province and from the Park. All frogs were measured and subjected to parasitological examination. Microscopic observation were carried out on fresh and stained skin scraping, blood smear and fresh mount of intestine scraping. Moreover the intestinal tract and the muscle were dissected looking for helminths, that were fixed in 70% ethanol, clarified with Amman lactophenol and observed under light microscope for morphological identification.

RESULTS: No ectoparasites and muscle metacercariae were found. Sixteen blood smears out of 23 (69.6%) were positive for one or more parasite: in 13 smears, intraerythrocytic inclusions, containing Rickettsiae referable to *Aegyptianella* spp. were found; other inclusions referable to meronts of *Dactylosoma* sp. were present in 5 smears and extracellular *Trypanosoma* sp. in 4. Microfilariae were observed in one sample. In particular in one large sized female frog, *Aegyptianella sp.*, *Dactylosoma* sp., *Trypanosoma* sp, and microfilariae were present in the same individual host. Microscopic observation of the intestine scraping smears showed the presence of larvae of Nematodae in 7 specimens and Trichodinae in 3. In 15 specimens (65.2%), adult nematodes were found in the intestinal tract: in 13 frog the presence of nematode Cosmocercidae (presumably *Cosmocerca ornata* Dujardin, 1845) was registered. The parasite density ranged between 1 and 7 (mean intensity 3.3; mean abundance 1.8). In one frog also 3 small nematoda were observed together with *C. ornata*. In one specimen, a male referable to *Oswaldocruzia filiformis* was detected.

CONCLUSIONS: In our study an unexpected wide variety of parasites were observed in the blood of the frogs examined, especially in one of the largest specimen, probably related to the longer exposure to vectors, according to Barta JR, Desser SS (1984, J Wildlife Dis, 20: 180-189). To our knowledge no other survey has been carried out in amphibian haemoparasites in Italy. *Dactylosoma* spp. is widely distributed in a variety of hosts and the well-known species *D. ranarum* is probably a species complex (Manwell RD, 1964, J Protozool, 14: 726-731). The identification at species level of this genus cannot be based only on the morphology but a molecular approach is needed (Barta JR 1991, Adv Parasit, 30: 1-37). The microfilariae observed in one specimen could be referred to the species *Foleyella*, a relatively common filarial worm of amphibians (Barta JR and Desser SS, 1984, l.c.) or to *Icosiella neglecta* common in European frogs (Desportes, C, 1941, Ann Paras Hum Comp 18: 46-66). *Aegyptianella* sp. (probably *Aegyptianella bacterifera*) was found in 56,3% of the specimens; this value is similar to the one (68%) obtained by Barta JR et al (1989, Trans Am Micr Soc, 108: 6-20) in *Rana esculenta* from Corsica. With regard to the intestinal parasites, the species *Cosmocerca ornata* was the more frequent nematode found. The morphological characters of this genus can vary within the species in different hosts and could be confused with *Neyraplectana schneideri* Travassos, 1931 if only female are present, as in our case (Grabda-Kazubska B, 1986, Acta Par Pol, XXXI: 7-23). Both these species have been already described in Italy in *Bufo bufo* (Galli P et al, 2001, *l.c.*).

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A survey on plerocercosis by *Diphyllobothrium latum* (Cestoda: Pseudophyllidea) in Northern Italy

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AIM: Diphyllobothriasis is a parasitic fish-borne zoonosis desrcibed in Italian sub-alpine lake districts due to the consumption of raw, cold-smoked or undercooked freshwater fish parasitized by the plerocercoid larvae of the pseudophyllidean cestode *Diphyllobothrium latum*. In recent years there has been documented a recrudescence of cases of human Diphyllobothriasis in Switzerland, France and Italy, indicating the persistence of this parasite in fish populations from lake environments. In order to update the epidemiological data on *D. latum* plerocercosis in fish populations from Italian sub-alpine lakes, a parasitological survey has been carried out on perch (*Perca fluviatilis*), pike (*Esox lucius*), burbot (*Lota lota*), whitefish (*Coregonus lavaretus*) and shad (*Alosa fallax lacustris*) from Como, Iseo, Maggiore, Garda, Monate and Comabbio Lakes.

MATERIALS AND METHODS: From July 2008 to June 2011 a total of 1,606 fish were examined: 684 from Como Lake (509 perch, 13 pike, 29 burbot, 96 whitefish and 37 shad), 319 from Iseo Lake (271 perch, 2 pike, 5 burbot, 7 whitefish and 34 shad), 306 from Lake Maggiore (195 perch, 24 burbot, 64 whitefish and 23 shad), 212 from Garda (185 perch, 5 pike, 2 burbot, 20 whitefish), 57 perch from Comabbio Lake and 28 whitefish from Monate Lake. All the fish were weighed, measured and subjected to parasitological examination by visual inspection of fillets (by naked eye and candling) and of visceral organs. The parasites referable to cestode plerocercoid larvae were isolated and fixed in 70% ethanol. Identification was carried out at genus level by morphological study on the basis of the key proposed by Andersen KI, Gibson DI (1989, Syst Parasitol, 13: 6-9), then at species level by molecular analysis. The primers used were 82F (CAG TAG TCA 5.-TAT GCT TGT CTC AG-3) and 81R (TTC ACC TAC 5.-GGA AAC CTT GTT ACG-3), amplifying a fragment of 2500 bp of the 18S rDNA. The positive samples were sequenced and then compared with those deposited in GenBank using BLAST software. Prevalence (P) and Mean Intensity (MI) values were calculated as suggested by Bush AO et al (1997, J Parasitol, 83: 575-583).

RESULTS: Plerocercoid larvae were found from Maggiore, Como and Iseo Lakes in perch, pike and burbot (see Table 1), in both fillets and visceral serosa. All the fish examined from the two small lakes of Monate and Comabbio as well as from Lake of Garda were negative for the presence of *Diphyllobotrhium* spp. plerocercoids. All the plerocercoid larvae found were identified as *Diphyllobothrium latum*.

	Maggiore Lake		Como Lake		Iseo Lake	
	p/e (P)*	MI	p/e (P)*	MI	p/e (P)*	MI
P. fluviatilis	7/195 (3.59%)	1	153/509 (30.06%)	1.6	46/271 (16.97%)	1.3
E. lucius	-	-	6/13 (46.15%)	13.3	1/2 (50%)	4
L. lota	0/24	-	5/29 (17.24%)	9.4	0/5-	-

*p/e (P)= No. of fish positive/No. of fish examined (Prevalence)

CONCLUSIONS: The results of this survey indicate a widespread presence of *D. latum* plerocercoids in perch populations of Lake Como and, to a lesser extent, in perch from Iseo and Maggiore Lakes, confirming this species as elective second intermediate host of *D. latum* in Italy. The occurrence of *D. latum* plerocercoid larvae in the lateral muscle of pike and burbot caught in Lake Como is an important finding since these two paratenic hosts usually show infections by *Diphyllobothrium* larvae only at the level of visceral serosa. This factor may greatly increase the risk level in consuming these two fish species as undercooked dishes. Furthermore it should be emphasized that all the examined whitefish and shad, among the most traded fish species on the local market and often consumed smoked and/or marinated, were negative and then could be considered safe with regard to *D. latum* transmission.

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Red fox (*Vulpes vulpes*) as wild reservoir of *Opisthorchis felineus* (Digenea: Opisthorchiidae) in Italy

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AIM: Opisthorchiasis by *Opisthorchis felineus* (Digenea: Opisthorchiidae) is gaining an increasing importance in Italy for the recent occurrence of several human cases due to the consumption of raw or undercooked/marinated tench (*Tinca tinca*), so far known as the more suitable second intermediate host of the parasite in Italian country. The "classic" life cycle involves the domestic cat (*Felis catus*) as definitive host assuming the infective stage (metacercaria) of *O. felineus* by the consumption of infected fish remains, a freshwater snail belonging to *Bithynia* genus as first intermediate host and cyprinid fish as second intermediate host. Man is a suitable definitive host, such as dog and other fish eating mammals. Recent surveys (Crotti D et al, 2007, G It Microbiol Med Odont Clin, 11: 20-23; De Liberato C et al, 2010, Vet Parasitol, 177: 67-71) have shown, in endemic areas of Central Italy, *O. felineus* eggs prevalence values in cat feces of 31.8%, 36.6% and 73.3% around Trasimeno, Bolsena and Bracciano Lakes respectively.
Although in European and former U.S.S.R. countries the occurrence of a life cycle of *O. felineus* linked to wild environment is reported (World Health Organization, 1995, Control of Foodborne Trematode Infection. WHO Technical Report Series: 125-126; , Parasitol Res, 85: 142-146; Adams AM, 2006, in: Foodborne parasites, Ortega Ed, Springer Science, New York, USA), in Italy only two cases of opisthorchiasis in European polecat *Mustela putorius* in Pisa province, Tuscany, have been reported (Macchioni G, 1963, Ann Fac Med Vet Pisa, 16: 238-247), in the same area where *O. felineus* was reported for the first time by Rivolta in XIX century in cats and dogs (Rivolta S, 1884, G Anat Fisiol Patol Anim, 16: 20-28). With the aim of defining the possible role of wild mammals in *O. felineus* epidemiology in Italy, a parasitological survey has been undertaken on fecal samples collected from foxes in Tuscany.

MATERIALS AND METHODS: Five fecal fox samples collected during 2007 in the framework of a research project on epidemiology of Trichinellosis in red foxes of Tuscany (Magi M et al, 2008, Hystrix, 19: 31-38) in Cascina and Bientina areas in Pisa province and stored in 10% buffered formalin were subjected to coprological examination by sedimentation and flotation with zinc sulphate $ZnSO_4$ (s.g. 1.350), searching for Opisthorchiid eggs. Microscopically positive fecal samples were subjected to DNA extraction with QIAamp DNA Stool Mini Kit (Qiagen) and to nested PCR, amplifying the ITS rRNA (Luton K et al, 1992, Mol Biochem Parasitol, 56: 323-328). The PCR product was sequenced in both direction by ABI 3730 DNA Analyzer at StarSEQ GmbH (Mainz, Germany).

RESULTS: One out of five fecal fox samples was positive for the presence of digenean eggs, showing measures and morphological features consistent with eggs of Opistorchiidae trematodes. The sequence obtained showed 100% identity with *O. felineus*.

CONCLUSIONS: The possible role played by red fox as wild reservoir of Opisthorchiasis in Italy needs further investigations, already undertaken by our research groups in different Italian regions. The presence of a "wild" life cycle in addition to the "classic" one linked to the cat as main reservoir could complicate the epidemiology and, as a consequence, the control of this parasitic zoonosis in Italy.

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Parasitofauna of loggerhead sea turtles (*Caretta caretta*) stranded in Northern Adriatic Sea

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There are significant helminth community dissimilarities between loggerhead sea turtles, *Caretta caretta*, collected from different localities of the Mediterranean Sea, the overall pattern being compatible with the hypothesis that parasite communities reflect the ontogenetic shift that juvenile loggerheads undergo from oceanic to neritic habitats (Santoro M et al, 2010, Parasitol Int, 59: 367-375).

AIM: The aim of this report was to check the parasitofauna of *C. caretta* specimens stranded along the Northern Adriatic coastlines; this is part of a more extensive research to detect the causes of death and to collect biological and anatomo-pathological data about loggerhead sea turtles stranded in this area.

MATERIALS AND METHODS: From June 2009 to September 2011, thirty five loggerhead sea turtles stranded along Northern Italian Adriatic coast were necropsied at the Faculty of Veterinary Medicine of Padova. Parasitological survey was carried out on 20 (57.14%) animals, due to condition of the carcasses. The collected parasites were stored in 70% alcohol until identification. Smears of faeces were stained with the modified Ziehl Neelsen technique. To confirm the *Cryptosporidium* infection, positive faecal samples were processed by PCR (Polimerase Chain Reaction) technique, using primers RLB R2 -5'-CTAAGAATTTCACCTCTGACAGT-3' and RLB F2 5'-GACACAGGGAGGTAGTGACAAG-3' (Centero-Lima S et al, 2003, Trop Med Int Health, 8: 760-764), and the amplified PCR products subsequently sequenced. Tissue samples of the major organs were also fixed in 10% buffered neutral formalin, paraffin-embedded, cut and routinely stained with hematoxylin and eosin (EE) and PAS for microscopic examination.

RESULTS: The parasitological survey has permitted to find on the skin and carapace the presence of epibionts barnacles (Cirripedia, Thoracica) (90%) and *Ozobranchus margoi* (Hirudinea) (10%). Five species of endoparasite Digenea helminths *Rhytidodes gelatinosus* (30%), *Orchidasma amphiorchis* (25%), *Plesiochorus cymbiformis* (25%), *Pleurogonius trigonocephalus* (16%), *Enodiotrema* sp. (10%) and larvae and adults of the Nematoda *Sulcascaris sulcata* (10%) have been found.

The presence of spirorchiid trematodes eggs was an occasional finding in histological sections of lungs, spleen and pancreas of one animal and they were associated with multifocal granulomatous inflammation of tissues; neither gross lesions nor adult worms were observed. Mycotic pneumonitis was diagnosed in 5 loggerheads; these were characterized by multifocal granulomas, with multinucleate giant cells, and by the presence of septate hyphae, probably belonging to *Fusarium* or *Aspergillus* spp. *Cryptosporidium* infection has been detected in one loggerhead sea turtle.

CONCLUSIONS: Overall the results confirm data reported by other Authors in regards to the parasitofauna of the loggerhead sea turtles stranded along Italian coastlines (Piccolo G, Manfredi MT, 2001, First Mediterranean Conference on Marine Turtles, Rome; Scaravelli D et al, 2005, XII Convegno Nazionale S.I.P.I., Cesenatico, FC). Further studies could be useful to assess the prevalence and associated pathological lesions caused by Spirorchiids in sea turtles of the Mediterranean Sea, since these trematodes are implicated as an important cause of stranding and mortality in sea turtles in other parts of the world (Stacy BA et al, 2010, Dis Aquatic Organ, 89: 237-259). *Cryptosporidium* infections have been reported in various species of chelonians; in sea turtles this protozoan has been described only in *Chelonia mydas* from Hawaiian islands (Graczyk TK et al, 1997, Appl Environ Microbiol, 63: 2925-2927).

Genetic identification and diagnostic morphological characters among the three cryptic species of the *Anisakis simplex* complex (Nematoda: Anisakidae)

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AIM: Three cryptic species (i.e. *A. simplex sensu stricto*, *A. pegreffii* and *A. simplex* C) (Nascetti G et al, 1986, Int J Parasitol, 16: 633-640; Mattiucci et al, 1997, J Parasitol, 83: 401-416) were formerly included within the *A. simplex* (Rudolphi, 1809) *sensu lato* complex. The aim of this study was to identify by means of nuclear and mitochondrial markers adult specimens of the three cryptic species so far included in the *A. simplex* complex in order to investigate the morphological and morphometric characters of these species, to provide a differential morphological analysis, and support with morphological evidences the results obtained with genetic/molecular methods.

MATERIALS AND METHODS: Several adult specimens of *Anisakis* spp. collected from stranded cetaceans (*Globicephala melaena, Stenella coeruleoalba, Balaenoptera acutorostrata*), of the South Pacific Ocean, North-East Atlantic Ocean, and Mediterranean Sea, were genetically identified, based on allozymes and sequences analysis at mtDNA *cox-2*. Phylogenetic analysis of the obtained sequences was performed by Maximum Parsimony using PAUP* (Swofford D, 2003, Sinauer Associates). Morphological and morphometric analyses were carried out on the same adult specimens previously identified genetically. Log-transformed data sets obtained at several morphological traits were elaborated using the library Principal Component Analysis (PCA)-Methods by the software R (R Dev Core Team, 2010, R Found Stat Computing).

RESULTS: Genetic/molecular markers allowed the identification of the nematodes studied. Mixed infection by *A. pegreffii* and *A. simplex* C was found in three individuals of *G. melaena* from South Pacific Ocean; no F1 hybrids have been found, confirming the reproductive isolation of the two cryptic species. Specimens of *B. acutorostrata* were identified as belonging to the species *A. simplex s.s.*, while those collected from individuals of *S. coeruleoalba* of the Mediterranean Sea were found to belong to the species *A. pegreffii*. PCA analysis showed a significant different distribution pattern of the morphometric characters in the three species.

CONCLUSIONS: Genetic analysis confirm the reproductive isolation between the sibling species in sympatric condition (i.e. *A. pegreffii* and *A. simplex* C in *G. melaena*)Some morphological characters of diagnostic value between *A. pegreffii* and *A. simplex s.s.* were previously suggested (Quiazon KM et al, 2009, Parasitol Int, 57: 483-489). New diagnostic have been here proposed between the two taxa. This is the first detection of diagnostic structural differences to distinguish the three species of the *A. simplex* complex. Similarly, the discovery of diagnostic morphological characters accompanied the molecular detection of other species of the genus *Anisakis* (i.e. *A. nascettii* vs *A. ziphidarum; A. paggiae* vs *A. brevispiculata* and *A. physeteris)* (Mattiucci S et al, 2005 Syst Parasitol, 61: 157-171; 2009 Syst Parasitol, 75: 199-217).

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Genetic diversity of the two sibling species, *Contracaecum osculatum* D and C. *osculatum* E (Nematoda: Anisakidae) and their parasitic infection levels in fish from the Ross Sea (Antarctica): an indicator of temporal stability of the marine food-web?

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AIM: Two sibling species of the anisakid nematodes of the *Contracaecum osculatum* species complex were detected genetically in the Antarctic area; they are *C. osculatum* D and *C. osculatum* E (Orecchia et al, 1994, Int J Parasitol, 24: 367-377; Mattiucci & Nascetti, 2008, Adv Parasitol, 66: 47-148). It has been suggested that the genetic diversity and parasitic infection levels of anisakid nematodes from different hosts of the Antarctic area, could be used as an indicator of ecosystem food-web stability (Mattiucci & Nascetti, 2007, Vet Parasitol, 148: 43-57). The scope of the present study has been the study of the genetic diversity of the anisakid nematodes *C. osculatum* D and *C. osculatum* E and their infestation levels in fish species of the Ross Sea (Antartica), at the temporal scale level.

MATERIALS AND METHODS: A parasitological survey has been carried out on a total of 107 fish specimens belonging to different species of the Families Channichthydae (*Chionodraco hamatus)* Bathydraconidae (*Gymnodraco mawsoni*) and Notothenidae (*Trematomus bernacchii, T. hansoni* and *T. newnesi*) fished during the XXVII Italian Expedition to Antarctica (2011-2012). The identification of the collected larvae has been carried out by means of allozymes according to the procedures reported by Mattiucci et al, 2008 (Parasite, 15: 408-419). The genetic diversity has been estimated at both nuclear (allozymes) and mitochondrial (sequences analysis of the mtDNA *cox2*) level, according to the procedures given in Mattiucci et al, 2008 (cit.ref.). Various appropriate programs have been used for the estimation of genetic diversity values and the parasitic infection levels. A comparative analysis of the present data sets concerning the genetic diversity estimates and parasitic infection levels has been performed with respect to the Antarctic populations of the same anisakid species previously collected from the same fish species during the Italian Expedition to Antarctica on 1994.

RESULTS: Allozymes markers and mtDNA *cox2* sequences analysis allowed to identify larval stages of the two sympatric species, *C. osculatum* D and *C. osculatum* E. Relative frequencies so far obtained of the two species in the different fish species indicate that they have a differential distribution in benthic and mesopelagic fish hosts. A substantial congruence of the parasitic infection estimates in the fish hosts by the present (years 2011-2012) and previously collected populations (year 1994) belonging to the two species has been so far observed. Preliminary estimates at allozymes level suggest a similar level of polymorphism observed in the present populations as those previously reported of the two taxa previously analyzed. Similarly, at mitochondrial level, a high level of nucleotide and haplotype diversity has been so far observed in the two Antarctic anisakid species.

CONCLUSIONS: The possible use of the combination of genetic diversity estimates and parasitic infection levels of these nematodes as a possible indicator to monitor, at temporal and spatial scale level, the stability of Antarctic food webs, is discussed.

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Metazoan gills parasites of *Auxis rochei* (Osteichthyes: Scombridae) from the western Mediterranean Sea

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The bullet tuna *Auxis rochei* (Risso, 1810) is a small tuna widely distributed in tropical and subtropical waters. It is one of the most abundant tuna in the Mediterranean Sea, where it is exploited by artisanal fisheries. Little is known about its biology and migrations in this area (Macías D et al, 2006, Collect Vol Sci Pap ICCAT, 59: 571-578). Currently it is accepted the existence of two species within the genus *Auxis*: *Auxis rochei* (BLT) and the frigate tuna *Auxis thazard* (Lacepède, 1800) (FRI), both worldwide distributed including the Mediterranean Sea (Collette BB, Aadland CR, 1996, Fish Bull, 94: 423–441). However, recent genetic and morphometric studies on the Mediterranean BLT populations seem to support the old idea of ichthyologist about the existence of only one species of *Auxis* in the Mediterranean Sea and adjacent areas of the Atlantic Ocean (Orsi Relini L et al, 2009, Collect Vol Sci Pap ICCAT, 64: 2200-2210), and it could re-open the debate on the taxonomy of this genus.

AIM: To study the metazoan gill parasites of BLT and to evaluate their possible use as biological tags to improve the knowledge of the host biology and ecology.

METHODS: The gills of 63 specimens of BLT (33-43 cm fork length) were analysed for metazoan parasites. Fish were caught in traditional trap fishery (*almadraba*) of La Azohía (Spain, western Mediterranean Sea), in May 2008 and 2011. Gills were excised, stored individually in plastic bags and frozen at -20° C. Thawed gills were examined by naked eye and under a stereomicroscope for metazoan parasites. The location of the parasites was recorded according to Mele S et al (2012, Dis Aquat Org, 97: 219-225).

RESULTS: Five parasite species were found on the gills of BLT: three polyopisthocotylean monogeneans (*Allopseudaxine macrova*, prevalence=17%; *Churavera triangula*, 6%; and *Hexostoma auxisi*, 6%), one didymozoid trematod (*Didymozoon auxis*, 59%), and one caligid copepod (*Caligus bonito*, 13%). Moreover 52% of fish harboured post larval stages of unidentified didymozoids. *D. auxis* was the dominant species. Platyhelminthes showed high site specificity: *D. auxis* was found encapsulated in the outer margins of the gill filaments, and the monogeneans were mainly located between the gill filaments (i.e., basal and central part of them); while *C. bonito* infected both arches and gill filaments.

CONCLUSIONS: The records of *A. macrova* and *C. triangula* are new for the Mediterranean Sea. In spite of the commercial interest of BLT, no parasitological data are available from the Atlantic Ocean, while *D. auxis* and *H. auxisi* have been previously reported in *Auxis* spp. from the western Mediterranean Sea (Dollfus RP, 1926, Ann Parasit Hum Comp, 4: 148-161; Palombi A, 1949, Arch Zool Ital, 34: 204-408). It has to be stressed that all the parasite species found in the Mediterranean BLT have also been reported in the congener FRI from the Atlantic and Pacific Oceans (Mogrovejo C et al, 2004, Rev Bras Zool, 21: 201-206 and references therein). These results point out the need to deepen the knowledge on the taxonomy and parasitofauna of the genus *Auxis* worldwide, in order to contribute to the understanding of the population spatial dynamics of both host and its parasites.

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Epidemiology and molecular identification of larval *Anisakis* spp. in commercial fish caught off northern Sardinia (western Mediterranean Sea): an update

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AIM: Nematodes of the genus *Anisakis* have a global distribution among a wide variety of marine fish and cephalopods that serve as paratenic hosts. Their presence in fishery products is a hazard to human health and causes economic losses. The aim of this study is to investigate the presence of *Anisakis* spp. larvae and to identify them at the species level fromcommercial fish caught off northern Sardinia.

MATERIALS AND METHODS: Between 2008 and 2009 a total of 777 specimens of 10 commercial fish species (Table) were collected from the Gulf of Asinara (northern Sardinia, western Mediterranean Sea) and examined for *Anisakis* sp. larvae through visual inspection and peptic digestion. Larvae were identified as type I or II (*sensu* Berland, 1961 Sarsia, 2: 1-50) and stored in 70% ethanol for molecular analysis. A subsample (33%) of *Anisakis* type I larvae and all the type II were identified to the species level by means of: 1) a single species-specific PCR for *Anisakis pegreffii* (APEF) and *Anisakis physeteris* (APHF) for the amplification of a fragment of the ITS region (Fang et al, 2010, Exp Parasitol, 124: 197-201); 2) PCR-RFLP of the ITS region of nuclear rDNA (ITS-1, 5.8S and ITS-2) using the restriction enzymes *Hinf*I and *Hha*I (D'Amelio S et al, 2000, Int J Parasitol, 30: 223-226; Pontes T et al, 2005, J Parasitol, 91: 1430-1434); and 3) DNA sequencing of the partial ITS region (ITS-1, 5.8S, and ITS-2).

RESULTS: A total of 1286 *Anisakis* sp. larvae were found in 218 out of the 777 fish examined (total prevalence 28%, total mean intensity of infection 5.9). After morphological analysis, 1272 (99%) larvae were identified as type I and 14 (1%) as type II. The species-specific PCR showed that all type I larvae examined were *A. pegreffii*, and all type II *A. physeteris*. The results were confirmed by the analysis of the ITS region, and the nuclear ribosomal ITS sequence confirmed clustering within *A. pegreffii* and *A. physeteris* clades. The levels of infection of *Anisakis* spp. are shown in the Table. Monospecific infections by *A. pegreffii* were found in 208 specimens, by *A. physeteris* in 2 specimens of *M. poutassou*, and both *Anisakis* spp. in 8 specimens (4 *M. poutassou*, 2 *T. trachurus*, 1 *M. merluccius*, 1 *S. colias*). Although *Anisakis* spp. larvae were mainly located in the body cavity, 6% of fish (excluding *P. blennoides* and *S. viridensis*) harboured *A. pegreffii* larvae in the muscle.

		Anisakis pegreffii			*Anisakis physeteris*		
Host	N	P (%)	Im	range I	P (%)	Im	range I
Merluccius merluccius	96	37.5	1.3	1-3	1.0	1.0	1-1
Micromesistius poutassou	57	66.7	3.9	1-50	10.5	1.5	1-4
Phycis blennoides	46	0.0	-	-	0.0	-	-
Engraulis encrasicolus	38	65.8	2.8	1-5	0.0	-	-
Sardina pilchardus	252	13.1	1.2	1-3	0.0	-	-
Sardinella aurita	30	13.3	1.0	1-1	0.0	-	-
Scomber colias	29	96.6	15.1	1-46	3.4	1.0	1-1
Sphyreana viridensis	140	5.7	2.3	1-8	0.0	-	-
Trachurus mediterraneus	52	13.5	2.1	1-5	0.0	-	-
Trachurus trachurus	37	100.0	13.6	1-12	5.4	1.5	1-2
TOT	777	27.8	5.9	1-50	1.3	1.4	1-4

CONCLUSIONS: The present study confirms the widespread occurrence of *A. pegreffii* in commercial fish caught off Sardinia and in the western Mediterranean Sea, and the occurrence in the same area of *A. physeteris* (Mattiucci S et al, 2007, J Helminthol, 81: 117–127; Farjallah S et al, 2008, Parasitol Res, 102: 371-379; Meloni M et al, 2011, J Parasitol, 97: 908-914). No hybrids between *Anisakis simplex* sensu stricto and *A. pegreffii*, previously identified in the Iberian, Sardinian, Tyrrhenian, Aegean, Tunisian and Japanese Seas, were detected in the present study.

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Temporal changes of metazoan parasite infections on the gills of wild *Thunnus thynnus* (Osteichthyes: Scombridae) from the western Mediterranean Sea and the north eastern Atlantic Ocean

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Atlantic bluefin tuna, *Thunnus thynnus*, (BFT) is a pelagic fish inhabiting the Atlantic Ocean and the Mediterranean Sea. A number of studies suggested the use of its metazoan parasites as biological tags to better understand its migratory spatial patterns (Rodríguez-Marín E et al, 2008, Aquat Living Resour, 21: 365-371; Mele S et al, 2011, Studi Trent Sci Nat, 89: 153-156). These studies generally explore geographical differences during a definite time period. However, replications in the same localities for more than one sampling time are needed in order to explore the possible variability of the parasite infections through the years (Ferrer-Castelló E et al, 2007, J Helminthol, 81: 169-178).

AIM: The interannual variability of the metazoan parasite infections on the gills of the Atlantic bluefin tuna from the north eastern Atlantic Ocean and the western Mediterranean Sea is analysed.

MATERIALS AND METHODS: Parasites were studied in 26 tunas (160-249 cm fork length) caught in the traditional trap fishery (*tonnara*) of Sardinia (western Mediterranean) in 2006, 2009 and 2011 (BFT-Med); 40 tunas (160-245 cm fork length) caught in the trap fishery (*almadraba*) of the Gulf of Cadiz (Spain, north eastern Atlantic), in 2005 and 2010 (BFT-Atl). Prevalence and mean intensity of infection of each parasite species were calculated, and the differences between years evaluated (Reiczigel J, Rózsa L, 2005, Quantitative Parasitology 3.0, Budapest).

RESULTS: Sixteen parasite species/taxa were found in the BFT-Atl specimens: *Capsala magronum*, *C. onchidiocotyle*, *C. paucispinosa*, *Hexostoma thynni*, *Cardicola* sp., *Copiatestes thyrsitae*, *Didymocystis reniformis*, *Didymocystis* sp. 3 (*sensu* Rodríguez-Marín *et al.* 2008), *Didymosulcus wedli*, *Didymosulcus* sp. 2 (*sensu* Rodríguez-Marín *et al.* 2008), *Didymosulcus* sp. 3, *Didymozoon pretiosus*, *Wedlia bipartita, Eurhyphorus brachypterus, Pseudocycnus appendiculatus* and Isopoda gen. sp. Three of these species, *Cardicola* sp., *D. pretiosus* and Isopoda gen. sp., were not recorded in the BFT-Med fish.
The parasitic infections of BFT-Atl tunas showed significant differences between 2005 and 2010 in prevalence (*H. thynni*, *Didymocystis* sp. 3, *D. wedli, Didymosulcus* sp. 3, and *E. brachypterus*) and mean intensity (*D. wedli*). Those of the BFT-Med tunas showed significant differences in prevalence between 2005 and 2011 (for *Didymocystis* sp. 3 and *Didymosulcus* sp. 2) and between 2009 and 2011 (*D. reniformis*, *Didymocystis* sp. 3, *Didymosulcus* sp. 2 and *Didymosulcus* sp. 3).

CONCLUSIONS: The present results show that the levels of infection of the parasites of BFT can vary between sampling years, and such temporal variations should be taken into account for characterization of the fishing grounds. The temporal changes in the levels of infection could be due to changes in environmental conditions, or to migration of tunas from different areas with different parasite faunas. The parasite infection differences observed should not be related to host size or fishing locality, because host size was homogeneous and the tuna traps are in permanent locations. Further investigations are necessary to understand which factors trigger the temporal changes observed.

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SESSIONE 6

DIAGNOSTICA

DELLE MALATTIE PARASSITARIE

Usefulness of several excretory/secretory antigens and ELISA for the diagnosis of Iberian ibex (*Capra pyrenaica*) *Oestrus* spp. infestation

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AIM: Oestrosis is a myasis caused by flies belonging to the genus *Oestrus* (Diptera: Oestridae) with a worldwide distribution. Larvae cause obligate myiasis in mammals, living within host tissues and body cavities, and which have a great impact on the productivity and welfare of domestic and wild animals. Up to now, diagnosis of oestrosis involves invasive methods such as necropsy, which makes difficult among wild animals.

MATERIALS AND METHODS: Larvae belonging to *Oestrus ovis* were recovered at a local abattoir (Lugo, Spain), washed in phosphate buffered saline, classified into L1, L2 and L3 stages, and finally incubated in RPMI culture medium. That is how we obtained *O. ovis* excretory/secretory antigens L1OES (from L1 larvae), L2OES (L2) and L3OES (L3). The antigenic composition was analyzed using Experion ™ Pro260 Analysis Kit (Bio-Rad). The immune humoral response (IgG) against the *O. ovis* excretory/secretory larval antigens in 32 male sera from Iberian ibexes (*Capra pyrenaica*) from Sierra Nevada Natural Space (southern Spain) has been analyzed by ELISA. These animals hunted between April and June 2010 were immobilized with a mixture of xylazine and ketamine. The blood samples were collected by jugular punction. Necropsy was considered as the gold standard.

RESULTS: L1OES showed 10 bands with several molecular weights (25, 29, 32, 36, 45, 52, 70, 74, 101 and 153 kDa). L2OES presented 6 bands (25, 29, 32, 36, 38 and 45 kDa). Meanwhile, L3OES showed only 3 bands (32, 38 and 45 kDa).
The best results by using the immunoenzymatic assay were obtained by the investigation of IgG antibodies against the L1OES antigens (specificity = 89%; sensitivity = 100%; positive predictive value = 100%; negative predictive value = 57%). The IgG seroprevalence against L1OES was 78% (95% confidence interval (CI) = 64-92%). The percentage of ibexes with *Oestrus* larvae was 88% (95% CI 76=99%).

CONCLUSIONS: The analysis of IgG antibodies against excretory/secretory antigens collected from L1 *O. ovis* larvae provides a very useful and non-invasive procedure for the reliable diagnosis of oestrosis. A control program for reducing or preventing the oestrid infestation in the natural space is required to avoid the infection in wild animals.

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Assessment of an ELISPOT test to evaluate IFN-γ responses in cattle infested by *Hypoderma* spp.

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AIM: The enzyme-linked immunospot (ELISPOT) assay is one of the most sensitive techniques for the *ex vivo* quantification of cytokine secreting cells after stimulation with an antigen *in vitro*. The secreted cytokine is captured by the antibodies coated on the ELISPOT plates, avoiding their diffusion and dilution on the supernatant, as occurs in the ELISA. In this study, an ELISPOT test was standardised for the detection of cattle interferon- (IFN-γ) in order to study the immunomodulatory effect of a crude larval extract (CLE) and the purified fractions hypodermin A (HyA, HyB and HyC), obtained from first stage larvae (L1) of *Hypoderma lineatum*. The frequencies of IFN-γ secreting cells (SC) and the levels of secreted IFN-γ in cellular cultures from cattle naturally infested by *Hypoderma* were compared.

MATERIALS AND METHODS: Three Frisian cows presenting warbles on their back were bled in heparinised tubes by caudal venipuncture and peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation using Ficoll (specific gravidity 1.077 $g.mL^{-1}$). Washed cells from each animal were resuspended at 2×10^5 cells in RPMI 1640 complete medium and were added by triplicate on ELISPOT plates coated with the capture antibody. PBMCs were stimulated with the mitogen phytohemagluttinin (PHA) at 30µg/ml and/or different antigens (CLE, HyA, HyB and HyC) obtained from *H. lineatum* first instars at 2.5µg/ml as final concentrations. The viability of the cells was determined by Trypan Blue dye exclusion. Cells were cultured at 37ºC in 5% CO_2 for 24h. Alkaline phosphatase was used as streptavidin conjugate. Colour was developed with BCIP-NBT as substrate and the spots were counted with an ELISPOT scanner (A.EL.VIS GmbH, Germany). IFN-γ levels in cell culture supernatants were evaluated by a capture ELISA test described by Dacal *et al*, (2009, Vet Immunol Immunpathol, 131: 59-64), using the same antibody pair than in ELISPOT.

RESULTS: The number of IFN-γ SC hardly oscillated with the addition of the HyA to PHA stimulated cultures, whereas the incubation with HyB reduced slightly the number of CSC and on the contrary, the HyC provoked an important increment in the frequency of IFN-γ SC. Coestimulation with the antigen HyA and the mitogen induced approximately 17-fold higher numbers of CSC than in absence of the PHA, however, this difference was 4-fold higher for the HyB and HyC. Surprisingly, the CLE completely inhibited the production of this cytokine, regardless of the presence of the mitogen. There was a relation between the number of IFN-γ SC and the cytokine levels obtained by ELISA. However, the IFN-γ levels detected in cell culture supernatants were very low with all the antigens, especially in absence of the mitogen.

CONCLUSIONS: We have observed as previously done by other authors as Panadero *et al,* (2009, Parasite Immunol, 31: 72-77), that *H. lineatum* larval secretions cause an immunomodulatory effect on PBMC from infested cattle. This modulation is characterized by a suppression of the production of the IFN-γ, especially with the CLE, whereas the collagenase HyC. Our results also demonstrated that the ELISPOT is more sensitive than the ELISA to detect the production of the IFN-γ because the cytokine is captured directly onto a solid phase before having he chance to be diluted in the culture supernatant, degraded by proteases or captured by cytokine receptors on adjacent cells. Further studies aimed to detect different cytokines secreting cells such as IlL-10 and Il-4 should be developed to better understand the mechanism of susceptibility or resistance to *Hypoderma*.

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Mini-FLOTAC, a new tool for copromicroscopic diagnosis of *Toxocara canis*, *Ancylostoma caninum* and *Trichuris vulpis* in dogs

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AIM: Mini-FLOTAC is a new apparatus which comprises two physical components, namely the base and the reading disc. There are two 1-ml flotation chambers, which are designed for optimal examination of faecal sample suspensions in each flotation chamber (total volume = 2 ml) and which permits a maximum magnification of ×400. The Mini-FLOTAC can be used for performing the three techniques (basic, dual and double), which are variants of a single technique but have different applications. The seven operating steps of the *Mini-FLOTAC basic technique* (MFBT) are: (1) weigh the faecal sample [2 g for dogs, cats and humans (+ 2 ml of formalin 5% if needed); 10 g for herbivores]; (2) add the flotation solution (FS) using a dilution ratio of: (a) 1:20 for dogs, cats and humans with an analytic sensitivity of 10 parasitic elements per grams (PEG = eggs, larvae, oocysts and cysts); (b) 1:10 for herbivores (analytic sensitivity = 5 PEG); (3) homogenize, (4) filter, (5) fill the two Mini-FLOTAC flotation chambers; (6) wait 10 minutes, (7) translate and examine under a microscope.

The present study was aimed at comparing the MFBT with other three copromicroscopic techniques, namely: direct smear (DS) (Foreyt W J, 2001, Vet Parasitology, Blackwell Pub, Iowa, USA), flotation in tube (FT), and Wisconsin (WS) (MAFF, 1986, Manual of Veterinary Parasitological Laboratory Techniques, London, UK), in terms of efficiency (eggs per gram (EPG) of faeces), using fresh and fixed dog faecal samples naturally infected by *Ancylostoma caninum*, *Toxocara canis* and *Trichuris vulpis*. For all the flotation based techniques two FS were used: FS2, sodium chloride (density = 1.20) and FS7, zinc sulphate (density = 1.35).

MATERIALS AND METHODS: Faecal samples from 23 naturally infected dogs were collected. Each fresh sample was thoroughly homogenized and used to perform 3 replicates of (i) DS, (ii) FT and (iii) WS. Then, from each fresh sample one aliquot of 20 g was weighted and fixed with 20 ml of formalin 5% (dilution ratio of 1:2). Two aliquots of 4 ml (= 2 g of faeces) of each fixed sample were used to perform 3 replicates of (iv) MFBT for each FS. The remaining part of fixed feces (36 ml = 18 g) was diluted with water to reach 360 ml (faecal dilution = 1:20), thoroughly homogenized and filtered through a 250 m wire mesh. From the filtered suspension, 12 aliquots of 10 ml were placed in 15 ml tubes and then centrifuged for 3 min at 170 × g. The tubes were then randomly assigned to the following techniques: (v) FT and (vi) WS to have 3 replicates for each FS used for each technique. The arithmetic mean EPG were calculated for each parasite and each technique. Statistical analyses (Mann-Whitney U-test , ANOVA) were carried out using STATA 10.0 software (Stata Corp., Texas 77845, USA).

RESULTS: The results are summarized in the following table. Significant differences for different letters ($P < 0.05$)

CONCLUSIONS: The results showed that regarding EPG of *T. canis* and *A. caninum*, MFBT was more efficient than DS, FT and WS. In conclusion, the present study suggest that the MFBT is a promising technique for detecting and counting helminth eggs in dog feces, and can be used in place of the FLOTAC techniques (Cringoli G et al, 2010, Nat Protoc, 5(3): 503-155),, the "Gold standard", in laboratories where the centrifugation step cannot be performed.

Parasite	Fresh feces (mean EPG)					Faeces fixed in formalin 5% (mean EPG)					
	Direct Smear (DS)	Flotation in tube (FT)		Wisconsin (WS)		FT Flotation in tube (FT)		Wisconsin (WS)		Mini-FLOTAC basic technique (MFBT)	
		SF2	SF7	SF2	SF7	SF2	SF7	SF2	SF7	SF2	SF7
T. canis	0.2[a]	5.8[a]	7.4[a]	20.5[a]	27.0[a]	5.3[a]	6.2[a]	66.6[a]	80.2[a]	118.3[b]	129.3[a]
A. caninum	0.1[a]	32.1[a]	17.4[a]	41.9[a]	22.8[a]	29.1[a]	4.9[a]	55.5[a]	30.2[a]	124.8[b]	72.9[a]
T. vulpis	0.4[a]	4.6[a]	6.2[a]	28.6[a]	36.8[a]	5.6[a]	3.5[a]	43.0[a]	53.1[a]	82.3[a]	73.3[a]

Elucidating poorly known morphological and biological features of *Eucoleus aerophilus*

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Eucoleus aerophilus (syn. *Capillaria aerophila*) parasitizes wildlife, dogs and cats across Europe and other regions. Also, this trichuroid displays, albeit seldom, a relevant zoonotic potential. Adults live in the trachea, bronchi and bronchioles of the infected hosts and cause different respiratory distresses (Traversa D et al, 2010, Parasit Vectors, 3: 62). Animals become infected by ingesting environmental larvated eggs or infected earthworms. Although the number of diagnosed clinical cases of pulmonary capillariosis in pets is increasing, several aspects of this infection are still poorly known.

AIM: With the aim to fill gaps in basic knowledge of *E. aerophilus*, the present work provides new insights into morphology and morphometry of its eggs and into their *in vitro* development.

MATERIALS AND METHODS: Eggs were collected from four dogs and one cat with natural infections, pooled and divided in different batches for light microscopy (LM), scanning electron microscopy (SEM) and *in vitro* development. Eggs of *E. aerophilus* from the cat and dogs were compared by LM. Eggs of the canine intestinal whipworm *Trichuris vulpis* were also collected and microscopically processed for comparison with *E. aerophilus* eggs. LM was performed by using a Leica DM LB2 microscope and a Leica DFC425 digital camera supported by the software Leica LAS V3.7m. For SEM, eggs were washed in distilled H_2O, air-dried, mounted on Al stubs and sputtered with palladium. Observation was done with a FEI Quanta 200 SEM. Egg batches were also suspended in 1 % (v/v) saline solution at the same day of shedding and cultivated at environmental temperature and humidity for two months. Cultured eggs were microscopically examined after two weeks, at days +35, +45 and after two months.

RESULTS: No differences between *E. aerophilus* eggs from dogs and the cat were found. LM of all eggs showed a typical morphology: barrel-shape, asymmetry of bipolar plugs with absence of thickening at the basis, and outer shell with anastomosing ridges and bridges. The lengths of major and minor axes of all eggs were 64.91 (± 1.11) 65.04 (± 1.50) m and 34.89 (± 3.34) 36.96 (± 3.15 m), respectively. Eggs of *T. vulpis* were bigger, with a size ranging from 72–94 m long and 31–42 m wide, and possessed a thick and brownish wall, with symmetrical plugs having rings at their basis. At SEM analysis, eggs of *E. aerophilus* presented a typical outer densely striated and net-like surface, with depression and ridges irregular in distribution, whereas *T. vulpis* had a smooth shell with no ridges or pits. After two weeks of coproculture at 20 ± 1 °C and 80–85% humidity no eggs of *E. aerophilus* were mature, while developing rate increased after 35 and 45 days (~ 30% and ~ 50–70%, respectively). At day +60, most eggs contained a viable larva.

CONCLUSIONS: Lung capillariosis is not commonly included in differential diagnosis in current practice. The parasite is neglected and most often the eggs are misdiagnosed with those of intestinal whipworms (Traversa D et al, 2010, Parasit Vectors, 3: 62). In fact, the major diagnostic hindrance is lack of awareness in veterinarians on significance of capillariids in dogs and cats. Indeed, the net-like wall of *E. aerophilus* eggs can be difficult to appreciate by conventional LM being the size and the plugs aspects the most important key features to identify these eggs. When trichuroid eggs are found in canine faeces, a thorough morphometric and morphological analysis is required. Feline whipworms are only confined in scattered areas, where they occur very rarely, thus not causing relevant diagnostic dilemmas. In any case, a careful morphological analysis allows differentiating eggs of *Trichuris* spp. and *E. aerophilus* in cats as well. The present results on the *in vitro* development indicate that after two months the vast majority of *E. aerophilus* eggs become infective. These information represent a first step towards elucidating further issues of the biological cycle, which need to be still clarified, e.g. actual role of earthworms in transmitting the infection and possibilities of cross-infections among different host species.

Utility of an immunoassay with *Anoplocephala* spp. metabolic antigens for the detection of equine cestodoses

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AIM: To improve the diagnostics of equine cestodoses (*Anoplocephala* spp.) by using an immunoassay with *Anoplocephala* spp. excretory/secretory antigens.

MATERIALS AND METHODS: Adult *Anoplocephala* spp. specimens collected from 4 horses slaughtered at a local abattoir (A Estrada, Pontevedra, Spain) were washed in PBS (phosphate buffered saline, pH 7.4). The tapeworms were incubated for 72 h at 37°C and 5% CO_2 in RPMI medium containing 0.5 mM PMSF, and finally the *Anoplocephala* excretory/secretory antigens (AnES) were extensively dialyzed against water and lyophilized.
The analysis of the utility of the AnES-ELISA consisted on the evaluation of the humoral IgG immune response in 135 horses from Galicia (NW Spain). Faecal and blood samples were individually collected from each equine. The stools were analyzed by means of the flotation copromicroscopical procedure. Sera were faced to the AnES by using an ELISA.
The flotation test was considered as the gold standard probe. Two procedures were performed for assessing the cut-off value in the serological test. Firstly, positive values were determined as the mean optical density of sera from 45 foals (9-month old) plus three standard deviations; none of these foals resulted positive to the flotation test. Secondly, the ROC (Receiver Operating Characteristic) curve analysis was applied for estimating the best cut-off value by taking into account the sensitivity and specificity values.

RESULTS: Twenty-four out of 135 horses passed *Anoplocephala* spp. eggs by faeces. By means of the sera from the foals negative by flotation, a cut-off value of 0.41 was calculated. The statistical values were 100% sensitivity (S), 57% specificity (Sp), 33% predictive positive value (PPV), 100% negative predictive value (NPV), 2.3125 positive likelihood ratio (PLR), 0 negative likelihood ratio (NLR) and 0.318 kappa. The seroprevalence of cestodoses was 53%.
The ROC analysis showed the best cut-off value was 0.6705. In this way, the statistical values resulted 88% S, 78% Sp, 47% PPV, 97% NPV, 4.047 PLR, 0.165 NLR and k= 0.491. A percentage of 33% seroprevalence of cestodoses was detected in this way.

CONCLUSIONS: A notable agreement between the coprological and serological probes by using *Anoplocephala* spp. excretory/secretory antigens was reached. The estimation of the cut-off value by using the ROC curve analysis allows us to improve the statistical values collected in comparison to the flotation test and thus we strongly counsel this procedure.

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Diagnosis of *Eucoleus boehmi* (syn. *Capillaria boehmi*) by optical and scanning electron microscopy

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AIM: Dogs can be infected by several nematodes of the Trichuridae family. Trichuridae eggs have a similar morphology and misdiagnosis among different species is possible. *Eucoleus boehmi* (syn. *Capillaria boehmi*) lives in the nasal cavities and paranasal sinuses of foxes, wolves and dogs. This parasite was identified for the first time as a new species in silver foxes from Moravia and Austria (Supperer R, 1953, Z Parasitenk, 16: 51-55) and then reported in wild animals in several European regions (Zarnowsky E and Patyk W, 1960, Acta Parasitol Pol, 8: 205-213; Sréter T et al, 2003,Vet Parasitol, 115: 329–334; Davidson RK et al, 2006, Vet Parasitol, 136: 307-316); in dogs few cases are reported in Europe (Zarnowsky E and Patyk W, 1960, Acta Parasitol Pol, 8: 205-213; Gajewska A et al, 2004, Zycie Weterynaryjne,79: 208-212; De Liberato C, 2009, Parassitologia, 51: 43-45) and in north America (Campbell BG and Little MD, 1991, JAVMA, 198: 1520-1523; Schoning P et al, 1993, Vet Res Commun, 17: 277-281; Baan M et al, 2011, JAAHA, 47: 60-63). *E.boehmi* may cause nasal discharge, sneezing and anosmia (PiperisovaI et al, 2010, Vet Clin Path, 39: 121-122). The life cycle is yet not completely clear (Conboy GA, 2009, Vet Clin North Am Small Anim Pract, 39: 1109-1126); eggs are released into the environment via hosts' faeces, reaching the infective stage after 7-8 days (Pérez Tort G, 2010,Vet Focus, 20: 44-48).
The aim of our work is to report the presence of *E.boehmi* in dogs in Italy and to describe the eggs using optical and scanning electron microscopy (SEM).

MATERIALS AND METHODS: From January 2010 to March 2011, 347 canine faecal samples were collected in Liguria (Imperia and Savona districts), north west Italy, and examined by centrifugal flotation with $ZnSO_4$ (s.g. 1.350). Eggs were identified with an optical microscope (LEICADM75) using morphologic and morphometric characteristics (Campbell BG, 1991, Comp Cont Ed Pract Vet, 13: 769-778; Schoning P, et al, 1993, Vet Res Commun, 17: 277-281). For SEM, eggs were isolated by flotation in $ZnSO_4$ solution and sieved, modifying the technique of Al-Sabi MN et al (2010, Parasitol Res, 107: 135–140), then mounted on aluminum stubs, air dried, gold coated with the sputtering technique and observed with JEOLJSM5410 SEM. Sterile cotton tip swabs were inserted into each nasal cavity for nasal examination.

RESULTS: Among the 347 examined faecal samples, 6 dogs (1.7%, 95% confidence interval: 0.3-3%) were positive for *E. boehmi.* At light microscopy, *E. boehmi* eggs measured 55-59×32-34µm (average 58×34µm), were clear to golden in colour, barrel-shaped with polar plugs and contained a multicellular embryo. These eggs, when released into the environment, are already partially embryonated; the enclosed embryo retracts from the shell, leaving a characteristic space between itself and the egg wall. SEM highlighted the characteristics of the egg shell: the surface presented a dense network with a fine mesh, surrounding irregularly distributed small pits. Nasal swabs performed on all six dogs positive for *E.boehmi* were negative, but the low sensitivity of this test has already been reported (Campbell BG and Little MD, 1991, JAVMA, 198: 1520-1523; Schoning P et al, 1993, Vet Res Commun, 17: 277-281).

CONCLUSIONS: The description of the characteristics of *E. boehmi* eggs with optical and scanning electron microscopy highlights some differences with *E. aerophilus*, the other respiratory Trichuridae of dogs. The eggs of *E. aerophilus* are on average larger than *E. boehmi* eggs, measuring 60-72 x 26-34 µm (average 67 × 29 µm), have an elliptic shape with asymmetrical polar plugs, and are entirely filled by a one- or two-cell embryo. The shell of the eggs of *E. aerophilus* by SEM looks like a thick mesh with wide depressions (Magi et al. personal communication; Campbell BG, 1991, Comp Cont Ed Pract Vet, 13: 769-778; Traversa et al, 2011, Parasitol Res, 109: 97-104). SEM gives a detailed image of the egg shell wall, which is one of the most reliable distinctive characteristic of the species, and thus allows an accurate diagnosis. However, since it cannot be routinely used, and since by optical microscopy Trichuridae eggs may frequently be misdiagnosed, the development of biomolecular diagnostic methods is desirable.

Magnetic Resonance Imaging in sheep affected by chronic *Coenurus cerebralis*

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AIM: to document skull and brain MRI findings in sheep naturally infected by chronic *Coenurus cerebralis* (CC).

MATERIALS AND METHODS: thirty-three CC-affected sheep and 10 control sheep received brain Magnetic Resonance Imaging (MRI). The volume of the cranial cavity (CrC), rostral fossa (RF), and caudal fossa (CF) was measured and compared in the two groups. In affected animals, the number, location and volume of the cysts were measured and the percentage of each cranial cavity occupied by the cyst was calculated. Focal and diffuse abnormalities of the bone were used for the evaluation of the skull. Perilesional edema, hemorrhage, and signs of increased cranial pressure (ICP) were used for the evaluation of brain parenchyma. Data were statistically evaluated by Stata 11.2 software.

RESULTS: MRI showed that the volume of the cranial cavities was significantly larger in CC-affected versus control animals ($P<0.001$). The cysts occupied 4.40 – 46.93 % of the volume of the CrC, between 4.12 – 51.53 % of the volume of the RF, and between 15.24 – 68.30% of the volume of the CF. Moderate to severe diffuse bone alterations and ICP were observed respectively in 21 and 24 sheep. These findings were positively correlated to the volume of the cyst.

CONCLUSIONS: Significant volumetric and morphological changes observed in this study might represent a failure or an adaptation of the bone tissue formation as a response to the chronic ICP caused by the development of the cyst.

FLOTAC for the detection of parasitic elements in reptiles faeces

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AIM: The aim of the present study was to investigate the occurrence of parasitic and pseudoparasitic elements in reptiles, using the FLOTAC technique (Cringoli G et al, 2010, Nat Protoc, 5: 503-515).

MATERIALS AND METHODS: A total of 212 faecal samples collected from 134 snakes, 25 lizards and 53 tortoises were analyzed. All faecal samples were stored in 5% formalin and subsequently examined in the laboratory using the *FLOTAC pellet technique*. This quantitative technique has been developed for fixed faecal samples with an unknown weight and or an unknown proportion between faeces and fixative (as reported in the FLOTAC manuals). Two different flotation solutions were used: FS2 (sodium chloride solution) (density = 1200) and FS7 (zinc sulphate solution) (density = 1350). Parasitic elements (eggs, larvae, oocysts and cysts) were identified in accordance with the guidelines reported in the literature.

RESULTS: Out of the 212 samples examined, 123 (58.0%; 95% confidence interval (CI) = 51.0-64.7%) showed the presence of parasitic elements (eggs, larvae, oocysts and cysts), specifically 16/25 (64.0%; 95% CI= 42.6-81.3%) of the lizards, 54/134 (40.3%; 95% CI=32.0-49.1%) of the snakes and 53/53 (100%; 95% CI=91.6-99.8%) of the tortoises. The most frequent parasites in lizards and snakes were oxyurids, *Rhabdias*, *Kalicephalus*, *Capillaria* and Eimeriidae, whereas in tortoises oxyurids, *Nyctotherus*, *Balantidium*, *Entamoeba*, ascarids and *Isospora* spp. Pseudoparasitic elements also were detected by the FLOTAC technique. In lizards and snakes eggs of *Myocoptes musculinus*, *Trichuris muris*, *Hymenolepis nana*, *Aspiculuris tetraptera* and *Syphacia obvelta* were found, whereas mite eggs were found in tortoises.

CONCLUSIONS: Reptiles have become increasingly common domestic pets. As a consequence of their popularity, the interest of the scientific community in these animals has increased; however, little is known about their infections, including parasites. While several reptile species sold as pet animals are bred in captivity, most of them are taken from the wild or are the offspring of wild-caught parents. These animals can harbor a wide variety of parasitic and pseudoparasitic elements due to their extremely diverse diet. As result, the faeces of reptiles often contain artefacts such as plant and invertebrate parts, and parasite eggs of the animals used as food; this makes problematic the copromicroscopic diagnosis. The results of the present study showed that the FLOTAC technique was a rapid and sensitive test to improve diagnosis and acquire new information on the parasitological fauna of reptiles, furthermore it has demonstrated to be highly sensitive also when we tested samples of little or even unknown weight (Rinaldi L et al, 2012, Exp Parasitol, 130: 282-284). In conclusion, FLOTAC can be considered a promising tool for detecting parasitic elements in these exotic animals.

Performance of a commercially available immunocromatographic test (SNAP®4Dx) for serodiagnosis of *Anaplasma phagocytophilum* infection in horses

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AIM: Equine granulocytic anaplasmosis (EGA), a tick-borne disease (TBD) with a worldwide distribution and global significance, represents a veterinary medical problem of increasing concern in several European countries, including Italy. Traditionally, infection in horses is diagnosed basing on: 1) the morphologic appearance of the characteristic morulae in peripheral blood smears, 2 the presence of anti-*Anaplasma phagocytophilum* antibodies detected by Indirect Fluorescent Antibody Assay (IFAT) and, more recently, 3) the detection of microorganism's DNA by PCR assays. However research efforts for rapid, practical and accurate detection methods to be applied in the field; a qualitative in-clinic immunocromatographic kit (ICT, SNAP®4Dx, IDEXX Laboratories, Westbrook, ME, USA) developed for the identification of antibodies against a *A. phagocytophilum* peptide of the immunodominant p44 protein in canine serum is commercially available. Although a formal validation for the use in horses is scanty, different Authors reported a strong reactivity with equine sera (Hansen GB et al, 2010, AVS, 52:3). Aim of the present work was to evaluate the performance of the SNAP®4Dx test for the detection of antibodies against *A. phagocytophilum* in serum samples of horses.

MATERIALS AND METHODS: Two hundred asymptomatic horses and 244 animals showing clinical signs compatible with TBDs and rearing in Central Italy were selected to be included in a cross-sectional survey. Venous blood samples were collected from each horse into sterile tubes with and without EDTA for buffy coat (BC) and serum collection respectively. All the horses were tested for the presence of anti-*A. phagocytophilum* IgG antibodies using an IFA assay (Passamonti F et al, 2010, CIMID, 33: 73-83) and the SNAP®4Dx kit, according to the manufactures instructions. Moreover serum and BC samples of symptomatic horses were respectively tested for evidence of *A.phagocytophilum* active infection through detection of IgM by IFAT and amplification of a specific region of the 16S rRNA gene by PCR analysis (Barlough JE et al, 1996, Vet Parasitol, 63:319-29). Symptomatic horses were considered to be affected by active EGA based on criteria combining serological and biomolecular results (IFAT IgG titer≥1/640 or IgG titer < 1/640 but IgM and/or PCR positive). Concordant and discordant results between IFAT and ICT overall results were assessed by computing K statistic, assuming IFAT (for IgG) as the comparative test. The relative agreement of ICT with regard to IFAT was also estimated in symptomatic and asymptomatic animals as well as the relative sensitivity (Se) and specificity (Sp). Furthermore ICT accuracy exhibited in active EGA infection was evaluated.

RESULTS: Forty five on 444 horses (10.13%) tested positive for IFAT (27 symptomatic and 18 asymptomatic) whereas antibodies against *A. phagocytophilum* by the SNAP®4Dx were identified in 40 (9%) horses (25 symptomatic and 15 asymptomatic). The overall concordance between SNAP® 4Dx and IFAT was 98.4% (98.4% in symptomatic and 98.5% in asymptomatic animals), with K>0.9; ICT showed to have very high performance index (Se: 86.67%; Sp: 99.75%) and almost equal for symptomatic and asymptomatic animals. Concerning the symptomatic animals with as certained presence of the parasite (no 36), 13 horses (5.33%) were found to be affected by active EGA and 23 (9.42%) to be exposed (no. 16 IFAT IgG<1/640 and no. 7 only PCR positive). Among these 36 horses, the ICTcorrectly identified 24 animals, 11 (45.83%) belonging to the group affected by active EGA with 2 negative discordances and 13 (54.16%) to the exposed group, with 10 negative discordances.

CONCLUSIONS: The present study indicates that SNAP®4Dx even though could represent a feasible in-field test for practicing veterinarians, need of confirmatory analysis due to the fact that in more than 50% of the cases is not able to distinguish infected to exposed animals. However the ICT, showing an almost perfect agreement with IFAT, could represents a very good screening method for epidemiological survey. Despite the SNAP®4Dx has been already validated for epidemiological survey on equine *B. burdorferi* infection (Chandrashekar Ret al, 2008, Intern J Appl Res Vet Med, 6:145-150) the authors suggest the assessment of an ICT able to reveal co-infections with further tick borne pathogens such as *Babesia caballi* and *Theileria equi*.

Anatomical distribution of *Oestrus ovis* instars, macroscopic lesions, nasal discharge and IgG immune response in a naturally infested flock of Sarda breed sheep

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AIM: In order to add practical recommendations on the diagnostic approach to ovine oestrosis, a field study was conducted in a Sarda breed sheep flock. Data on anatomical distribution of *Oestrus ovis* larval burden and macroscopic lesions were collected and compared with indirect ELISA and nasal discharge.

MATERIALS AND METHODS: The study was carried out on 92 sheep of Sarda breed, aged years. The sheep were treated subcutaneously with 200mcg of Moxidectin/kg BW (0.1 ml/5 kg BW Cydectin® injection of 1%) in August 20, 2010. In July 17, 2011, prior to slaughtering, blood samples were collected from jugular vein from each sheep and nasal discharge score was individually evaluated in the whole flock, using the protocol of our previous studies (Mula PP et al. 2011, 19th Congress Proceedings FeMeSPRum, 164-170). Blood serum samples were stored at -20 °C and then analyzed by indirect ELISA according to Suárez JL et al (2006, Vet Parasitol, 134: 153-158) to estimate the percentage of IgG. At slaughterhouse skull were opened and count and localization of instars larvae in the nasal and sinuses cavities were performed. Data on the presence of inflammation in the mucosa of the same districts were also macroscopically evaluated and classified as following: i) absent, ii) presence of mucus, and iii) presence of mucopurulent exudation. Parasitological data on each localization of the larvae and percentage of IgG were then compared through Pearson correlation. Nasal and mucosal exudation were also compared and used as factors in the Anova test to evaluate their relation with parasitological and immunological data. Statistical significance was considered for $P<0.05$.

RESULTS: Number of positives ewes, prevalence (Prev.) and mean intensity (M.I.) of infestation according to the anatomical localization for each of the three *O. ovis* instars (L1, L2 and L3) are shown in the following table.

The group which harboured L1 larvae had an higher mean percentage of IgG than the L1 negative ewes. In the whole study a positive correlation was found between IgG percentages and L1 intensity of infestation ($R= 0.277$; $P = 0.007$). First instar in the frontal sinus, in the nasal cavities and in the ethmoidis had a low positive correlation with antibody percentage with a tendency to significance ($P=0.07$). Sheep with mucosal exudation in the maxillary sinuses showed also higher levels of IgG, even if in this localization the lowest values of abundance were registered for all the instars. The overall mean intensity of second instars was found related to the number of localizations in which the mucus production was detected (Anova, $P= 0.000$). Nasal discharge score was significantly related to L2 instars in the ethmoid ($R= 0.227$; $P= 0.029$), and to the total number of larvae in the same district ($R= 0.327$; $P= 0.001$).

CONCLUSIONS: Our preliminary results seem to point how the presence of several development instars (particularly in the ethmoid) seems to provoke an exudative response in the mucosa which is visible as nasal discharge. Further findings in the different seasons could complete the scene and so, when the effect of concomitant development instars in the same ewes is less evident (diapauses), this could add more findings on the effective role of first instar.

	Nasal cones and meatus				Ethmoidis				Frontal sinuses				Maxillary sinuses			
	Pos.	Neg.	M.I.	Prev.	Pos.	Neg.	M.I.	Prev.	Pos.	Neg.	M.I.	Prev.	Pos.	Neg.	M.I.	Prev.
L1	41	51	2.4	45.0%	25	67	2.2	27.0%	2	90	1.5	2.0%	1	91	1	1.0%
L2	39	53	2.5	42.0%	48	44	3.4	52.0%	51	41	3.8	55.0%	8	84	1.6	9.0%
L3	8	84	1	9.0%	2	90	1	2.0%	13	79	1.2	14.0%	1	91	1	1.0%

Histopathological and immunohistochemical findings in goat and muflon affected by cryptococcosis

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AIM: Cryptococcosis is a disease of human, domestic, and wild animals caused by two fungal species: *Cryptococcus neoformans*, which includes serotype A (variety *Grubii*) and serotype D (variety *neoformans*), and *Cryptococcus gattii* which is divided in serotypes B and C (Gago S et al, 2011, J Clin Microbiol, 49: 3663-3666). These two species are included in the so called *Cryptococcus* species complex. The present study was aimed to define the histopathological and immunohistochemical aspects of *Cryptococcus* species complex infection in central nervous system and lung of goats and muflon.

MATERIALS AND METHODS: From three goats and one muflon with neurological and/or respiratory signs, brain, spinal cord and lung were sampled at necroscopy and then fixed in 4% buffered formalin to be embedded in paraffin. Sections of brain, spinal cord and lung were stained with Haematoxylin-Eosin (HE) and Alcian Blue&Period Acid-Shiff (AB&PAS) for histological examinations. For determining the *Cryptococcus* serotype, selected section from the same organs were stained immunohistochemically by using mAb 471, 302, F10F5 and 1326 as primary antibodies (Krockenberger M B et al, 2001, Med Mycol, 39: 523-533). In addition CD immunophenotyping of inflammatory cells was performed to define the local immune response related to *Cryptococcus* species complex infection.

RESULTS: No gross changes were observed in brain and lungs of the animals under study. The prominent histological change was the presence of aggregates of *Cryptococcus* spp. organisms giving "soap bubble" appearance disseminated mainly around blood vessels in the brain and spinal cord and within the alveolar spaces in the lung. *Cryptococcus* spp. appeared as numerous spherical organisms surrounded by a non-staining or blue capsule by using HE or AB&PAS, respectively.
In the goats, perivascular lesions seem to be more severe and coalescing at the levels of basal nuclei and mesencephalon sections with meninges resulting the most affected district throughout the whole brain. In the lungs, interstitial chronic pneumonia with infiltration of mononuclear cell were observed. CD cell immunophenotyping demonstrated the presence of $CD163^+$ macrophages, $CD3^+$ T cells, and $CD79^+$ B cell, with the later being less prominent. The severity of the lesions did not correlate with the magnitude of the infiltrate. Interestingly, serotyping by immunohistochemistry identified *C.gattii* serotype B in goats, and *C. neoformans* serotype A in muflon.

CONCLUSIONS: It is well known that in small ruminants *Cryptococcus* spp. causes sporadically mastitis (Pal M and Randhawa H S, 1976, Sabouraudia, 14: 26-261), pneumonia and meningoencephalitis (Barò T et al, 1998, J Clin Microbiol 36: 458-461). However, to the authors' knowledge this is the first report in Italy of cryptococcosis affecting goats and muflons.
Cryptococcus infections in human occur mostly in immunodeficient individuals, including patients affected by the human immunodeficiency virus (HIV), but recently also in immunocompetent patients (Reis F et al, 2011, Arq Neuropsiquiatr 69(5): 851). In our cases goats did not show clinical and histopatological finding related to other etiological agents. In this respect, an experimental study in healthy immunocompetent goats showed that intrammammary inoculation of *C. neoformans* caused mastitis (Singh M et al, 1994, Mycopathologia 126: 147-155).
Histopathological changes in the central nervous system of the goat and muflon under study are similar to those previously described in cattle (Riet-Correa F et al, 2011, J Vet Diagn Invest, 23: 1056-1060). The lack of correlation between the magnitude of the inflammatory reaction with the severity lesion may be related to the antiphagocytic and immune suppressive properties of cryptococcal polysaccharide capsule (King J Wand DeWitt M L, 2007, Cryptococcosis).
In our study we observed that, in brain and lung affected by *C. gattii* serotype B and *C. neoformans* serotype A, T cells and $CD163^+$ macrophages are the predominant immune host cells, as reported in humans. This suggest an innate immune response, however the co-presence of $CD79^+$ B-cells could support the hypothesis of the involvement of an important humoral response.

Critical points in morphological descriptions of adults and larvae of Strongylidae in equids

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AIM: Strongylidae, which includes the most common parasites of equids, have been historically difficult to identify. Molecular methods, whose use is taking place in recent years, have the disadvantage of being expensive and have been developed only for the most prevalent strongylid species. That is why, more extensive studies of the strongylids by classical morphological methods are still necessary. The aim of this study is to update the knowledge about the species of nematode parasites affecting Italian donkeys and give a critical review about identification keys for larvae (Euzeby J, 1981, Diagnostic experimental des Helminthoses animales, Information Technique des Services Veterinaires, Ministère de l'Agricolture, Paris, F) and adults (Lichtenfels JR et al, 2008, Vet Parasitol, 156: 4-161) of Strongylidae.

MATERIALS AND METHODS: Between November 2009 and April 2010, the intestine of ten donkeys was collected from a slaughterhouse. All animals, five male foals and five adult females, belonged to an organic farm and never had anthelmintic treatments. Following a proven technique for parasites' isolation in Equidae (Bu Y et al, 2009, Acta Parasitol, 54: 263-268; Matthee S et al, 2000, J of Parasitol, 86:756-762), after dilution in water, 10% of the whole intestinal content was collected by each intestinal tract and repeatedly washed before parasite isolation. Adult parasites were identified following Lichtenfels' keys. If available, a faecal specimen was collected from rectum for coprocolture (seven animals); 100 larvae were identified for each faecal specimen following Euzeby's keys.

RESULTS: Adult parasites: 21 species of Strongylidae were identified (*Strongylus vulgaris*, *Strongylus edentatus* (first report in Italian donkeys: frId), *Triodontophorus serratus*, *Triodontophorus brevicauda* (frId), *Triodontophorus minor* (frId), *Cyathostomum alveatum*, *Cyathostomum catinatum*, *Cyathostomum tetracanthum, Coronacyclus coronatus, Coronocyclus labratus, Cylicocyclus adersi* (frId), *Cylicocyclus asini* (frId), *Cylicocyclus auriculatus, Cylicocyclus leptostomum, Cylicocyclus nassatus, Cylicocyclus radiatus, Cylicostephanus calicatus, Cylicostephanus goldi, Cylicostephanus longibursatus, Cylicostephanus minutus, Cylicodontophorus bicoronatus*). Critical points verified in Lichtenfels' keys and descriptions were the following:

- the key for the identification on the genus *Cyathostomum* report that the insertion point of the internal leaf crown is at about ¼ - ½ of buccal capsule depth; actually it is not true for *C. alveatum*, as clearly appears from the figures and description of this species;
- the dorsal gutter of *C. asini* is described as short, little, button-like. It is clearly a mistake as, consistently with other descriptions of this species (Matthee S *et al*, 2002, Syst Parasitol, 51: 29-35) and with the photos and pictures of Lichtenfels himself, the dorsal gutter is long and well developed;
- the photos of *C. radiatus* are not consistent with its descriptions;
- *C. calicatus* and *C. minutus* are very difficult to distinguish as the only morphological difference regards the number of external leaf crown elements (12-18 vs. 8).

Larvae: 3 types of strongylid larvae were observed. According to Euzeby's descriptions they were *Cyathostomum* spp. *sensu lato*, *Oesophagodontus* spp. and *Strongylus vulgaris*. However the following critical points, verified comparing larval identification with adult parasites isolated in the same host, were observed:

- larval length of *Strongylus vulgaris* was constantly less than the length reported; it is remarkably critical given that the larval length is the starting dichotomous character for identification in Euzeby's keys;
- larvae with 16 intestinal cells couldn't be *Oesophagodontus* spp. larvae, as suggested by Euzeby, as there were no adult specimens in the corresponding faecal samples; they probably belong to the species *Triodontophorus serratus* (isolated among the adult worms) as confirmed also by other authors (Bowman DD, 2009, Georgis' Parasitology for Veterinarians, Saunders Elsevier, St Luis, USA; Lichtenfels JR *et al*, 2008, Vet Parasitol, 156: 4-161);

CONCLUSIONS: A moderate revision of the excellent paper of Lichtenfels is needed. However the major concern is about larval identification. Euzeby's keys are indeed aged, incomplete and unreliable, so that a total revision is needed.

Use of a commercial ELISA Kit for the detection of anti-*Toxoplasma gondii* antibodies on swine meat juice

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AIM: Indirect Fluorescent Antibody Test (IFAT) is considered the golden standard for the determination of *Toxoplasma gondii* antibodies in swine serum (Garcia JL et al, 2006, Exp Parasitol, 113: 100-105) but other techniques, such as Enzyme Linked Immunosorbent Assay (ELISA) and Modified Agglutination Test (MAT), showed good accuracy and a better applicability for monitoring purposes (Garcia JL et al, 2008, Res Vet Sci, 84: 237-242). Recently, meat juice has been suggested to be an easy to obtain and constantly available matrix alternative to serum for the detection of antibodies against *T. gondii* in swine (Dubey JP et al, 2005, J Parasitol, 91: 1082-1093; Bergen-Schoch AE et al, 2011, Zoonoses Public Health, 177: 290-297). Several studies are available on the application of ELISA for the diagnosis of infection by *T. gondii* using meat juice, but they adopt different antigens and cut-off or test different muscles and productive categories (Dubey JP et al, 2005, J Parasitol, 91: 1082-1093; Gamble HR et al., 2005, Vet Parasitol, 128: 177-181; Hill DE et al, 2006, Vet Parasitol, 141: 9-17). Aim of the present study was to evaluate the possible application of a commercially available indirect ELISA kit, commonly used for detection of anti- *T. gondii* antibodies in serum, in meat juice samples of naturally-infected swine.

MATERIALS AND METHODS: The ELISA Kit "ID Screen® *Toxoplasmosis* indirect" (IDVET, Montpellier-France) consisted of a purified peptide of the main P30 *T. gondii* protein as antigenic substrate and a multi-species peroxidase as conjugate. To evaluate the precision and accuracy of the commercial ELISA kit, intra-assay and inter-assay coefficients of variation (CVs) were preliminary assessed on previously obtained 5 positive and 5 negative meat juice samples with the respective replicates (4 for each). Subsequently, 50 IFAT positive and 50 IFAT negative meat juice samples, previously obtained from diaphragm muscles of heavy pigs (Ranucci D et al, 2012, Foodborne Path Dis, 9: 75-78), were tested for IgG antibodies against *T. gondii* by the ELISA commercial kit accordingly to manufacturer's instructions. The optical density values obtained by the spectrophotometer lecture, were converted into a percentage values (S/P %). The results of the ELISA test were compared with those obtained by IFAT, assumed as standard comparison test, using the McNemar's chi-squared test. The concordance between IFAT and ELISA results was assessed by K statistic and percentages of positive and negative agreement (Ppos and Pneg) were calculated. All the statistical analyses were performed using WINPEPI software (www.epi-perspectives.com/content/1/1/6) setting the p-value at 0.05.

RESULTS : The results showed a good accuracy and precision of the ELISA kit (CV< 10%), resulting the intra-assay CVs of 7.5% for positive and 5% for negative samples and the inter-assay CVs of 9.35% and 5.83% for positive and negative samples respectively. Antibodies against *T. gondii* were detected by ELISA in 45/50 IFAT positive and in 7/50 IFAT negative meat juice samples (Ppos: 88.2%, 95% confidence interval (CI) = 80.4-93.7%; Pneg: 87.8%, 95% CI = 79.4-93.3%). No significant differences were detected between the two assays (p= 0.56) and K value was "almost perfect" (K= 0.96).

CONCLUSIONS : Considering the difficulties in the producing of new antigens and in the development of new diagnostic kits, the precise definition of discriminatory power possessed from those already commercially available can represent a rational approach. The results obtained reveal that the ELISA kit has a good precision and accuracy on meat juice and can be considered a good test for toxoplasmosis epidemiological survey on swine although the comparison with IFAT shows the necessity of further studies to reduce the presence of false positives.

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Validation of *Calicophoron daubneyi* excretory/secretory antigens purified by Fast Protein Liquid Chromatography (FPLC)

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AIM: To estimate the usefulness of *Calicophoron daubneyi* excretory/secretory antigens purified by liquid chromatography for the diagnosis of rumen fluke infection in cattle.

MATERIALS AND METHODS: Paramphistomidae (*C. daubneyi*) specimens were collected in the rumen and reticulum of bovine slaughtered at a local abattoir (Lugo, Spain), and washed in PBS (phosphate buffered saline, pH 7.4). The flukes were incubated for 24 h at 37°C and 5% CO_2 in RPMI medium containing 0.5 mM PMSF. Finally, the *C. daubneyi*-excretory/secretory (CES) antigens were purified through an FPLC automated system.
The usefulness of the peaks was analyzed by the study of the humoral IgG response in cattle. Serum samples from 62 cattle (44 passing Paramphistomidae-eggs by faeces) were used for validating the ELISAs. The analysis of cross-immunity was conducted by means of sera from 70 cattle (39 passing *Fasciola hepatica*-eggs by faeces).

RESULTS: Four protein peaks of 57, 43-30, 25 and <13 kDa were solved by the FPLC and labeled C1-C4. The ROC analysis showed the highest area under the curve (AUC) was observed with the C4 and the lowest with the C3. By using the antigen peaks, values of 78% sensitivity and 95% specificity were recorded, with the best results by using the C3 products. By estimating the kappa statistics, the best agreement between the coprological and the immunoenzymatic probe was observed when using the C4 (= 0.773, *P*= 0.001).
The percentage of cattle monoinfected by *F. hepatica* and positive to the ELISA oscillated between 5% (C3, C4) and 15% (C1). Only 1 of the bovines negative to the shedding of *F. hepatica* was positive to the immunoassay (C3).

CONCLUSIONS: The FPLC provides a very useful one step procedure for collecting antigens from *C. daubneyi* flukes. By means of this method, four antigenic peaks helpful for the immunodiagnosis of infection by Paramphistomidae trematoda have been collected. The best results were given by a <13 kDa protein.

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Evaluation of Iscom ELISA for *Neospora caninum* detection in sheep milk

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AIM: The aim of the present work was to evaluate the Iscom Elisa for the detection of antibodies of *Neospora caninum* in sera and milk of Sardinian dairy sheep (Italy). Milk and tank bulk milk has been used in the past with Iscom ELISA for the serodiagnosis of Neospora in cattle; in the present note we report the first survey in sheep milk.

MATERIALS AND METHODS: Iscom Elisa survey for *Neospora caninum* on individual serum and milk samples was carried out in five different sheep farms of North Sardinia (A, B, C, D, E). Of these farms the last four were with negative anamnesis for *Neospora caninum* and any case of abortion while in the farm A was from September 2010 to March 2011 on outbreak of abortion was recorded involving about 20% of the adult sheep and the totality of the primiparous ewes.
In June 2011, 493 animals were bled from jugular vein and the sera were separated and stored at -20°C. Individual milk samples were collected from lactating ewes of the five selected farms.
Milk samples were skimmed, mixed with the contralateral and stored at -20°C before use. Sera and milk samples were then analysed using ISCOM Elisa kit following the manufacturer's instructions, using a percentage of positive value (PP) of 20 as cut-off.
Serum samples were diluted 1/100 and skimmed milk 1/2 in buffer solution before analysis. *N* sera *(n* negative and *n* positive) and n milk samples were tested three times by the same operator and the same equipment, in order to estimate the repeatability index. The percentage of antibodies was calculated using the following formula [(o.d.sample/o.d mean O.D positive control)*100]. Samples were considered positive when % Ab was >20.

RESULTS: Seroprevalences values found in the farms through analysis of blood serum and milk were reported in the following tables:

SERA

Farm	N°	Mean % ab	N.pos	N.neg	Prev
a	154	62.5%	103	51	66.9%
b	77	4.1%	0	77	0%
c	85	7.3%	9	76	10.6%
d	81	5.6%	8	73	9.9%
e	96	6.2%	0	96	0%

MILK

Farm	N°	Mean % ab	N.pos	N.neg	Prev
a	154	89.4%	103	51	66.9%
b	77	5.5%	7	72	6.5%
c	85	9.5%	8	77	9.4%
d	81	13.8%	8	73	9.9%
e	96	10%	10	87	9.4%

Seroprevalences for Neospora Ab found in sera for the different farms were statistically significative (^{2}= for trend with 4 df = 224.93; p<0.00001), as those found in milk in the same farms (^{2}= for trend with 4 df = 174.83; p<0.00001). Milk samples and blood serum show a correlation of % Ab Neospora sera and % Ab Neospora milk of 0.941 (P-Value = 0.000). with a concordance index of 0.96.

CONCLUSIONS: Iscom ELISA carried out on blood and milk showed an excellent correlation. thus confirming also for the first time in sheep the possibility of carrying out serodiagnosis of Neospora in milk. The high seropositivity found in the farm A. compared with "normal" Ab values against Neospora recorded in other farms (B. C. D. E) shows how this protozoa could have determined repeated abortions in sheep of this flock. Even further deepening should be done as well direct isolation of the parasite through PCR, milk samples screening with Iscom ELISA were quite interestingly, with an high correlation with sera and should be used for massive bulk milk survey on diary sheep farms as done in the past for diary cattle by the same authors (Varcasia et al. 2006; Parasitol Res 98(3):264-267).

SESSIONE 7

SISTEMATICA MOLECOLARE
ED EVOLUZIONE

Genetic variability and phylogeny of a new species of onchocercid infesting dogs: *Cercopithifilaria* sp.

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Microfilariae of *Cercopithifilaria* sp. *sensu* Otranto et al., 2011 (hereinafter referred as *Cercopithifilaria* sp.) is a recently studied filarioid infesting dogs (Otranto D et al, 2011, Vet Parasitol, 182: 221-229) transmitted by the brown dog tick, *Rhipicephalus sanguineus* (Brianti E et al, 2012, Vet Parasitol, 183: 330-337). Since its first retrieval, microfilariae of this filarioid have been found in the skin of dogs from different areas of the Mediterranean basin (e.g., Italy, Greece and Spain), suggesting that it is well spread among canine populations of Europe (Otranto D et al, 2012, Parasit Vectors, 5: 1). The lack of information on *Cercopithifilaria* sp. is due to the difficulties in finding dermal microfilariae in tissues/skin. Consequently, there are still many questions regarding its biology and ecology that need to be addressed.

AIM: To assess (i) a PCR protocol for the specific diagnostic of *Cercopithifilaria* sp. (ii) the genetic variability this filarioid based on cytochrome c oxidase subunit 1 (p*cox*1) partial gene sequences, and (iii) its phylogenetic relationship with other filaroids within the Onchocercidae family.

MATERIAL AND METHODS: Dog skin samples (n = 1387) and *R. sanguineus* ticks (n = 876) were collected at different time points from southern Italy (i.e., Apulia, Basilicata and Sicily), central Spain and eastern Greece. All samples were molecularly processed for specific amplification of partial cytochrome *c* oxidase subunit 1 gene (p*cox*1, ~304 bp) using primer pair CbCox1F-NTR specifically designed for *Cercopithifilaria* sp. (Otranto D et al, 2011, Vet Parasitol, 182: 221-229). The nucleotide variability was calculated and the evolutionary relationship among p*cox*1 sequences herein generated and those belonging to other species of Onchocercidae family available in GenBank™ were inferred using the Neighbor-Joining method (Saitou N, Nei M, 1987, Mol Biol Evol, 4: 406-425).

RESULTS: A total of 176 (12.5%) and 24 (2.7%) p*cox*1 amplicons were produced from 1411 skin and 876 tick samples, respectively, being all (n = 200) sequenced. Sixteen haplotypes were found, of which haplotypes I (n = 146, 73.0%) and X (n = 27, 13.5%) were the most prevalent, followed by haplotype VIII (n = 10, 5.0%) and other 13 haplotypes (n = 17, 8.5%). Three haplotypes (II, V and VI) were found exclusively in ticks. The overall intraspecific nucleotide variation among p*cox*1 haplotypes ranged from 0.4 to 3.5% (mean = 1.2%). Phylogenetic analysis of the nucleotide sequence data showed a clustering of *Cercopithifilaria* sp. with the other *Cercopithifilaria* species (with strong statistical support) with the exclusion of other onchocercid genera.

CONCLUSIONS: This study provides a new tool for the molecular diagnosis of *Cercopithifilaria* sp., as an alternative to traditional parasitological methods. Significant genetic variation among sequences found in samples coming from different areas of the Mediterranean basin might be explained by the complex ecology and transmission patterns as well as the high mutation rate of the mitochondrial DNA and/or inbreeding associated with hosts and their vectors. Furthermore, the relationship between genetic variability and vector susceptibility was probably due to the feeding behaviour of ticks, which can take multiple blood meals either on the same or on different dogs, being three haplotypes (i.e., II, V and VI) found exclusively in ticks. The topology of the inferred tree by p*cox*1 was efficacious in resolving this species of *Cercopithifilaria* sp. within the Onchocercidae family and within the clades of the genus *Cercopithifilaria* (Otranto D et al, 2011, Vet Parasitol, 182: 221-229). Further studies on this nematode are needed to elucidate its potential pathogenic role for dogs.

Large-scale sequencing of parasitic helminths: the next generation

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In the last few years, there has been a massive expansion in the demand for and access to low cost, high-throughput sequencing, attributable predominantly to the establishment of next-generation sequencing (NGS) technologies, which allow massively parallelised sequencing of genomes (i.e. all of the DNA in the cell) and transcriptomes (i.e. all of the mRNA encoded by the genome) (Metzker MF, 2010, Nat Rev Genet, 11: 31-46). NGS platforms, such as 454 Life Sciences/Roche (Margulies M et al, 2005, Nature, 437: 376-380) and Solexa/Illumina (Bentley DR et al, 2008, Nature, 456: 53-59), have transformed parasite genomics by decreasing the cost, time and performance limitations of previous approaches. This situation has resulted in an explosion of the number of nucleic acid sequences deposited in public databases; most of these sequences still require detailed functional annotation. The annotation of large datasets produced by NGS has demanded major advances in computing capacity and performance as well as new bioinformatic tools to draw biologically meaningful information or interpretations from such datasets. Clearly, user-friendly and flexible bioinformatic pipelines are needed to assist researchers from different disciplines and backgrounds in taking full advantage of the advances in NGS. Increasing access to high-throughput sequencing will benefit a range of areas of parasitology. Profound explorations of genomic, transcriptomic and proteomic datasets of parasites will have major implications for improving our understanding of their development and reproduction, survival in and interactions with the host, virulence, pathogenicity, immunobiology, the diseases that they cause and drug resistance (Cantacessi C et al, 2012, Biotech Adv, 30: 469-488), and have the potential to pave the way to novel approaches for treatment, diagnosis and control. This presentation will provide a brief account of key sequencing and bioinformatic technologies, and describe recent advances in the establishment and applications of semi-automated bioinformatic pipelines for the assembly and annotation of genomic and transcriptomic datasets, with a view toward an improved understanding of parasites at the molecular level and the development of new methods of intervention for parasites.

Mosquito/microbiota interactions: from basic research to biotechnological perspectives in mosquito borne diseases control

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AIM: Mosquitoes show a great ability to adapt to very different types of habitats. It is very likely that their microbiota have provided the mosquito a great capacity to adapt to many different environments. Since some years we have started a study for the identification of mosquito symbionts. In particular we have identified and focused our interest on two of them:
1) The acetic acid bacterium *Asaia*; 2) The yeast *Wickerhamomyces anomalus.* We have identified these microorganisms for the first time as symbionts of mosquito vectors (Favia G et al, 2007, Proc Natl Acad Sci USA, 104: 9047–9051.). They show all required features for their use in a paratransgenic or symbiotic control of mosquito borne diseases. Both were found in some malaria and dengue virus mosquito vectors. *Asaia*, the most prevalent bacterium in both natural and lab mosquito populations, has been detected in the midgut, salivary glands, the same anatomic districts of *Plasmodium* (fig.1), and reproductive organs (Favia G et al, 2007, Proc Natl Acad Sci USA, 104:9047–9051). Consequently, it has been proposed to express anti-pathogen(s) molecules directly in the midgut of the mosquito to exert an inhibitory effect against pathogens. Its presence in the reproductive organs permits a vertical transmission thus providing the basis for the introduction of engineered bacteria into mosquito populations in the field (Damiani C et al, 2008, Curr Biol , 18:1087–1088). *W. anomalus* is present at all the developmental stages of mosquito, where it localizes in the midgut and reproductive organs (fig.2). Some strains of *W. anomalus* produce killer toxins with antimicrobial activities against several human pathogens including *Leishmania* spp. (Ricci I et al, 2011, Environ Microbiol , 13:911-921; Ricci I et al, 2011, J Applied Entomol, 135:487-493). The finding that *W. anomalus* associates with some mosquito vectors of several human parasites led to the proposition to its use to control mosquito-borne diseases.

MATERIAL AND METHODS: The identification of *Asaia* and *W. anomalus* in malaria vectors, was performed through the use of different techniques such as PCR, TEM, ISH and FISH. Using recombinant strains of *Asaia* expressing fluorescent proteins, we have demonstrated the ability of the bacterium to colonize the same organs colonized by *Plasmodium.* Finally by IFA and western blot approaches we the *W. anomalus* killer toxin with antimicrobial activity.

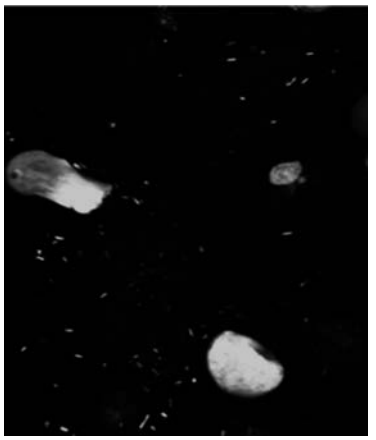

Fig. 1. Co-localization of florescent recombinant *Asaia* (green) and *Plasmodium* (green) in midgut of *An. stephensi*.

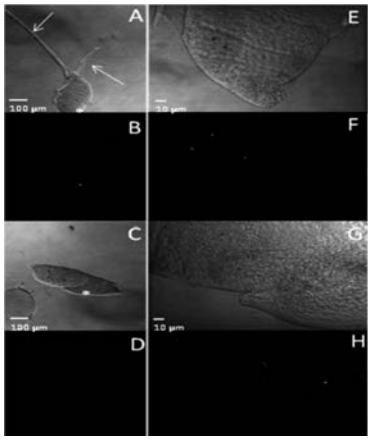

Fig. 2. *W. anomalus* FISH of in the male mosquito reproductive organ. In parels A, C, E, and G are shown confocal microscope merged images (phase contrast and red-Cy3-oligonucleotidic probes); panels E and G are enlarged particulars of image A and C respectively, where as terisk indicates testicles and arrow the spermatic ducts. In panels B, F, D and H are visible *W. anomalus* specific red signals in dark field.

RESULTS: Experiments performed, with engineered bacteria expressing fluorescent proteins, have provided the feasibility of using *Asaia* to express anti-parasite effectors to control malaria and others mosquito-borne diseases. At the present we have focusing on the "function(s)" that *Asaia* is exerting on adult mosquito vitality.

We have already described that *Asaia* reduce the time required from the larvae to develop in pupae (Chouaia B et al, 2012, BMC Microb, 12:(Suppl 1): S2). Preliminary observations indicate that the *W. anomalus* strains isolated from a mosquito also produce killer toxins, thus suggesting the possible use of the yeast in the control of mosquito-borne diseases. Studies for the biochemical characterisation of this protein are in due course.

CONCLUSIONS: The field of mosquito symbiosis is providing an important contribution to the knowledge of the biology of mosquitoes. Our studies contribute to a better knowledge of the relationships between mosquito and symbionts as well as a proof of principle about the feasibility of these approaches in mosquito borne diseases control.

Deepening the evolutionary relationships in *Ascaris* spp.

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Due to their significant morphological and biological similarities, the taxonomic identity of *Ascaris lumbricoides* (from humans) and *Ascaris suum* (from pigs) still represents a debated scientific issue. Several hypotheses have been proposed to explain the taxonomic status and origin of the ascarids above (Leles D et al, 2012, Parasit Vectors, 5:42), namely: a) *A. suum* and *A. lumbricoides* are two valid species; b) *A. suum* is the ancestor of *A. lumbricoides*; c) *A. lumbricoides* is the ancestor of *A. suum*; d) *A. suum* and *A. lumbricoides* are conspecific.

The main aim of the present study was to investigate the genetic variation of nuclear and mitochondrial target regions (ITS and *cox*1, respectively) within and among *Ascaris* populations of human and pig origin, which were collected from a range of geographical regions and compared at local and global scale, in order to infer the evolutionary and phylogenetic relationships among samples.

A total of 130 *Ascaris* spp. worms from pigs and humans were examined (77 from Italy and 53 from Eastern European countries) using a PCR-RFLP approach on nuclear ITS rDNA that allow the differentiation of the two *Ascaris* species. A representative geographical sub-sample was also analysed by sequencing *cox*1 mtDNA (barcoding), to compute variability at population level. Data were compared to GenBank retrieved sequences from endemic regions (i.e., Brazil, Japan, Zanzibar and China), to infer pattern of genetic differentiation using phylogenetic and phylogeographic approaches.

The overall results showed no fixed differences between human and pig *Ascaris*. The RFLP analysis confirmed that pigs were the source of human infection in non-endemic area (i.e., Italy) and the presence of hybrid genotype between the two species, circulating in both host species. The analysis of molecular variance carried out twice using two different criteria in grouping samples (endemic-non endemic origin and host affiliation) showed that "endemic-non endemic" factor is more relevant than "host-affiliation" in shaping genetic variability. Results from phylogenetic analysis described a puzzled scenario, with no evident host affiliation pattern. The phylogeographic networks indicated a genetic differentiation of the Slovak sample with respect to the other area of collection and to database retrieved sequences.

These results are in agreement with previous evidences about the taxonomic status and evolutionary picture of *A. suum* and *A. lumbricoides* (Anderson TJC, 2001, Trends Parasitol, 17: 183; Peng and Criscione, 2012, Infect Genet Evol, 12:227; Liu GH et al, 2012, Gene, 492:110), suggesting the existence of gene flow between the two taxa, with relevant implications on the systematics, transmission and control programs.

The mitochondrial genome of *Ixodes ricinus*: an evolutionary scenario from the living fossil *Lymulus polyphemus* to modern ticks

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AIM: determination of parasite mitochondrial (mt) genome sequences is useful for phylogenetic reconstructions, identification of molecular diagnostic targets, determination of metabolic networks, and development of novel antiparasite molecules. The mt genome of the hard tick *Ixodes ricinus* has not yet been determined, despite the characteristics of this arthropod, which is the main vector of Lyme borreliosis and other diseases in Europe, and harbors a bacterium capable of invading mitochondria. The mt genome of *I. ricinus* has recently been determined in our laboratories, with the aim of investigating the mitochondrial phylogenomics of ticks and other chelicerates, and of setting the bases for the reconstruction of metabolic interactions among the tick and its symbonts.

MATERIALS AND METHODS: an adult female of *I. ricinus* was collected from the field and DNA extraction was performed. Partial sequences of *I. ricinus* genes *cox1*, *cob* and *rrnS* as well as the complete mt genome sequences of other *Ixodes* species were obtained from GenBank. Using these sequences and the multi-alignment of mt genomes, we designed 17 mitochondrial-specific primers that were used in several combinations for mt fragment amplifications. Mitochondrial fragments were amplified by PCR. Positive and unambiguous PCR products were directly sequenced by ABI technology (Applied Biosystems), corrected and assembled using Geneious Pro 5.3. Phylogenomic analyses were performed on the amino acid and nucleotide sequences of the 13 protein coding gene sequences (PCGs) from the 10 previously sequenced Ixodida mt genomes, using Maximum Likelihood and Bayesian inferences.

RESULTS: the mitochondrial genome of *I. ricinus* is a circular molecule of 14566 bp with AT-content of 78.7%. The genome contains all the 37 genes usually found in metazoan mtDNAs. Thirteen genes are protein coding (PCG); 2 ribosomal RNAs and 22 tRNAs were also recorded on the two strands. The overall GC- and AT-skews of the major strand (J-strand) are -0.26 and -0.023 respectively. PCGs total length is 10882 bp for a total of 3617 amino acids. Six of the 13 PCGs present an incomplete stop codon (U--). The J-strand encodes for 13 tRNAs and for 9 PCGs. The minority strand (N-strand) encodes for 4 polypeptides, 9 tRNA and the two ribosomal RNAs. All the 22 tRNAs present in the genome show a typical cloverleaf secondary structure, with tRNASer(AGN) lacking the DHU arm. Phylogenetic analyses conducted on the 13 protein-coding genes using different methods and datasets confirm previous topology within the Prostriata group of the Ixodidae, while the relationships within Metastriata are not fully resolved by some methods/datasets. The mt gene order of *I. ricinus*, which is shared with the Argasidae and with part of the Ixodidae, is the same as that of the living fossil *Lymulus polyphemus.*

CONCLUSIONS: genome comparisons, including basal chelicerates and representative from other arthropod lineages, revealed the presence of plesiomorphic character states in the mtDNA of *I. ricinus*, likely preserved along over 400 million years of evolution. The complete sequences of *cox1*, 12S rDNA, a possible control region and other genome portions will provide useful markers for species identification and for the study of the genetic structure of the European sheep tick populations.

Genetic characterization of carnivore capillarids

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Capillarinae is a large and taxonomically complex group of nematodes that includes more than 300 species infecting mammals, birds and amphibians worldwide and three zoonotic species: *Capillaria hepatica* (syn. *Calodium hepaticum*), *C. philippinensis* and *C. aerophila* (syn. *Eucoleus aerophilus*). Dogs and wild carnivores can be infected by several Capillarinae nematodes: *C. aerophila* and *C. boehmi* (syn. *Eucoleus boehmi*) are respiratory parasites, while *C. plica* (syn. *Pearsonema plica*) lives in the urinary bladder, *C. putorii* (syn. *Aonchotheca putorii*) in the stomach and small intestine and *C. hepatica* in the liver. Diagnosis of capillarid infections often relies on the identification of the nematode eggs, but since the eggs of these species are very similar, morphological diagnosis can be challenging. For these reasons, the development of biomolecular diagnostic methods is desirable, but very little is known on Capillarinae from a genetic point of view. The only partially characterized gene sequences are the small subunit (18S) rRNA of bird capillarids (Honisch M and Krone O, 2008, J Helminthol, 82: 129-133) and the mitochondrial cytochrome oxidase subunit I (COI) of rodent capillarids and of *C. aerophila* (Zhu X et al, 2000, Int J Parasitol 30: 933-938; Di Cesare A et al. 2012, J. Clin. Microbiol. doi:10.1128/JCM.00103-12).

AIM: The aim of this work was to characterize the small subunit (18S) rRNA gene of several species of Capillarinae that can affect dogs, cats and wild mammals.

MATERIALS AND METHODS: Adult worms of *C. aerophila* and *C. plica* were obtained from red foxes (*Vulpes vulpes*) from north-west Italy and Switzerland, and specimens of *C. putorii* from red foxes from north-west Italy. Eggs of *C. hepatica* were obtained from dissected liver parenchyma of one fox and one vole (*Arvicola terrestris*), those of *C. boehmi* were isolated from the faeces of two dogs using a combination of flotation and sieving (as described in Al-Sabi MN et al, 2010, Parasitol Res, 107: 135–140). Species were identified following morphological keys and on the basis of their localization in the host (Moravec F, 2000, Acta Soc Zool Bohem, 64: 271-304; Skrjabin KI et al, 1970, Trichocephalidae and Capillaridae of animals and man and the diseases caused by them, Keter, Jerusalem). For DNA isolation, individual worms were washed in PBS, triturated in a 1.5 ml Eppendorf tube, and genomic DNA was extracted using a commercial kit (DNA mini kit, Qiagen, Hombrechtikon, Switzerland) following the manufacturer's instructions. DNA of concentrated eggs was also isolated with this kit following some previously described modifications (Fahrion AS et al, 2010, Vet Parasitol, 177, 186-189). Part of the small subunit (18S) rRNA gene was amplified with AmpliTaq Gold® using primers 18S 965 and 18S 1573R (Powers TO et al, 2009, Molec Ecol, 18: 985-996). Purified amplicons were directly sequenced by a private company (Synergene, Zurich, CH).

RESULTS: Part of the 18S (~620 bp) rRNA gene was amplified from the genomic DNA of *C. aerophila*, *C. boehmi, C. plica*, *C. hepatica* and *C. putorii,* yielding the first such sequences of these 5 capillarid nematodes, and no intraspecific variation was observed. The 18S rRNA gene sequences of these species are highly conserved: *C. aerophila* and *C. boehmi*, e.g., differ at only 3 polymorphic sites. Sequences of the same species from different geographical origin (see *C. aerophila* and *C. plica*) and from different hosts (see *C. hepatica*) were identical. Moreover, 18S rRNA gene sequences were also highly similar to sequences available in GenBank from the same taxonomic group, the bird capillarids *Eucoleus dispar* (accession nr. EU004821) and *Capillaria tenuissima* (accession nr. EU004822), confirming the high homology at this site.

CONCLUSIONS: An accurate diagnosis of capillarid infections is important for epidemiological studies and assessing control efforts. The amplified gene locus is highly conserved among different species and shows too low variability to be useful as a specific diagnostic target, but could represent a first step for a better understanding of the taxonomic relations in the Capillarinae group. In fact, the analysis of the differences between the species reflects the latest taxonomic revision (Moravec F, 1982, Folia Parasitol, 29: 119-132). Further studies will be needed to identify loci with higher variability, e.g. the "DNA barcoding region" (mitochondrial COI gene), and the potential of this gene as a species-specific marker in this nematode group is currently under evaluation.

Dissecting the genetic basis of differentiation and speciation in recently radiated species of the *Anopheles gambiae* complex

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AIM: The seven cryptic mosquito species of the Afrotropical *Anopheles gambiae* complex (Coluzzi et al., 2002, Science, 1415: 8) - and the M and S molecular forms within *An. gambiae* s.s. (della Torre, et al., 2005, IBMB, 755:69) - represents an emblematic example of fast adaptive radiation and incipient speciation, but the recent origin of this group creates relevant constraints to the reconstruction of its phylogenetic history. This speciation process strongly affects malaria epidemiology in sub-Saharan Africa, due to different ecology, behaviour and contribution to malaria transmission of the various species and forms. In fact, among them, some are major human malaria vectors, such as *An. gambiae* s.s. M and S forms, while others are minor or locally important vectors and non-vectors. Here are summarized the substantial progresses made in the understanding of the inter- and intra-specific diversification in this species-complex and some of the recent efforts to reconstruct their phylogenetic history.

MATERIALS AND METHODS: Analyses on structural variations in polytene chromosomes (i.e. chromosomal paracentric inversions), estimation of genetic distances in sexual and autosomal loci and genotyping approaches through wide-genome scan (based on the *An. gambiae* reference genome; Holt et al., 2002, Science, 129: 49) were carried out in natural populations of the *An. gambiae* complex. Statistical analyses were performed to infer phylogenetic trees and to highlight population genetic structures.

RESULTS: Data gathered so far on chromosomal inversions contributed to depict an evolutionary frame for the species complex (Ayala & Coluzzi, 2005, PNAS, Suppl. 1, 6535: 42; Sharakhov et al., in press), although it partially contradicts affinities expected on the basis of their ecology and adaptations. Moreover, chromosomal phylogeny is also not often consistent with reconstructions obtained with other molecular markers, due to shared ancestral polymorphisms and introgression events (reviewed in White et al., 2011, Annu. Rev. Ecol. Evol. Syst., 42: 111–132). Recent results also highlighted genomic areas and loci under positive selection that could be involved in the ongoing speciation process within *An. gambiae* s.s. (Lawniczak et al., 2010, Science, 512: 4) and that, thus, could have played instrumental roles in the ecotypic differentiation both within and across the semipermeable boundaries of M and S molecular forms.

CONCLUSIONS: Despite the large amount of data obtained on the genetic divergence within the *An. gambiae* complex, the reconstruction of a stable phylogeny among species and forms is still hindered by discrepancies between chromosomal and genetic data. Recent advances in genomic technologies will hopefully provide novel tools to resolve the conflicting results in the phylogeny of this group. A reliable reconstruction of the evolutionary history would allow to better understand the inheritance of evolutionary traits and adaptive abilities of these vector species, thereby helping in comprehending malaria epidemiology and optimizing effective vector-mediated control measures.

Investigating the evolutionary link between malaria and autoimmunity: a large scale immunogenetic study in two West African populations

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AIM: Despite equivalent exposure to infection and comparable use of protective measures, the Fulani of West Africa have been shown to mount stronger immune responses to *Plasmodium falciparum* antigens and to be less susceptible to infection and mild disease than sympatric populations (Modiano et al. 1996, PNAS). The Fulani also show a higher response to other pathogens, and both their Th1 and Th2 responses are enhanced, suggesting that their resistance to malaria could result from a generally stronger immune activation. Key genes related to T regulatory cell function are indeed down-regulated in the Fulani (Torcia et al. 2008 PNAS). This disorder of immune homeostasis could be driven by genetic factors positively selected by *P. falciparum* and may underlie the higher susceptibility of the Fulani to diseases with autoimmune pathogenesis reported in the literature (Fish et al. 1987, Diabetologia; Mahe et al. 1996, Br J Dermatol; Brieger et al. 1997, Trop Med Int Health). The general aim of the proposed investigation is to explore the genetic basis of the lower susceptibility to malaria observed in the Fulani, and in particular to evaluate the role of autoimmunity loci.

MATERIALS AND METHODS: To investigate this hypothesis, we conducted a large-scale epidemiological study in rural villages of Burkina Faso inhabited by Fulani, Mossi and Rimaibe communities. The field study lasted 2 years (2007-8) and consisted in a combination of cross sectional and longitudinal surveys. At each survey we collected parasitological (*P. falciparum* index and parasite density), clinical (fever, anemia, spleen size) and serological data (IgG levels against *P. falciparum* and self antigens). We genotyped 363 Single Nucleotide Polymorphisms (SNPs) on 2186 samples using the Sequenom System, based on allele-specific primer extension and MALDI-TOF Mass Spectrometry. SNPs included polymorphisms previously shown to be involved in resistance to severe malaria, in resistance to infection and/or in antibody production, as well as polymorphisms at autoimmunity loci. We conducted population genetic analyses and genetic association analysis with parasitological, clinical and serological phenotypes using the free software package R.

RESULTS: Principal component analysis revealed that Mossi and Rimaibe (Non-Fulani) are not genetically distinct among themselves, whereas the Fulani are a clearly distinct group, in agreement with data obtained on HLA class I-II alleles (Modiano et al. 2001, Tissue Antigens; Lulli et al. 2009, Hum Immunol). We therefore compared allele frequencies and calculated Fst, a measure of population genetic differentiation, between Fulani and Non-Fulani. We observed that the proportion of autoimmunity SNPs with Fst>0.05 (indicating moderate/high differentiation and corresponding to at least a two-fold difference in allele frequency) is 20%, versus 10% shown by other loci (p=0.03). Genetic association analysis of susceptibility to infection and infection levels showed association signals among genes involved in resistance to severe malaria (*TNF, DDC, ABO, IFNG-IL22, GNAS, MECP2, G6PD*). Furthermore we observed strong signals of association, both in Fulani and Non-Fulani, in the 5q31 region of the genome, which has been previously linked to *P. falciparum* infection levels (Rihet et al. 1998, Am J Hum Genet; Mangano et al. 2008, Genes Immun). Finally, association signals were also observed among genes related to T regulatory cell function and/or involved in autoimmunity (*TGFBR3, CD25, FCGR2A, CR1, IL1R1L-IL18RAP, IL1A-IL1B, IL21, BLK, ORMDL3, TGFB1*).

CONCLUSIONS: The results of our investigation support the hypothesis that malaria has exerted a selective pressure on the immune system and has affected is evolution, and provide evidence that common gene regulatory networks could underlie susceptibility to malaria and to immunological disorders such as autoimmune diseases.

SESSIONE 8

MALATTIE TRASMESSE DA ARTROPODI

Molecular investigation on tick-borne pathogens of zoonotic concern in ticks from Emilia Romagna region

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AIM: In the last decades, the prevalence of tick-borne zoonoses has been increased, because of a higher number of ticks and of the interest of people in outdoor activities. The aim of this study was to assess the prevalence of tick-borne agents, such as piroplasms, *Anaplasma phagocytophilum* and *Borrelia burgdorferi* s.l, in ticks from parks of Emilia Romagna and from animals and humans from the same areas.

MATERIALS AND METHODS: The survey was carried out from 4 sites located in three parks of the provinces of Bologna and Ravenna: two sites were in the park "Gessi Bolognesi and Calanchi dell'Abbadessa" and one in the park "Monteveglio Abbey" (both in the province of Bologna) one site was located in the park "Carnè" (in the province of Ravenna), usually attended by people. In each site questing ticks were collected by flagging every 15 days from April to October 2010; feeding ticks were sampled from different animals (13 dogs, 13 horses, a cat and a cattle) and three humans by private vets and owners. Ticks were stored in alcohol 70% and identified by taxonomic keys (Manilla, 1998, Fauna d'Italia Vol. XXXVI. Acari Ixodida, Ed. Calderini, Bologna; Iori et al., 2005, Parte III Zecche d'Italia, In: Cringoli G. (Ed.) Mappe parassitologiche 6-Zecche, Rolando Editore, Napoli). For molecular analyses, ticks were processed in pools of 10 larvae or 5 nymphs (from the same site and sampling time) or individually when adults. DNA was extracted by commercial kit (Nucleo Spin Tissue, Macherey NagelGmbH & Co. Germany), according to the manufacturer's instructions, increasing the period of lysis of ticks from 10 to 30 minutes. A total of 393 samples obtained from 330 larvae, 1165 nymphs and 127 adults were tested for the presence of piroplasms by the amplification of 18S rRNA (Armstrong et al., 1998, Am J Trop Med Hyg, 58: 739–742). Only the samples of nymphs and adults (360) were analyzed for the presence of *A. phagocytophilum* and *B. burgdorferi* s.l. Taqman real-time PCR were performed for msp2/p44 gene of *A. phagocytophilum* (Drazenovich et al., 2006, Vector Borne Zoon Dis, 6: 83-90) and for 16S rRNA gene of *B. burgdorferi* (Barbour et al., 2009, Am J Trop Med Hyg, 81: 1120-1131) considering positive a Ct-value < 40. The PCR positive samples for piroplasms were purified with Nucleo-Spin Extract II (Macherey-Nagel) and sequenced by BMR Genomics (Padova).

RESULTS: The PCR recognized piroplasms in 30 (7.6%) out of 393 samples. At sequence analysis, 17 samples (4.3%), 2 from feeding ticks and 15 from environment, were positive for *Theileria buffeli/sergenti/orientalis* group; 11 samples (3.4%) of questing ticks collected in all sites were positive for *Babesia* EU1 and 2 samples (0,6%) of questing ticks from Carnè park were positive for *B. divergens/capreoli*. All the positive questing ticks were *Ixodes ricinus* species, whereas the 2 positive feeding ticks were adults of *Hyalomma marginatum marginatum,* sampled from a dog and a horse. Thirty-three samples out of 360 (9.2%) were positive for *A. phagocytophilum*. Among these, all the 23 positive questing ticks were *I. ricinus* species collected from all sites but not in the park "Carnè"; the 10 positive feeding ticks included 6 *I. ricinus* from dogs (2), cat (1) and human (1), one *Ixodes acuminatus*, one *Rhipicephalus turanicus*, one *R. bursa* and one *R. sanguineus* collected from 3 dogs. Concerning *B. burgdorferi* s.l, 78 positive ticks (21.6%) out of 360 were obtained; only *I. ricinus* questing ticks from all the sampling sites resulted infected. Sixteen samples, from all sites, were co-infected with at least 2 pathogens.

CONCLUSIONS: This study revealed the presence of zoonotic agents, such as *Babesia* EU1, *A. phagocytophilum* and *B. burgdorferi* s.l, in ticks in all these parks of Emilia Romagna, indicating a potential risk for people visiting these areas. These results enhance the knowledge on the distribution of these agents in areas of Emilia Romagna not yet studied.

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Investigations on the presence Haemoparasites in cattle of Caprivi strip (Northern Namibia)

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AIM: In January 2010 started a project on ticks and Tick Borne diseases coordinated by Istituto "G. Caporale", the Italian reference centre for the study and assessment of exotic disease of animals and funded by Italian Ministry of Health. The project is being conducted as a collaboration of the three countries within which the area of study falls: Namibia, Botswana and Zambia.
The aim of the project was to study the ecology, biology, ethology and seasonal dynamics of hard ticks in domestic animals as well as the epidemiology of tick borne diseases occurring in eastern Caprivi (Zambia, Namibia and Botswana) and Southern Caprivi (Botswana and Namibia). This work describes the results obtained by testing cattle for Babesioses and Theilerioses in a survey performed in Caprivi strip (Namibia).

MATERIALS AND METHODS: Caprivi is a strip of land running east to west in the northeastern corner of Namibia, having a depth varying between 30 and 100 km bordering with Zambia in the north, Zimbabwe and Botswana in the East and South respectively. In January 2011 during the FMD vaccination campaigns in Caprivi, 174 cattle were sampled from 6 crush-pens. Each animal selected was also checked for ticks in dewlap and belly, ears and perianal region. From the same area and with the same protocol other 100 animals were sampled in January 2012. Specific Real time PCRs for 5 *Theileria* species (*T. parva, T. velifera, T. mutans, T. taurotragi* and *T. annulata*) and for *Babesia bigemina* and *Babesia bovis* were set up and tests were carried out on blood samples collected.

RESULTS: The following ticks were recorded; *Rhipicephalus* (*Boophilus*) *decoloratus*, *Rhipicephalus appendiculatus/zambesiensis*, *Rhipicephalus evertsi*, *Amblyomma variegatum* and *Hyalomma truncatum*. The prevalence of each of the seven tick-borne pathogens detected by Real time PCR ranged from 0% to over 88%. In particular, no animal was positive for *T. parva*, the most important bovine theilerial species in sub-Saharan Africa, and for *Babesia bovis,* which is generally more pathogenic than *B. bigemina*. Conversely, 88% and 69% of the animal were positive for *T. mutans* and *T. velifera* respectively, which are considered mild or non-pathogenic species.

CONCLUSIONS: Tick borne diseases are one of the most important causes of livestock loss in Sub-Saharan Africa, among these Babesioses and Theilerioses were prominent. They are thus of particular relevance for any measure aiming to improve livestock production. On the other side, high activities of *Amblyomma variegatum* ticks can be considered the reason of the high prevalence reported for *T. mutans* and *T.velifera*. The negative results obtained by Real time PCR for *T. parva* need to be investigated considering that in 2009 survey carried out in Caprivi in free ranging African buffaloes detected a prevalence of 56% for the parasite. The reason of this apparent discrepancy is likely linked to the ecology of the disease in that specific environment and mainly to the host, parasite and vector relationships. Similarly the absence of *B. bovis* could be due of the lack in Caprivi of the vector, *Rhipicephalus* (*Boophilus) microplus,* that is reported in bordering countries. Despite the high number of ticks and the high level of prevalence for *B.bigemina*, clinical cases of tick borne disease reported are few, suggesting that endemic stability and the resistance of local breed may play a role in protecting animals from the clinical diseases. Development of control strategies necessary has to be transboundary since the study area can be considered epidemiologically as one entity where the environmental conditions affecting the biology of the tick vectors of different diseases are shared between different countries.

Candidatus Neoehrlichia mikurensis: a retrospective study in *Ixodes ricinus* ticks collected in north-eastern Italy, 2006-2011

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AIM: In the last decade, a new pathogen belonging to the family of *Anaplasmataceae* was detected in several countries from *Ixodes* ticks and rodents. The bacterium was characterised in 2004 from *Rattus norvegicus* and *Ixodes ovatus* in Japan (Kawahara M et al, 2004, Int J Syst Evol Microbiol, 54: 1837-1843) and proposed as "*Candidatus Neoehrlichia mikurensis*" (*CNm*). In 2003 an Ehrlichia-like 16S rRNA gene sequence was found in a *I. ricinus* tick removed from a human in Belluno Province (Veneto region, north-eastern Italy), provisionally named "*Candidatus Ehrlichia walkerii*" (Brouqui P et al, 2003, Ann N Y Acad Sci, 990: 134-140). This sequence was later recognised as *CNm* and represents the first report of this new species in Italy. In 2010 several human cases were reported in immune-compromised people, i.e. two cases from Germany (von Loewenich F D et al, 2010, J Clin Microbiol, 48: 2630-2635), one from Sweden (Welinder-Olsson C et al, 2010, J Clin Microbiol, 48: 1956-1959) and one from Switzerland (Fehr J S et al, 2010, Emerg Infect Dis, 16: 1127-1129). A clinical case in a dog has also been reported (Diniz P et al, 2011, J Clin Microbiol, 49: 2059-2062). The aim of this work was to evaluate retrospectively the presence and prevalence of *CNm* in *I. ricinus* ticks collected in north-eastern Italy during the previous years.

MATERIALS AND METHODS: A total of 1118 *I. ricinus* collected from several sites located in Friuli-Venezia Giulia and Veneto Regions from 2006 to 2011 were analysed for the detection of *CNm*. Ticks were divided into three groups: (i) 66 ticks removed from humans since 2006; (ii) 192 adult questing ticks collected from 2006 to 2008, and (iii) 860 questing ticks collected in a restricted area of Belluno Province in 2011. Adult ticks were analysed singularly, while nymphs and larvae were grouped into maximum 10 and 20 specimens per pool, respectively. All the ticks removed from humans were processed singularly. A conventional PCR targeting the groEl gene (1024 pb) was used for the detection of *CNm* (Diniz P et al, 2011, J Clin Microbiol, 49: 2059-2062), and the positive results were confirmed by DNA sequencing.

RESULTS: Overall, 29 samples (2.6%) were found positive for *CNm*. The results according to each group and development stage are summarised in the following table:

	adults		nymphs		larvae		
	tested	pos (P)	tested	pos pool (MIR*)	tested	pos pool (MIR*)	Total
Ticks from humans	19	2 (10.5%)	38	1 (2.6%)	9	-	66
Ticks 2006-2008	192	20 (10.4%)	-		-	-	192
Ticks 2011 (BL)	70	3 (4.3%)	475	3 (0.6%)	315	-	860
Total	281	25 (8.9%)	513	4 (0,8%)	324	-	1118

** MIR=minimum infection rate (considering that at least 1 tick is positive in a positive pool)*

Adults were significantly more infected than nymphs (chi-square test; $p<0.01$), and larvae were never found positives. The positive ticks removed from humans were collected in 2008 and 2009. Ticks from group (ii) were found positive all over the years of collection (2006 to 2008). The 26 nucleotide sequences obtained were all identical to each other.

CONCLUSIONS: In this study the circulation of *CNm* in *I. ricinus* ticks of north-eastern Italy was demonstrated since 2006. *CNm* currently represents the third pathogen, after Lyme diseases agents and *Rickettsia* spp., most likely transmitted to animal and humans by adult and nymphal ticks in this area. However, some authors found that although a fifth of the people had removed at least one tick infected with *CNm*, none displayed symptoms, suggesting that its transmission may not be immediate and/or that immunocompetent individuals may not be affected (Richter D, Matuschka FR, J Clin Microbiol. 2012, 50(3): 943-7). The negativity of the larvae suggests that the transovarial transmission does not occur or occurs with very low efficiency. In conclusion, *CNm* can be regarded as an emergent agent in Italy too as for Europe (Andersson M, & Råberg L, Emerg Infect Dis 2011, 17(9): 1716-8) and deserve attention and further research. (*This work was supported by the Veneto Region).*

Apparent tick paralysis by *Rhipicephalus sanguineus* (Acari: Ixodidae) in dogs

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Rhipicephalus sanguineus (Acari: Ixodidae), commonly known as the "brown dog tick", is the most widespread ixodid infesting dogs. This tick species has a cosmopolitan distribution in tropical, subtropical and even temperate regions (Dantas Torres F, 2008, Vet Parasitol 152: 173-185). Its primary importance in medical and veterinary sciences mainly relies on the role *R. sanguineus* plays as vector of pathogens causing diseases in dogs and humans. Certain species of ixodid ticks (e.g., *Ixodes holocyclus*) can induce a rapidly ascending flaccid paralysis in animals (Edlow JA, Mc Gillicuddy DC, 2008, Inf Dis Clin North Am 22: 397-414). In spite of the broad distribution of *R. sanguineus*, the role of this tick species as cause of paralysis in dogs remains anecdotic. A single report from Venezuela describes cases of tick paralysis in dogs massively infested by *R. sanguineus* (Viloria PR, 1954, Rev Med Vet Parasitol 13, 66-70).

AIM: The aim of this paper is to present 14 cases of apparent tick paralysis in young dogs heavily infested by *R. sanguineus* in an endemic area of southern Italy.

MATERIALS AND METHODS: From May to June of 2011, 14 dogs were referred to the teaching hospital of the Faculty of Veterinary Medicine (University of Bari, Italy) with neurological disorders. At admission, none of them had a history of treatment with acaricides and a high tick infestation was recorded. The owner reported to have removed "many ticks" (not counted) from three animals (nos. 1, 3 and 6) one day before the presentation of clinical signs. At the clinical examination, ticks as possible were removed from each dog which were treated with a spot-on formulation of Fipronil 10%/(S)-Methoprene 12% (FrontLine Combo, Merial). The following haematochemical parameters were recorded. Blood and buffy coat smears were prepared and stained using the MGG Quick Stain (Bio Optica Spa, Italy) and stained-smears were examined under optic microscopy for the presence of intracellular inclusions (or free forms) of common tick-borne pathogens (Otranto D et al, 2010, J Clin Microbiol 48: 3316-3324).

RESULTS: Out of 14 animals, 10 died within 24 hours from the presentation whereas four recovered, within three days, following treatment with a spot-on formulation of Fipronil 10%/(S)-Methoprene 12%. At the very beginning of their clinical presentation, all animals showed hind limb incoordination, generalized weakness, and difficulty to move. These clinical signs evolved rapidly (within 12-24 hrs) to inability to stand and walk, quadriplegia, hypothermia, slow and labored respiration with fatal outcome in ten animals. Ten animals were positive by blood smear examination for *Hepatozoon canis* with a high parasitemia and two of them also for *Babesia vogeli*. Out of the four remaining dogs, two scored negative for these hemoparasites and for two dogs haematological samples were not processed. All dogs were massively infested by ticks (min. no. 63 max. 328), which were morphologically identified as *R. sanguineus*. Thrombocytopenia, hypo albuminemia, pancytopenia were the haematological alterations most frequently recorded.

CONCLUSIONS: Although *R. sanguineus* has only been implicated on one occasion (Viloria PR, 1954, Rev Med Vet Parasitol 13: 66-70) as an agent of tick paralysis, our findings may suggest a potentially higher toxicosis virulence of tick populations circulating in southern Italy. Other causes for neurological signs were excluded and the putative diagnosis of tick paralysis by *R. sanguineus* was corroborated *ex juvantibus*.

Entomological monitoring following the first case of West Nile Disease in Southern Sardinia

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AIM: The West Nile Disease (WND) is a zoonotic disease transmitted by vectors, which causes forms of meningo-encephalitis in wild and domestic birds, in horses and humans (Smithburn K C et al, 1940, Am J Trop Med, 20: 471-492). The West Nile virus (WNV) is an arbovirus belonging to the family Flaviviridae, genus *Flavivirus*, included in the serum-complex of Japanese Encephalitis, which is transmitted by different species of mosquitoes (Diptera: Culicidae), mainly belonging to the genus *Culex* (Campbell G L et al, 2002, Lancet Infect Dis, 2: 519-529). Following the first outbreak of WND that occurred in Italy and precisely in Tuscany in 1998 (Lelli R, et al, 1998, Atti Convegno SIDILV), the Ministry of Health, in 2002, approved a national plan of serological and entomological surveillance. The monitoring plan is managed by CESME (IZS dell'Abruzzo e del Molise) and coordinated by II.ZZ.SS., in collaboration with the local Veterinary Services and the Istituto Superiore di Sanità. For Sardinia was identified the S'Ena Arrubia wetland area (Cabras, Prov. of Oristano). The entomological activities carried out allowed to increase the knowledge on the populations of Culicidae in the risk areas (Toma L et al, 2008, Veterinaria Italiana, 44 (3): 483-497). In 2008, after ten years, WND makes its reappearance in Italy, affecting the regions of Emilia Romagna, Veneto and Lombardy. In the following years are also reported outbreaks in central Italy and in Sicily, involving horses and humans (Rossini G et al, 2008, Euro Surveill 13, 41). In September 2011, the WND makes its appearance in Sardinia. Outbreaks are recorded in the provinces of Oristano, Cagliari and Olbia-Tempio and there were three human lethal cases due to severe meningo-encephalitis. After the death of a horse for WND, occurred in a riding club in the Regional Natural Park Molentargius-Saline (Quartu S.E., Prov. of Cagliari), on 29th September 2011 the procedures in case of "Area with viral circulation" were started. The purpose of this monitoring was to know the wildlife culicidic area in question, especially for the presence of potential vectors of WNV, to assess the overwintering and to detect the virus on the samples by molecular tools (PCR).

MATERIALS AND METHODS: From September to December 2011, the entomological activities have been carried out by staff of Dip.to di Cagliari - IZS della Sardegna, in collaboration with OEVR and IZS dell'Abruzzo e del Molise. Forty-two samplings of Culicidae were carried out, by using traps to catch adults (CDC, BG Sentinel® and aspirators), and ovitraps to collect early stages and eggs. The specimens captured were identified and stored at -80 °C.

RESULTS AND CONCLUSIONS: During the study period, 152 adults (males and females) of the following species were found: 84 *Culex pipiens*, 1 *Culex* sp., 52 *Aedes albopictus*, 2 *Ochlerotatus detritus*, 1 *Ochlerotatus* sp., 10 *Culiseta longiareolata*, 2 *Cs. annulata*, and 4 larval stages of *Cs. longiareolata*, 3 of *Oc. detritus* and 5 of *Ae. albopictus*; 43 eggs of *Aedes albopictus* were also found. A total of 56 adult specimens were sent to CESME for WNV search: 18 *Cx. pipiens*, 34 *Ae. albopictus*, 3 *Cs. longiareolata* and 1 *Cs. annulata*. A total of 12 adults (1 *Culex* sp., 6 *Cx. pipiens* and 5 *Ae. albopictus*) were processed by PCR in the virology laboratory of IZS of Sassari, accompanied by engineers of IZS dell'Abruzzo e del Molise. Despite the species found are potentially involved in viral transmission, all samples processed by PCR (45% of the specimens found) were negative for WNV. The two *Culiseta* species detected have different characteristics: *Cs. longiareolata* is a strictly ornithophilic and batracophilic species that occasionally feeds on humans; it can share larval breeding sites with *Cx. pipiens* and could be possibly involved in the maintenance of the enzootic cycle. *Cs. annulata* shows a certain degree of m*ammophily*, anthropophily and endophily and in some cases could be a secondary "bridge-vector" between birds and mammals (humans included). This work shows important data and reasons to carry out the monitoring activities related to the WND National Plan and to apply both national and regional legislation.

Integrated Diagnosis of Cattle Tick-borne Haemoparasites in sub-Saharan Africa: A Nigerian Example

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AIM: The present study aims to assess the occurrence of tick-borne haemoparasite infections in cattle in Central Nigeria, by means of a Polymerase Chain Reaction (PCR)-based Reverse Line Blot (RLB) hybridization assay.

MATERIALS AND METHODS: The study was carried out on 792 indigenous (*Bos indicus*) cattle extensively reared, without any acaricidal treatment, in 10 villages belonging to three local government areas (i.e., Bokkos, Mangu, and Pankshin) in Plateau State, Central Nigeria. Animals were sampled in the late rainy season (October 2008), when the highest tick burdens and prevalence of clinically apparent tick-borne diseases (TBDs) are usually reported (Maina JA, 1984, Animal health in subhumid Nigeria, Proceedings of the Second ILCA/NAPRI Symposium, Paper 8, Kaduna, Nigeria). 100 l of whole blood were collected from each animal, spotted onto FTA™ filter paper (Whatman™ Bioscience, Cambridge, UK) and let to air-dry overnight. Five 3 mm circular portions of sample-saturated FTA™ matrix were then punched for each sample, to be subjected to a molecular processing including DNA purification, elution, PCR, and RLB hybridization. Three primer sets were employed to allow the simultaneous amplification of 5 different Genera of tick-borne microorganisms including *Babesia* and *Theileria* spp., *Anaplasma* and *Ehrlichia* spp., and *Rickettsia* spp. PCR products were then loaded on a Biodyne C blotting membrane (Pall Biosupport, Ann Arbor, Mi) to which catch-all and species-specific oligonucleotide probes were covalently linked, as previously described (Gubbels JM et al., 1999, J Clin Microbiol, 37: 1782–1789). The oligonucleotides used were chosen according to the existing knowledge on the epidemiology of bovine TBDs in West Africa (Leeflang P, Ilemobade AA, 1977, Trop Anim Health Prod, 9: 211–218).

RESULTS: Ten different tick-borne pathogens were detected, including *Anaplasma marginale*, *Anaplasma centrale*, *Ehrlichia ruminantium*, *Ehrlichia* sp. Omatjenne, *Rickettsia* spp., *Babesia bigemina, Babesia bovis*, *Theileria mutans*, *Theileria velifera*, *Theileria tautoragi*. High frequency of co-infections was diagnosed, with the majority of tested animals being positive for two or more microorganisms. *A. marginale* occurred more frequently than *A. centrale* as well as *B. bigemina* was more common that *B. bovis*.

CONCLUSIONS: The high prevalence found for *T. mutans, T. tautoragi, T. velifera*, and *A. marginale* suggests that these pathogens have endemically established in the study area. In particular, such a presence of *A. marginale* poses a threat to the introduction of exotic breeds. In contrast, the lower prevalence recorded for *B. bigemina*, *A. centrale*, and *E. ruminantium* suggests a picture of endemic instability for these pathogens. The detection of *Rickettsia* spp. DNA should be further investigated to ascertain the rickettsial species circulating in the area. Enabling the simultaneous detection of several Genera of cattle tick-borne haemoparasites, the study shows the usefulness of the RLB hybridization assay as an epidemiological tool in field studies carried out in an area of rural sub-Saharan Africa (SSA) at high risk for TBDs. In such contexts, the use of FTA card-based technology is also of great advantage, allowing the storage of samples at room temperature, with no need for refrigeration. Disclosing a complex scenario of multiple infections, this study highlights the potential risk of misdiagnosis amongst several tick- and other vector-borne (e.g., trypanosomiasis) diseases, when only clinical and cytological approaches are employed. Although costly to be initiated, the implementation of RLB can potentially be cost-effective in SSA, allowing the screening of ~40 samples for several pathogens at the same time. The use of a PCR+RLB hybridization approach is therefore advisable for prevalence surveys aiming to address the design of targeted control campaigns in these areas.

Detection of *Anaplasma phagocytophilum*, *Coxiella burnetii* and *Rickettsia* spp. from animals and ticks in a rural area of Latium Region

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AIM: The aim of the study was to investigate the presence of some tick-borne bacteria, acting as agent of zoonotic diseases, in a northern rural area of Latium Region. In particular, bacteria of *Anaplasma*, *Rickettsia* and *Coxiella* genus were investigated in bovine and equine using serological and molecular analysis and the occurrence of selected pathogens were also studied in ticks.

MATERIALS AND METHODS: Ticks: a total of 934 ticks from animals (n=325) or free living (n=609) were collected and morphologically identified. Of 934 ticks some were pooled cause their dimension and laboratory needs, giving a total of 151 tick samples. Five tick species were recognized, being *Ixodes ricinus* the most abundant (48.1%), followed by *Rhipicephalus bursa* (24.1%), *R.* (*Boophilus*) *annulatus* (22.2%), *R. sanguineus* (2.8%), *Hyalomma marginatum* (2.3%) and *Ripicephalus* spp. (0.5%). *R. sanguineus* was collected only free living, *B. annulatus* only on bovine and *R. bursa* on equine. PCR was performed to detect the presence of *Rickettsia* spp. (Roux V. et al, 1997, Int. J. Syst. Bacteriol. 252-261.), *Anaplasma phagocytophilum* (Massung R. F., Slater K.G. 2003, J. Clin. Microbiol. 41:717-722) and *Coxiella burnetii* (Parisi A. et al. Vet. Microbiol. 2006. 118:101-106.) on 934 ticks: 307 collected from bovine, 18 from equine and 609 free living (167 in meadow and 442 in wooden area).
Animals: a total of 281 blood samples were examined, 271 from bovine and 10 from equine. IFAT and ELISA serological tests were performed on bovine serum samples to detect antibodies against *A. phagocytophilum* and *C. burnetii*, while only *A. phagocytophilum* was investigated on equine samples. PCR was performed on buffy coat or blood coagulum to detect *A. phagocytophilum* and *Rickettsia* spp. DNA.

RESULTS: Serum samples examined scored positive for *C. burnetii* (4.5%) and *A. phagocytophilum* (49%) while PCR on buffy coat or coagulum were negative for *A. phagocytophilum* and *Rickettsia* spp. DNA. None of the equine blood samples showed positive results. Of 151 tick samples, 19 were found to be positive for *A. phagocytophilum* DNA in *B. annulatus* (3/151) and *I. ricinus* (3/151), *Rickettsia* spp. DNA in *I. ricinus* (3/151), *R. bursa* (1/151), *H. marginatum* (6/151) and *R. sanguineus* (1/151), *C. burnetii* DNA was detected only in *B. annulatus* (2/151). Minimum and maximum prevalence were calculated considering that some of the positive samples were pooled (see the table below).

Infection rate in ticks (PCR)						
	Bovine		Equine		free living	
	%		%		%	
	min	Max	min	Max	min	Max
A. phagocytophilum	1.0	5.9	0.0	0.0	*0.5*	4.8
Rickettsia spp.	2.9	10.8	0.0	0.0	*0.3*	2.0
C. burnetii	0.7	5.2	0.0	0.0	*0.0*	0.0

CONCLUSIONS: The results herein presented show that *A. phagocytophilum*, *C. burnetii* and *Rickettsia* spp. occur in the studied areas suggesting the different role played by tick species in pathogen transmission and risk for human and animal infection. Further studies would be necessary to corroborate data collected and to improve knowledge on presence and distribution of bacteria investigated.

Midichloria mitochondrii: the first Rickettsiales with a flagellar structure

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AIM: *Midichloria mitochondrii* is a Rickettsiales bacterium capable of invading mitochondria in ixodid ticks (Sacchi et al, 2004, Tissue cell 36:43-53). The recently sequenced genome of *M. mitochondrii* revealed the presence of 26 putative flagellar genes (Sassera et al, 2011, MolBiolEvol.28: 3285-3296). Open questions in relation to this observation are whether these genes are expressed and whether they possess the domains expected for the flagellar function. Thus, the aims of this work are: a) analyze the structural features and domains of the 26 putative flagellar proteins of *M. mitochondrii;* b) evaluate the expression of 7 putative flagellar genes; c) stain the protein coding for the flagellar cap FliD using an immunofluorescence assay and an immunogold staining. We have thus addressed specific questions related to the first evidence for a flagellar apparatus in a Rickettsiales, and we have also produced tools (recombinant rFliD and antibodies) that will facilitate the study of *M. mitochondrii*.

MATERIALS AND METHODS: A fragment of 325 amino-acids of the flagellar protein FliD of *M. mitochondrii* was expressed in recombinant form and purified (rFliD: MW: 38 kDa). Polyclonal antibodies anti-rFliD have been prepared. Ovaries of six *I. ricinus* and two *P. hexagonus* semi-engorged adult tick females were divided in four parts for: DNA extraction; transmission electron microscopy (TEM); immunogold staining and indirect immunofluorescence assay (using anti-rFliD). In addition, two pools of 50 eggs, five larvae, five nymphs and the ovaries from two semi-engorged adult females of *I. ricinus* were processed for RNA extraction and cDNA synthesis, for determining the expression of seven flagellar gene (*fliC*, *fliD*, *flgL*, *flgK*, *flgE, fliG, motA*) in different tick developmental stages.

RESULTS: The indirect immunofluorescence assay using polyclonal FITC-conjugated anti rFliD antibody on adult *I. ricinus* ovaries, lead to the observation of clusters of bacteria, that can be assumed to be *M. mitochondrii*. Moreover, anti-rFliD immunogold staining on *I. ricinus* ovaries revealed a specific pattern of colloidal gold deposit inside bacteria-like bodies and on the surface of these bacterial bodies. However, standard TEM did not lead to the observation of flagella. We thus decided to perform an in silico analysis of the 26 predicted flagellar proteins of *M. mitochondrii*. Results confirmed that these proteins actually possess the conserved domains and structural features required for their function in model bacteria. We thus decided to evaluate the expression of seven flagellar genes during the life cycle of *I. ricinus* (eggs, larvae, nymphs and adults). Results showed that eggs and adult samples express all the seven flagellar analyzed genes while larvae and nymphs present variable patterns of gene expression. It is reasonable to assume that intracellular bacteria like *M. mitochondrii* alternate trophic phases (in which flagella and motility are not required), with phases of the cycle in which a flagellar apparatus is used, for motility, or for other functions.

CONCLUSIONS: *M. mitochondrii* is the sole member so far described in the order Rickettsiales that possesses a complete set of genes coding for a putative flagellar apparatus. Whether *M. mitochondrii* uses a flagellar apparatus for motility is yet to be determined. However, considering the conservation of the flagellar proteins that we have analyzed and their expression at the RNA and protein level and the evidence for their ancestral origin we suggest that these genes have maintained their original function along the phylogenetic lineage leading to *M. mitochondrii*, and possibly in other Rickettsiales lineages.

West Nile Disease: spatio-temporal correlation between entomological and human surveillance in the Veneto Region, north-eastern Italy

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AIM: West Nile virus (WNV) is an arbovirus belonging to the family *Flaviviridae*, genus *Flavivirus*, which affects birds, domestic animals and humans; the vectors are mosquitoes, particularly *Culex* spp. (Weissenbock H. et al., 2010. Vet Microbiol 140: 271-280). Many species of wild bird are considered amplifying hosts, whereas humans, horses and other mammals are dead-end hosts. An outbreak of WNV has been ongoing in the Veneto region since 2008 (Busani L. et al., 2011, Epidemiol Infect; 139(6):818-25). Following the first clinical case of WNV, a regional surveillance program was implemented to track WNV activity in humans, animals and mosquitoes. In this study we compared the findings on WNV in mosquitoes with those in human clinical cases in order to evaluate whether positivity for WNV in mosquitoes could be predictive of disease in humans, including both West Nile Neurological Disease (WNND) and West Nile Fever (WNF).

MATERIALS AND METHODS: Entomological data were collected from 2009 to 2011. Twenty-four sites in 2009, 43 in 2010 and 49 in 2011 were monitored in the Veneto region, using CDC-CO_2 traps. Mosquitoes were collected every 15 days, identified and RNA was extracted from a pool of a maximum of 50 mosquitoes of the same species. RRT-PCR was used to detect Flaviviruses (Ravagnan S. et al., Proceedings of 5th Annual Meeting EPIZONE, France, 11-14 April 2011) and for subsequent amplicon sequencing. Prevalence of infection in mosquitoes was adjusted for the pooled sample and expressed as estimated rates of infection (ERI). Human cases of WND were identified in the framework of the regional surveillance plan and confirmed as described in Barzon et al, 2010 (Euro Surveill.; 16(33):19949).

RESULTS: A total of 226,145 mosquitoes were tested for Flavivirus: 20,060 specimens in 2009, 127,293 in 2010 and 78,792 in 2011. The main species was *Culex pipiens* (86.3%). In total, 15 pools of *Cx. pipiens* (ten in 2010 and five in 2011) were positive for WNV and the area most affected was the province of Venice. Six human cases were diagnosed in 2009, six in 2010 and ten in 2011. In the province of Venice, the first mosquitoes to test positive for WNV were detected in late July, while the first human case was identified at the beginning of September in 2010 and 2011, respectively. A similar trend was observed in the province of Rovigo in 2010. Conversely, in the province of Treviso, where circulation of the virus in mosquitoes was not detected until 2011, human cases were recorded before detection in vectors (end of August in man, mid-September in mosquitoes).

CONCLUSIONS: *Cx. pipiens* was confirmed to be widespread and to be the main vector of WNV in the region. Detection of the virus in mosquitoes before identification in humans was observed in areas where viral circulation had been established since at least the previous year (provinces of Rovigo and Venice). Where the virus had been more recently introduced, as the province of Treviso, entomological surveillance was not predictive of human cases. These findings may be partially attributable to the different distribution of the virus in the region. In endemic areas the virus is likely to be uniformly distributed (as suggested by serology on animals) and the location and intensity of vector monitoring affect the probability of virus detection to a lesser extent. In newly infected areas virus distribution is more likely to be spotted and hardly detectable in the absence of adequately intensive mosquito sampling and/or suitable trap location. However other variables, as climate and WNV reservoir density and distribution, should be considered.
Other valuable information besides WNV detection was gathered as part of entomological monitoring, which (i) enabled detection of other potential human pathogens, as Usutu Virus and Bunyavirus, (ii) defined high mosquito density areas where control needs to be implemented and (iii) provided the basic dataset to be included in models predictive of mosquito-borne disease.

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Variability within msp2 gene of *Anaplasma phagocytophilum* in *Ixodes ricinus* ticks from north-eastern Italy

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Anaplasma phagocytophilum is the agent of animal and human granulocytic anaplasmosis. It is transmitted by ticks, particularly by *Ixodes* species. In Europe and USA the msp2 gene, encoding the major surface protein 2, displays high antigenic variation in relationship to the bacteria and its circulation in vectors, reservoirs and hosts (Lin *et al.,* 2004, *Infect Immun* 72:3883-9).

AIM: The aim of this study was to evaluate the genetic variability in the msp2 gene fragment of *A. phagocytophilum* in north-eastern Italy.

MATERIALS AND METHODS: The study material consisted of 47 DNA samples isolated from *Ixodes ricinus* collected by dragging in Veneto and Friuli Venezia Giulia regions from 2006 to 2008 and in 2011. A fragment of the msp2 gene (334bp) was amplified by PCR (Levin *et al.*, 2002 *Vector Borne Zoonotic Dis 2:125-136)* and sequenced to investigate polymorphisms.

RESULTS - Fourteen variants (from 1-IrNEI to 14-IrNEI) were differentiated based on 11 nucleotide substitutions (Tab.1), with a genetic distance ranging from 0.5% to 4.6%. Eight of those variants were found in the sites of Belluno province with a high intraspecific difference up to 3.3% (Tab.1).

Table 1. Sequence polymorphism of the msp2 gene fragment of the 14 variants obtained from our sequences. Positions of polymerphisms was given according to the sequence with accession number AY166490. IrNEI=I.ricins north-eastern Italy.

Genotype	2035	2041	2042	2062	2122	2124	2125	2179	2191	2200	2203
1-IrNEI *	T	T	A	T	T	A	T	C	A	T	T
2-IrNEI *	A	T	A	T	T	A	T	C	A	T	T
3-IrNEI *	T	A	A	T	T	A	T	C	A	T	T
4-IrNEI *	G	T	A	T	T	A	T	C	A	T	T
5-IrNEI *	A	T	A	A	G	A	T	C	A	T	T
6-IrNEI *	T	T	A	T	G	A	C	C	A	T	T
7-IrNEI *	T	A	C	T	T	G	T	C	A	T	T
8-IrNEI	A	A	C	T	T	A	T	C	A	T	T
9-IrNEI	T	T	C	T	T	A	T	C	A	T	T
10-IrNEI	A	C	C	T	T	A	T	C	A	T	T
11-IrNEI	A	A	C	A	T	A	T	C	A	T	T
12-IrNEI *	T	A	C	T	G	A	T	C	A	T	T
13-IrNEI	A	T	C	A	T	A	T	T	G	A	T
14-IrNEI	A	T	C	T	T	A	T	C	A	A	A

**Genotypes of Belluno province*

Table 2. Comparison among our sequences and USA and Poland ones in polymorphic sites of the msp2 gene fragment.

Site in DNA	USA		Poland		Italy		
	Codon	AA	Codon	AA	Codon	AA	
2035	CTA	L	CTA	L	CTA	L	
	CTT	L	CTT	L	CTT	L	
					CTG	L	
2041	TCA	S	TCA	S	TCA	S	
	TCT	S	TCT	S	TCT	S	
	TCG	S			TCC	S	
2042	CAC	H	ACT	H	CAC	H	*
	AAC	N	ACA	N	AAC	N	
2062	ACT	T	CAC	T	ACT	T	
	ACA	T	AAC	T	ACA	T	
2113	GGC	G	GGC	G	GGC	G	
	GGT	G					
2122	GGT	G	GGT	G	GGT	G	
	GGG	G			GGG	G	
2124	TAT	Y	TAT	Y	TAT	Y	
2125	TAC	Y	TAC	Y	TAC	Y	*
					TGT	C	
2140	GCC	A	GCC	A	GCC	A	
			GTC	A			
2154	GAG	E	GAG	E	GAG	E	*
	GGG	G					
2179	ACC	T	ACC	T	ACC	T	
					ACT	T	
2182	AAG	K	AAG	K	AAG	K	
	AAA	K					
2186	ATT	I	ATT	I	ATT	I	
	GTT	V					
2191	AGA	R	AGA	R	AGA	R	
					AGG	R	
2200	GGT	G	GGT	G	GGT	G	
					GGA	G	
2203	AGT	S	AGT	S	AGT	S	*
					AGA	R	
2215	GAA	E	GAA	E	GAA	E	*
	GAT	V					

**Non synonymous substitution*

Our sequences were compared with the variants from USA and Poland (Rymaszewska *et al.,* 2010, *Folia Biologica* 56:269–75) (Tab.2) resulting in a genetic distance ranging from 0.5% to 3.5% and from 0% to 3%, respectively (the same variant 1-IrNEI was found in Poland in a dog). Our sequences presented more nucleotidic substitutions than Poland and some different nucleotidic substitutions with compared with those from USA (Tab.2). In our variants 3 substitutions at positions 2042, 2124 and 2203 were detected as missense mutations resulting in four variants of encoded proteins (3 of these were present in the sites of Belluno). In eight variants from this study, but also in USA and Poland sequences at position 2042, the first codon base was substituted (A C) and resulted in a change of *Asn* (N) into *His* (H) in the protein. In 7-IrNEI and in 14-IrNEI variants at position 2124, the second codon base was substituted (transition A G) resulting in the change of *Tyr* (Y) into *Cys* (C) and at position 2203 the third codon base was substituted (transversion T A) resulting in the change of *Ser* (S) into *Arg* (R) in the protein (Tab.2). The two missence mutations at position 2154 and 2215 were present only in the USA.

CONCLUSIONS: This study revealed a high genetic variability of *A. phagocytophilum* in a limited area as a more likely consequence of the evolutionary rate of this agent. Since genetic variants of *A. phagocytophilum* were segregated in specific natural hosts (Katargina *et al.,* 2012, *Clin Microbiol Infect* 18(1):40-6), the result of this study points towards the presence of different natural cycles in the area. However, the possibility of repeated introductions of new variants through migratory birds cannot be excluded. The pathogenicity of these variants and their reservoir deserve to be further addressed.

Rickettsia spp. infection rates and co-infections in *Ixodes ricinus* ticks from north-eastern Italy

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In Italy *Ixodes ricinus* is particularly abundant in pre-alpine areas of northern Italy and specifically in the north-eastern area.

AIM: The aim of this study was to assess the rates of infection and co-infections of *Rickettsia* spp. in *I.ricinus* ticks collected from well-known foci of tick-borne diseases and sites epidemiologically unknown in north-eastern Italy.

MATERIALS AND METHODS: From 2006 to 2008, ticks were collected by dragging in Veneto and Friuli Venezia Giulia (FVG) regions in 5 permanent sites, monitored monthly from April to November and 50 temporary sites, monitored once per year. Specimens were identified, DNA extracted and stored at -80°C. In this study all the adults collected through the years were screened singularly for *Rickettsia* spp.. In addition in the permanent site of Udine province pooled larvae and nymphs were also screened. *Rickettsia* spp. was searched by PCR (Márquez *et al.,* 1998, Am J Trop Med Hyg 58(5) 570-7) and species determined by sequencing. Rates of infection were calculated as Prevalence in adults and ERI (Estimated Rate of Infection; Cowling *et al.,* 1999 Prev Vet Med 39:211-25) in pooled samples. Prevalence differences in relation to tick's sex and stages were tested using χ^2 or Fisher's exact tests.

RESULTS: In total 192 adult ticks (95F; 97M) were collected in 31 sites and 32 ticks (16.6%) were found positives for *Rickettsia* spp.; in particular 24 ticks (12.5%) were found infected with *R.helvetica* and 8 ticks (4.2%) with *R.monacensis* ($p<0.01$). The rate of infection in female ticks was higher than in males (80% vs 20%; $p<0.01$) only for *R.helvetica.* Both species were found in FVG and only *R.helvetica* was found in Veneto sites.
The ticks collected in the permanent site of Udine and the results are shown in table 1.

			R. helvetica		*R. monacensis*	
Tick stages	Ticks collected	Pool/adult tested	pos (%)	ERI/P*	pool pos (%)	ERI/P*
larvae	1568	106	20 (18.87)	**1.40%**	7 (6.60)	**0.46%**
nymphs	978	139	40 (28.78)	**4.71%**	20 (14.39)	**2.18%**
adults	45	45	4 (8.89)	**8.89%**	0	-

** P= prevalence in adults; ERI= estimated rate of infection in pooled larvae and nymphs*

In this site, the rate of infection was significantly higher for *R.helvetica* than *R.monacensis* only in nymphs ($p<0.01$) and larvae ($p<0.05$). The majority of adult ticks showed a single infection (21/32; 65.6%). Out of 24 ticks infected with *R.helvetica,* 6 had a double infection (3 with *Borrelia garinii* and 3 with *Candidatus Neoehrlichia mikurensis*). Out of 8 ticks infected with *R.monacensis,* 3 had a double infection (with *B.afzelii, B.valaisiana* and *Ca.N.mikurensis*) and 2 a triple co-infection (with *B.afzelii/ Ca.N.mikurensis* and *B.burgdorferi s.s./ Ca.N.mikurensis*). Double co-infections were found in female ticks only, while the two triple co-infections were found in 1 female and in 1 male tick. Co-infections *R.helvetica/R.monacensis* were never observed.

CONCLUSIONS: This study confirms the circulation of at least two *Rickettsia* species in *I.ricinus* ticks of north-eastern Italy. *R.helvetica* is predominant and its relevant rate of infection poses a threat to human health as a cause of spotted fever. *R.monacensis* has been related to human clinical cases too (Jado I, *et al.,* 2007, Emerg Infect Dis 13(9):1405-7). The presence of *Rickettsia* spp. in questing larvae points the occurrence of transovarial transmission for both species, although with an apparent low efficiency, unlike other studies (Sprong H. *et al.,* 2009, Parasit Vectors 4:2(1):41), which found a comparable prevalence in the different tick stages. Our data suggest instead a considerable acquisition of infection from reservoir animals in the site monitored. Small rodents have been indicated as reservoir animals for both species, however the absence of co-infections between *R.helvetica* and *R.monacensis* in the vector suggests that the two species may have different rodent species as reservoir or other animals. The majority of co-infections of *R.monacensis* were with pathogens associated to small mammals (i.e. *B.afzelii, B.burgdorferi s.s.* and *Ca.N.mikurensis*), suggesting a role of these animals as reservoir hosts in this specific site. *(This work was supported by the Veneto region)*

Surface antigens and molecular markers of *Babesia bovis* Italian strains

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Babesiosis is one of the most common infections of free – living animals worldwide. It is caused by infectious intraerythrocytic parasites of the genus *Babesia*. *Babesia bovis* is a cattle pathogen and its transmission occurs through the specific tick vector *Rhipicephalus* spp. This parasite is one of the major causes of economic losses in the cattle industry in tropical and subtropical countries (Bock et al, 2004, Parasitology, 129, Suppl. S 247-99), causing in its hosts a severe anaemia, high fever and, sometimes, animal death. Current research addresses the study of *B.bovis* from a molecular point of view, in order to identify and study surface molecules that induce in the bovine a protective antigenic stimulus and that would be reasonable to include in a subunit vaccine useful for the control of the infection. Two proteins deeply studied thanks to their possibility to be included in a subunit vaccine are the Merozoite Surface Antigen 2c (Wilkowsky, SE, 2003, Mol Biochem Parasitol., Apr 3,127(2),133-41) and the Apical Membrane Antigen-1 (Gaffar FR et al., 2004, Infect Immun.72(5):2947-55).
Many efforts are also directed to the finding of *B. bovis* new molecular markers to track the provenience of pathogen strains. *Desmoyokin* and *85KDa* genes (Wilkowsky SE et al., 2009, Vet Parasitol. Apr 6; 161(1-2):9-18) are two molecular markers recently identified showing variation in number and sequence of tandem repeats among the different *B. bovis* isolates.

AIM: This work was aimed to the characterization of Merozoite Surface Antigen 2c (MSA-2c) and the Apical Membrane Antigen-1 (AMA-1) surface antigens in Italian *B. bovis* strains and to the investigation of their B-cell epitopes conservation between Italian and geographically distant *B. bovis* strains to evaluate the potential use of these antigens as vaccine and diagnostic tool. The research was also addressed to the analysis of Desmoyokin and 85KDa aminoacidic sequences in order to evaluate the repeats pattern in Italian strains.

MATERIALS AND METHODS: *B. bovis* infection was detected in two bovines died of suspected clinical babesiosis in Ragusa (Sicily, Italy) and Portici (Campania, Italy). Their spleens were removed post-mortem, genomic DNA was extracted from this tissue and *B. bovis* diagnostic PCR was carried out (Figueroa JV et al, 1993, Veterinary Parasitology, 50, 69-81)
Amplification of the *msa- 2c, ama-1, Desmoyokin* and *85KDa* genes from these *B.bovis* positive DNA samples was carried out by PCRs. Amplification was confirmed on ethidium bromide-stained agarose gels. PCR products were cloned and sequenced in the forward and reverse direction and sequences aligned using ClustalW2.0.10 and Bioedit (Tom Hall Ibis Biosciences). The software MEGA and DAMBE were used to calculate the percentage of similarity among each of the analyzed sequences. Each aminoacidic sequence was analysed to seek the presence of possible B-cell epitopes

RESULTS: Both MSA-2c and AMA-1 showed at aminoacidic level a very high percentage of identity each other (93.0 % for MSA-2c and 99.4% for AMA-1) and with the sequences annotated in GenBank (an average of 93.8% for msa2c and of 97.2% for AMA-1). Furthermore, six B-cells immunogenic peptides were identified in the MSA2c aminoacidic sequences by bioinformatics. Out of these, two were entirely conserved among Italian and geographically distant strains.
As regarding the molecular markers *Desmoyokin* and *85KDa*, the analysis allowed defining the pattern of the repeats of these genes. It was possible to obtain the first data regarding the repeats model of Italian strains. Interestingly, it was noted a conservation in the consensus pattern, but Italian strains showed a pattern never found in foreign strains.

CONCLUSIONS: The obtained results constitute the first information related to Italian strains of *Babesia bovis* and reinforce the hypothesis to use MSA2c and AMA-1 proteins as vaccine candidates and *Desmoyokin* and *85KDa* genes as molecular markers.

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Occurrence of *Babesia* spp. in wildlife and domestic animals from Northwestern Italy

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AIM: *Babesia* spp., are protozoa parasites of red blood cells, which infect a wide range of mammals, including humans, domestic animals and, wildlife. Recently, new zoonotic *Babesia* species (e.g. WA1, EU1, EU3) were described in USA and Europe (Herwaldt H et al, 2003, Emerg Inf Dis, 8:942-948). Although *Babesia* spp. infection is considered to be asymptomatic in wildlife, in the last few years an increasing number of fatal cases of babesiosis have been reported in free-ranging wild ungulates (Hoby S et al, 2007, Vet Parasitol, 148:341-345). Considering the changes occurring in the host-pathogen relationship and within tick-vector distribution and abundance (Hilpertshauser H et al, 2006, Appl Envirom Microbiol, 72: 6503-6507), we deemed interesting to assess, in regard of babesiosis, the epidemiological status of wildlife, cattle and dogs living in shared areas of Northwestern Italy (Piedmont Region).

MATERIALS AND METHODS: Genomic DNA was extracted from spleen samples of 799 wild animals (i.e. roe deer n= 370, red deer n=39, wild boar n=172, chamois n=10, red fox n=201, and wolf n=7), and from whole blood samples of 468 dogs, and 745 beef and dairy cattle. Cattle sampled, were grazed in summer on alpine grass land or in pasture patchy wooded areas. All samples were tested with a *Babesia/Theileria* catch-all PCR, as reported in literature (Gubbels JM et al, 1999, J Clinical Microbiol, 37:1782-1789; Schnittger L et al, 2004, Parasitol Res, 92: 189–196).

RESULTS: The overall prevalence of *Babesia* spp. in wild animals was 3.50% (IC 95% 2.38-5.09) from spleen samples. Prevalence data for each species examined, ranged from 1.16% (IC 95% 0.2-4.58) in wild boar and 1.62% (IC 95% 0.66-3.67) in roe deer, up to 48.7% (IC 95% 32.71-64.97) in red deer. Only one fox over 201 (P=0.5%, IC 95% 0.02-3.17) was found positive for *Babesia* spp. The prevalence of *Babesia* spp. in cattle was of 5.23% (IC 95% 3.79-7.15), and of 8.97% in dogs (IC 95% 6.62-12.03). Some of the positive samples were sequenced and identified as *Babesia divergens* (n=3 red deer), and *B. bigemina* (n=4 roe deer, n=1 red deer, n=2 wild boar), *B. canis* (n=3 dogs), *Theileria equi/B.microti* (n=2 cattle).

CONCLUSIONS: This is, to our knowledge, the first report in Italy of a fox infected with *Babesia* spp. Sequencing of the positive amplicons, revealed the presence in wildlife of the zoonotic *Babesia divergens,* and of species shared with domestic livestock such as *B. bigemina.* Further phylogenetic analysis are needed to establish the relationship occurring between *Babesia* spp. found in wildlife and those found in cattle and dogs. Preliminary sequencing data showed a possible overlap between *Babesia* specimens found in domestic and wild animals.

Applying Maxent species distribution model to *Babesia* spp. epidemiology in wildlife

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AIM: The distribution of a parasitic species is consistently related to the physical environment. Species distribution models (SDM) are tools that allow to conceptually link observations of species occurrence to environmental parameters. Traditionally, SDM focused on an animal or on a plant species, in order to reveal the causal drivers of species distribution or to make a prediction of distribution to new, unsampled sites (Elith J et al, 2009, An Rew Ecol Evol System, 40: 677-697). With these same aims we applied SDM to *Babesia* spp., a tick borne protozoa parasite of wildlife, using wild species distribution data only as one of the predictors for the target pathogen occupancy model.

MATERIALS AND METHODS: Considering the complex epidemiology of this multi-host, multi-species, vector-borne parasite, Maxent was chosen as reference Maximum Entropy Model in Northwestern Italy (Piedmont Region). It allows to be trained with multiple geographical and environmental predictors, which are ecologically relevant to the target species. As *Babesia* spp. is an intraerythrocytic protozoa, its occurrence in the environment depends upon the presence of vector ticks, and appropriate abundance of definitive mammalian hosts. Vector and host presence data have been modeled separately and then combined together into the definitive model (Oorebeek M, and Kleindorfer S, 2008,Parasitol Res 103:871–875). Density and richness of definitive hosts have been modeled from yearly updated, regional hunting censuses. The presence probability model for ticks was based on Normalized Difference Vegetation Index (NDVI) and Normalized Difference Moisture Index (NDMI), obtained from open-access Landsat images (Perret JL et al, 2003, J Exp Biol, 206: 1809-1815), as well as orographic data (altitude, slope steepness, roughness, and exposure). Temperature (since 1988) was also considered a key prior (Randolph SE, Storey K, 1999, J Med Entomol 36: 741-748). The study area overlapped with the sampling area, making the extension of our model limited but with high resolution, consistent with the available covariates. The high resolution of the model, allowed to make better inference on unsampled sites considered the complexity of parasite's life-cycle. Parasite's presence data were obtained from a parallel molecular study on *Babesia,* detected by PCR (Gubbels JM et al, 1999, J Clinical Microbiol, 37:1782-1789; Schnittger L et al, 2004, Parasitol Res, 92: 189–1996) on spleen of 799 hunted animals (chamois, red deer, roe deer and wild boar), animals culled within numerical restrain actions (red foxes), or accidentally found dead (wolf and all above species).

RESULTS: Preliminary results confirm the mathematical vigour of the accounted priors, and the good fitness of the model, verified by cross-validation and bootstrapping. The preliminary dataset is currently being expanded to obtain a more robust output.

CONCLUSIONS: Maxent combined to GIS, allowed to design an inferential model, with an easy-to-read graphical output, that has *Babesia* as dependent variable and environmental predictors as priors. The model will provide insights in the epidemiology of *Babesia* spp. and of its tick vector and mammalian hosts. Future application of this model will allow extrapolating predicted *Babesia* presence data to future climatic settings and to geographically and climatically similar areas (Franklin J, 2009, Mapping species distribution: spatial inference and prediction, CUP, Cambridge, UK). As more biological samples are tested, new priors will be analyzed and eventually added to the model, in order to include more specific variables for each of the species recorded as each *Babesia* species is associated preferably to certain ticks.

SESSIONE 9

EPIDEMIOLOGIA DELLE MALATTIE PARASSITARIE DEGLI ANIMALI DA REDDITO

Natural and experimental infection with gastrointestinal nematodes in dairy goats Alpine and Nera di Verzasca

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AIM: Gastrointestinal nematodes (GIN) cause significant economic losses to goat production and anthelmintic resistance is an increasing problem throughout the world. The research about alternative methods to control GIN infections is expanding and there is evidence that some breed are genetically and immunologically endowed with higher resistance and/or resilience to parasites (Chiejina SN, Behnke JM, 2011, Parasit Vectors, 4: 12-22). The purpose of this survey was to compare the response to GIN infection, both natural and experimentally induced, between two dairy goat breeds reared in northern Italy (province of Varese, Lombardy): Alpine and Nera di Verzasca (native breed originated from Italian-speaking Switzerland).

MATERIALS AND METHODS: Natural infection: 60 goats of the same herd (30 Alpine and 30 Nera di Verzasca) were treated with netobimin 15 mg/kg in November 2009. From January to December 2010 individual faecal samples were monthly collected. Goats were milked twice a day, they grazed free from April to November and received a diet supplementation during winter period. Experimental infection: in September 2011 15 dry goats of same age (8 Alpine and 7 Nera di Verzasca) were treated with netobimin 15 mg/kg and then infected with a doses of 6000 L_3 of *Haemoncus contortus* (day 0). From day 15 P.I. individual faecal samples were taken initially twice a week, then weekly until day 75 P.I.

RESULTS: All FECs were performed by FLOTAC *double technique* using NaCl 1.200 s.g. (Cringoli G, 2006, Parassitologia, 48: 381-4). A mixed model of variance analysis for repeated measures was applied to evaluate the effect of several factors (breed, time of sampling, age, parturition) as fixed effects on ln-transformed epg. FEC values in the different conditions of infection are shown in the figures below. In natural infection, epg rose to a peak in April in both breeds, then decreased until September and rose again in last months of the year, but with a great difference between the breeds to the advantage of Verzasca goats. Goats of Alpine breed showed epg values significantly higher than goats of Verzasca ($P<0.005$). Significant differences were also observed on epg counts among months of sampling ($P<0.0001$). In experimental infection epg showed an almost regular upward trend in both breeds; also in this case Nera di Verzasca goats expelled less epg than Alpine goats during the whole experiment period.

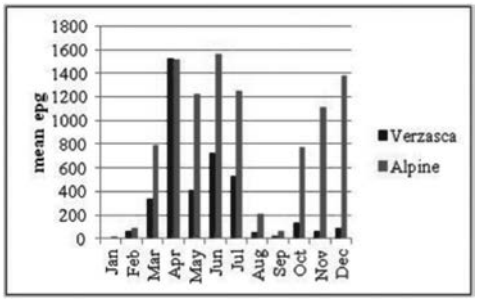

Fig. 1. Epg in the 2 breeds under natural infection.

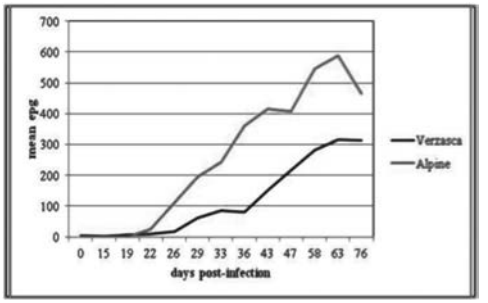

Fig. 2. Epg in the 2 breeds under experimental infection.

CONCLUSIONS: In previous studies Nera di Verzasca goats showed evidence of higher resilience than Saanen and Alpine goats: in fact, even though milk production in Nera di Verzasca is always lower, that was not affected by the parasite infection, as it happened instead in the other breeds (Alberti EG et al, 2012, Small Rum Res, in press). In the present survey Nera di Verzasca goats showed a lower emission of nematode eggs in every condition investigated. This suggests therefore that this breed could be characterized also by a higher resistance to gastrointestinal nematodes than Alpine. So it can be deduced that Nera di Verzasca is a strong breed, with a good ability to control gastrointestinal nematode infection, which is suitable especially in farming conditions with large use of pasture, as is usual in the region investigated.

A preliminary survey of liver trematodoses among cattle from Portugal

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AIM: The aim of this work was to investigate the current epidemiological status of liver trematodoses among cattle from Portugal.

MATERIALS AND METHODS: The prevalence of infection was determined both by parasitological examination of liver and by detection of serum antibodies. The survey was carried out at an abattoir located in Lugo (north-west Spain), where cattle (calves and adult cows) from northern Portugal and north-west Spain are slaughtered every week. A total of 102 animals from Portugal were examined for liver parasites.
Further, 158 blood samples of cattle from localities near Lisbon (Portugal) were collected and the humoral immune response analyzed in order to detect IgG anti-*Fasciola hepatica* and anti-*Dicrocoelium* respectively by means of an ELISA test where a recombinant protein (FhrAPS) and the excretory/secretory antigens obtained from adult *Dicrocoelium dendriticum* flukes (DdES) were used.

RESULTS: A percentage of 6% (95% C.I 0%, 14%) of slaughtered cattle had liver flukes, and *Fasciola hepatica* specimens were only identified. Seroprevalence in cattle from Lisbon area was of 41% (95% C.I 33%, 48%) and 11% (95% C.I 6%, 16%) with animals having IgG antibodies against *F. hepatica* and *Dicrocoelium* respectively.. No differences regarding the breed, age or management were obtained (P>0.05). ELISA showed that 9% (95% C.I 5%, 14%) of cattle had antibodies against the two trematoda, whereas 58% (95% C.I 50%, 65) resulted negative to the ELISAs.

CONCLUSIONS: Although the occurrence of infection by liver trematoda has been widely reported in cattle from NW Spain (Arias et al., 2011, Vet Rec, 168: 408-412; Arias et al., 2011, Parasitic diseases in livestock under different types of grazing management. Diagnosis and possibilities for their control. *In* T. Javed (Ed.), Rearing, Farming Practices and Diseases. Nova Science Publishers, Hauppauge NY, USA; Arias et al, 2012, Parasitol Res, 110: 1001-1007), there are few data on bovine trematodoses in Portugal. This preliminary investigation points a moderate risk of trematode infections

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Cystic echinococcosis in slaughtered cattle in Sardinia retrospective Space-Time analysis: result from the official data flow in Sardinia

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AIM: Our study was carried out to provide a detailed picture of the distribution of cystic echinococcosis (CE) in cattle by analysis of available official data. Records between 2009 and 2011, of all cattle slaughtered in Sardinian abattoirs were collected with the objective to find the prevalence of hydatid infection in different categories of slaughtered cattle and the distribution of infection in the municipality of Sardinia. Further, the data were transferred into a GIS software (MapInfo Professional®) to achivie thematic maps.

MATERIALS AND METHODS: The legislative act of the Sardinian Department of Hygiene, Health and Welfare (N° 23549 of 10/11/2008, amended by N° 852 of 01.19.2009) imposes the registration of the identification number of slaughtered cattle found positive for a disease during the post-mortem inspection by a veterinary officer. Lists of all positive slaughtered animals are sent to CeNRE and to the Epidemiological Veterinary Regional Centre (OEVR) on a monthly basis. An ID was given to all animals, which allows tracing information of the sex and age of animals and the farm of origin. Slaughtered cattle were aggregated into three groups according to the national classification.We used the identification number of the farms to aggregate the cases into municipality areas and to highlight the municipality with CE cases. Statistical analysis was carried out using the Poisson distribution. With the discrete Poisson model, the number of cases in each location is Poisson-distributed. Under the null hypothesis, the expected number of cases in each area is proportional to its population size. SaTScan™, version 8.2.1 was used for cluster analysis. The process was based on the design of a circular zone of variable size radius from zero up to a maximum specified by the user (Maximum Spatial Cluster Size) with the circle centre located on each centroid, this was fixed on points of geographical coordinates of Sardinia's municipalities. The default maximum radius, which contains 50% of the population at risk inside the circle, was used. The Maximum Temporal Cluster Size for space-time analyses can be specified in terms of a percentage of the study period as a whole or as a certain number days, months or years. The maximum must be at least as large as the length of aggregated time interval length. If specified as a percent, then for the Poisson models, it can be at most 90 percent. We used the recommended value of 50 percent. The best candidate cluster areas were evaluated. The circle with the maximum likelihood, and where there were a higher number of registered cases than expected, was designated the most likely cluster (MLC) (Kulldorff M. 1997. Com in Statist -Theory and Meth. 26, 1481-1496).

RESULTS: 1,360 cattle slaughtered in Sardinia in 2009, 925 in 2010 and 964 in 2011 were found to be positive for CE with a registered prevalence respectively of 4.2 %, 2.8% and 3.1%. The highest prevalence was registered among cows (26.2-19.5%), followed by bulls (16.2-13.5%). In calves (6-12 months of age) and baby beefs (12-24 months of age), the prevalence was considerably lower (0.8-0.5%). When accounted for at the municipality level, it was found that 282 out of 377 municipalities had CE-positive farms emphasizing that the disease is widely spread in Sardinia. Applying Discrete Poisson model adjusted for time by stratified randomization accounting for 3234 cases and the total population of 268267 cattle, study period from 2009/1/1 to 2011/12/3, the MLC was detected at latitude 39.435363 N and longitude 8.896053 E in a centroid of 39.57 km radius. Within this circle, 236 cases in the population of 20209 cattle were observed. The expected number was 69.3 giving the ratio of 3.4 for observed cases to expected cases. The relative risk was 3.6 and the Log Likelihood Ratio 126.938 (p-value << 0.0001). Time frame was from 2010/1/1 to 2010/12/31

CONCLUSIONS: The use of Mapinfo and software SatScan has allowed to realize thematic maps that give an immediate visual results. The data-flow model used for cattle provides useful epidemiological information with respect to CE. If applied to sheep, which have a higher relevance in epidemiology of this zoonosis, it would provide even better and more relevant information. A reliable assessment of the degree of intervention needed in Sardinia would be possible if the precise localization of farms hosting infested animals could be listed and analyzed in combination with the incidence data.

Environmental contamination by *Aspergillus* spp. of poultry farms for eggs production: a risk for public health?

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Aspergillus spp. are fungi worldwide distributed in soil, plants, water and other organic substrates. These fungi may act as pathogens causing severe illness in humans and animals (Tell LA, 2005, Med. Mycol, 43: 71-73). Generally, high concentration of spores in the environment and long-term exposure are necessary for animal and human infections (Tell LA, 2005, Med Mycol, 43: 71-73; Fairs A et al, 2010, J Investig Allergol Clin Immunol, 20: 490-498). It is well known that *Aspergillus* spp. is part of the environmental contaminants mostly in facilities where animals are housed (Tell LA, 2005, Med. Mycol, 43: 71-73; Fulleringer SL et al, 2006, Poult Sci, 85: 1875-80). Nonetheless, information on the occurrence and epidemiology of these fungi in poultry farms for eggs productions is meagre.

AIM: The aim of the present study was to determine the concentration of airborne *Aspergillus* spp. spores in poultry farms and to identify risk factors predictive for their occurrence. The isolation of *Aspergillus* spp. from poultry farm workers was also investigated.

MATERIALS AND METHODS: Fungal culture was performed from 57 air samples collected from 19 sheds (Group I), 69 samples of faeces (Group II), 19 samples of poultry feedstuffs (Group III) and three anatomical sites (i.e., nostrils, pharynx, ears) from 20 farm workers (total n= 60; Group IV).

RESULTS: The prevalence of *Aspergillus* spp. in different groups ranged from 31.6% (Group III) to 55.5% (Group IV), whereas the highest spore concentration (CFU) was retrieved in samples from Group II ($1.2x10^4$ CFU/m^3) and Group III ($1.9x10^3$ CFU/gr). The mean concentration of airborne *Aspergillus* spp. spores in poultry farms was of 70 CFU/m^3. *A. fumigatus* (27.3%) followed by *A. flavus* (6.3%) were the most frequently species isolated from all the groups sampled. The species of *Aspergillus* isolated from Group I was also retrieved in samples from Group IV.
Aspergillus spp. were isolated mostly from human nostril (40%) and ears (35%) ($p<0.05$). The prevalence of *Aspergillus* spp. was significantly higher ($p<0.05$) in shed located at >9m above sea level, with higher temperature (>29°C) and humidity (>50%) and in sheds in which poultry feedstuffs contained *Aspergillus* spp. spores>10^2CFU/gr. No fungal infections occurred in hens.

CONCLUSIONS: The results of this study provide basic knowledge into the epidemiology of *Aspergillus* spp. in poultry farms for eggs production, and demonstrate the relationship between the concentration of airborne *Aspergillus* spp. spores in poultry farms and human and animal health. Even if the concentration of airborne *Aspergillus* spp. spores (i.e., 70 CFU/m^3) here reported does not seem sufficient to trigger fungal infections in hens, it allows the *Aspergillus* spp. colonization of human tissues. The correct management of poultry farms (e.g., control of microclimate and/or the acquisition of more proper cleaning procedures) seems to be necessary to control the indoor environmental concentration of *Aspergillus* spp. spores thus reducing risk of animal and human infections.

Parasitological survey on cattle endoparasites from Abruzzo region of central Italy

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Gastrointestinal (GI) nematodes and coccidia are major threats for livestock industry all over the world. These endoparasites affect health and welfare of cattle and are causes of relevant economic losses (e.g. unthriftness, reduction in milk production, weight loss) especially in certain socio-economic settings (Charlier J et al, 2009, Vet Parasitol, 164: 70-79: Koutny H et al, 2012, Parasitol Res, 110: 1893-1901). Specifically, GI nematodes cause reduced growth, tissue edema, diarrhea, especially when broad spectrum anthelmintics are not sistematically administered (Hawkins JA, 1993, Vet Parasitol, 46: 159-173; Charlier J et al, 2009, Vet Parasitol, 164: 70-79). With regard to coccidia, around 13 different species of *Eimeria* are known to infect cattle and, in particular, clinical signs of heamorrhagic diarrhoea are likely associated with *Eimeria bovis* and *Eimeria zuernii* (Stockdale PH et al, 1981, Can J Comp Med, 45:3 4-37; Friend SC et al, 1980, Can J Comp Med, 44: 129-140). Worthy of note is that coccidian may greatly impair animal welfare and performances even in absence of clinical signs. Hence, a continuing monitoring of cattle parasitoses is crucial for appropriate control programs.

AIM: The present study aimed to evaluate the presence and occurrence of intestinal endoparasites in cattle farms from Abruzzo region of central Italy.

MATERIALS AND METHODS: In February- March 2012 a total of 500 faecal individual samples was collected from beef and dairy cattle of different breeds and age living in 10 farms (i.e. 50 heads/farm) located in Abruzzo region. Samples were collected directly from the rectum of the animals and examined for endoparasites using both qualitative and quantitative diagnostic methods. All faecal samples were examined using a standard flotation procedures and a modified McMaster technique with a zinc sulphate solution of 1.350 specific gravity. Slides were examined under a light microscopy at 100X, 200X and 400X magnifications and all parasitic elements were identified using key features (Sloss MW et al, 1994, Veterinary Clinical Parasitology, Iowa State University Press, Ames, Iowa, USA**;** Taylor MA et al, 2007, Veterinary Parassitology, Blackwell, Oxford, UK).

RESULTS: Of the 500 cows examined, 34 (6.8%) were positive for Strongylidae, 31 (6.2%) for *Eimeria* spp., 6 (1.2%) for *Trichuris* spp., 3 (0.6%) for Ascaridae, 3 (0.6%) for *Strongyloides* spp. and 1 (0.2%) for Paramphistomidae. Out of the 34 samples positive for Strongylidae, 30 were detected only by the flotation, 3 by the McMaster (with 50 epg values) and 1 with both techniques (with 50 epg value). Of the 31 samples which scored positives for *Eimeria* spp., 29 were detected by flotation, 3 by McMaster (range of 50-100 opg values) and 2 with both procedures (50 and 100 opg, respectively). One sample was positive for *Trichuris* spp. only at the McMaster method (50 epg) while the other parasites were found only at faecal floatations.

CONCLUSIONS: Though the small sample size, this survey indicate the presence of helminths and *Eimeria* infections in cattle from a selected area of central Italy, although with apparent low infection rates. Given that these infections are a potential threat for animal welfare and may cause relevant economic losses, further studies are warranted to investigate the distribution of these parasites in larger areas in both dairy and beef cattle and their actual impact on livestock production. Studies are presently ongoing to genetically identify at the species level the parasites retrieved, in order to provide data instrumental to control programs to be planned *ad hoc* according the different species circulating in cattle bred in the study area.

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Study of lesional aspects of cystic echinococcosis (CE) in sheep in Sardinia: organ distribution, fertility, morphology of hydatid cysts and protoscolex viability.

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AIM: Major transformations have taken place in Sardinia over the last decades, modifying the scenario of CE spread. A more entrepreneurial approach to animal raising, closed systems, landed investments co-exist with traditional extensive or semi-extensive sheep management, home slaughtering, large numbers of stray, community and sheep-dogs and poor disease awareness. Monitoring the disease in livestock is therefore essential for CE risk assessment, also because the severe economic downturn, emergence of other zoonoses, high production costs and low revenue from dairy products, have not only inhibited farmers ability to invest, but even to raise their animals. This investigation aims to determine organ distribution, fertility, cyst morphology and protoscolex (PSC) viability of CE cysts in Sardinian sheep, drawing comparisons with earlier surveys to gain an updated insight into changes in the spread of the zoonosis and into epidemiological factors currently associated with infection.

MATERIALS AND METHODS: A detailed analysis was carried out on 6200 CE cysts recovered from more than 1400 sheep raised in south Sardinia and slaughtered in the period 2005-10. The results were compared with data from a similar analysis of more than 10000 cysts from 1375 dairy sheep surveyed in 1995-97. Cysts were examined macroscopically, shape, size, cavity content and fertility evaluated to group cysts into 5 types according to our previous classification (Bortoletti et al., 2003, Ig. Moderna,100, 1401-15).

RESULTS. The most affected organ was the liver. Of the 6244 cysts recovered in the 2005-10 survey 64% were hepatic and 36% pulmonary, compared to 68% and 32% respectively for the 10326 cysts recovered in the 1995-97 survey. As much as 52% of infected sheep presented cysts in both liver and lungs, whereas 34% had only hepatic and 14% only pulmonary cysts. Overall 86% of infected animals harboured at least one hepatic cyst, whereas 66% hosted at least one pulmonary cyst. About 10% of the cysts were fertile, regardless of location. The morphostructural analysis revealed a significant difference in size between fertile and non-fertile specimens. Cysts were grouped into 5 different types: *Unilocular* cysts, accounting for ~10% of cases (~8% in the previous survey), show a single fluid-filled cavity. Ranging from 1 to >10 cm in size, these cysts were fertile, often with numerous brood capsules but with highly variable PSC vitality ranging from 1 to 100%. *Multivesicular* cysts, between 0.5 and 4-5 cm in diameter, but mostly less than 1 cm in the present survey, have cavity invariably divided into spheroidal chambers (3-10) that though fluid-filled are generally sterile or, in rare cases show very few PSC (from tens to some hundreds). Cyst wall has thick external adventitial layer and thin laminar layer with residual germinative membrane. Frequently found (31% of cysts vs 37% in the past) they represent a state of degeneration of the parasite. Further evolution of this cyst type results in the c*alcified* type, where internal chambers become almost virtual due to thickening of internal septa. Very small and sterile, they are commonly recovered in Sardinian sheep (52% of cases in both surveys), particularly in the liver, accounting for the final degenerative stage of parasite evolution. About 5% of recovered cysts (3% in the past), but more than 11% of pulmonary cysts appeared as *caseous*, similar in shape and size to unilocular type, but the cavity filled with a thick yellowish matrix of creamy consistency. Seldom do the *hyperlaminated* cysts account for less than 2% of the total (<1 in the past). More frequent in the lungs (3.7%), these relatively large cysts (up to 10 cm) have virtual cavity filled with extensively folded and overlapping sheets of laminated tissue.

CONCLUSIONS. The *unilocular* cysts, almost all fertile and able to assure the continuation of CE life cycle, appear to have increased slightly compared to the previous survey, suggesting a reversal in the downward trend observed over time for this type of cyst. In fact frequency of sheep harbouring at least 1 fertile cyst, about 70% in Sardinia 30 years ago, ranged between 25 and 40% in the 1980s, falling to 12.5% in 1995-97 is 14% at present. This finding warrants attention and further evaluation as it may be an early warning sign of an upsurge in disease spread after the improvements in the past.

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Parasites of the digestive tract of sheep in Sardinia: an epidemiological update

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AIM: The parasitic infections of the digestive tract of sheep are a widespread health problem in Sardinia (Garippa G. et al., 2008, Large Animal Rev, 14: 181), and it is one of the main factors limiting the livestock production. Their presence and intensity of infection in a geographical area, apart from the factors related to the host, is conditioned by farm management, environmental factors and climate. The collection of the data representing the areas of a region is the basis for the knowledge of the different epidemiological patterns and for the implementation of targeted control strategies. In this work, based on a project funded by the Autonomous Region of Sardinia for the monitoring of parasitism of small ruminants, the first results on the spread of parasitic infections of the digestive tract of sheep in Sardinia are reported.

MATERIALS AND METHODS: The Region of Sardinia was divided into 90 quadrants (20 x 20 km) using the software ArcView GIS 9.2 (ESRI, Inc), those with farms with more than 50 animals (78) were considered for the sampling. In each quadrant the farm closest to the quadrant centroid was selected. From February to December 2011, 67 out of the 78 farms were sampled, and 1310 individual faecal samples analysed (1005 ewes and 305 flock replacement). Ewes were present in all quadrants, while flock replacements in 61. In each farm 15 adults and five flock replacement were sampled, and four pools of 5 g of faeces (each made by equal parts of five individuals) were composed: three of adult sheep and one of flock replacement. The samples were processed using the FLOTAC® dual technique, with flotation solution FS2 (sg 1.20) and FS7 (sg 1.35) with a sensitivity of 6 EPG (Cringoli G et al, 2010, Nature Protocols, 5: 503-515).

RESULTS: A synthesis of the results is shown in the following table. The farms positive to gastrointestinal nematodes (GIN) were 64/67 (95.5%) and 53/61 (86.9%), for adults and flock replacement, respectively. Strongylidae showed the highest and most variable EPG in the different localities, ranging in ewes from 6 to 197 in 45 farms, 232-486 in 12, and 536-2084 in 6. In flock replacement, Strongylidae EPG ranged from 6 to 170 in 29 farms, 220-490 in 10, and 564-4000 in 10. The highest mean EPG of Strongylidae (232 and 215) were found in the provinces of Nuoro and Sassari, and the lowest (38 and 89) in those of Olbia-Tempio and Ogliastra. Strongylidae mean prevalences ranged from 80% to 100%, except in the province of Cagliari (55%).

	Ewes						Flock replacement	
	% farms positive	Repartition of P% per farm				Mean EPG	% farms positive	Mean EPG
		0%	33%	66%	100%			
Eimeria spp.	97.0	2	0	2	63	-	91.8	-
Strongylidae	94.0	4	3	14	46	152.4	80.3	273.1
Nematodirus sp.	35.8	43	18	5	1	3.0	31.1	33.5
Trichuris sp.	37.3	42	18	6	1	4.2	40.9	10.0
D. dendriticum	26.8	49	6	5	7	7.6	8.2	2.5
F. hepatica	1.5	66	0	1	0	0.3	1.6	0.1
Paramphistomidae	4.5	64	1	2	0	1.2	0.0	0.0
Moniezia sp.	65.6	23	15	13	16	-	49.1	-

CONCLUSIONS: The results confirmed that coccidiosis, GIN infections and dicroceliosis are still a sanitary problem in Sardinia (Capelli G. 2000, Atti Simposio Giasone, XIV Congr. Naz. SIPAOC, 2: 21-48; Garippa et al, 2010, Parassitologia 52: 316). Concerning GIN, the results did not differ markedly from those reported by Garippa et al (2008) for the whole Island. As regards the infections by trematodes, it is not easy to state a reduction of the diffusion of *F. hepatica* and an increase of *D. dendriticum*, in consideration of the different sampling protocols and diagnostic methods employed in the previous studies. Nevertheless, the lower diffusion of fasciolosis could be due to the reduction of risk areas accompanied by a good availability of specific and effective treatments; conversely the uniform distribution of intermediate hosts in the territory and the lack of specific drugs can still explain the high diffusion of *D. dendriticum* infections.

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Preliminary data on the occurrence and genotyping of *Prototheca zopfii*, a cause of bovine mastitis in dairy cattle farms in Veneto region

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AIM: Bovine mastitis due to unicellular, achlorophyllous algae of the genus *Prototheca* is a serious and complex ailment that accounts for high economic losses in the dairy industry (Buzzini P. et al., 2004,Mycopathologia 158: 427–430). In the last two years the increasing of diagnosis of bovine mastitis due to *Prototheca* species in Veneto region revealed that this opportunistic pathogen is more widespread than expected (unpublished data). Bovine protothecal mastitis has been almost exclusively associated with only one species, *Prototheca zopfii.* Recently, based on the 18S rDNA sequence analysis, *P. zopfii* has been divided into three genotypes (1–3), of which *P. zopfii* genotype 3 has been given the status of a new species *Prototheca blaschkeae* sp. nov. (Roesler U., 2006, International Journal of Systematic and Evolutionary Microbiology 56: 1419–1425).
The aim of this study was to investigate the occurrence and the genotypic composition of the population of *P. zopfii* bovine mastitis isolated from dairy cattle farms in Veneto region.

MATERIALS AND METHODS: From August 2011, 15 dairy farms from Vicenza province grouped in 3 groups were investigated for the presence of *Prototheca* sp. Groups were described as number of lactenting cows: group A (≤ 50), group B (50-100) and group C (≥ 100). Samples examined were milk from cows with a somatic cells count (SCC) > 2,000,000 cells/ml, milk from Bulk-Tank milk, specimens of cow-barn surroundings as bedding and surface swabs from milking machine. All samples were cultivated on *Prototheca* isolation medium (PIM) at 37°C from three to five days. Identification to the species level were reached by morphological methods and genotype discrimination by extraction and sequencing of 18S rDNA amplicons. Nucleotide BLAST of the sequences obtained were aligned in the GenBank database.

RESULTS: Overall 4 (26.7%) of the 15 dairy farms were positive for *Prototheca* sp. In particular *Prototheca* grew from cultures of individual milk (n=3), composite milk (n=4) and swabs (n=7) from group A and B, while in group C the occurrence of algae was only from the bedding (n=1). *P. zopfii* gen 2 was isolated from all type of samples but bedding, while *P. blaschkeae* was isolated from bedding and composite milk but individual milk.

CONCLUSIONS: These preliminary data suggest that bovine mastitis due to *Prototheca* species could be more common than reported by literature. In particular this study confirms the possible role of *P. zopfii* genotype 2 as the main mastitis pathogen and support the hypothesis of other Authors (Ricchi M. et al,2010, J Dairy Sci, 93(10):4625-4631) that such pathology could be caused occasionally by *P. blaschkeae.* Our next goal will be to focus on *Prototheca* positive farms to better understand the transmission pattern of the pathogens in the environment in order to prevent the exposure of animals from sources of infections. *Prototheca* has poor sensitivity to the conventional antibiotics and antifungal treatments (Rakesh R et al, 2006,Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, CAB Reviews 1, No. 017) and consequently preventive measures need to be implemented for controlling the disease.

Investigation of the zoonotic potential of *Cryptosporidium* in a diarrhoeic foal

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AIM: Diarrhoeal disease is very common in foals, especially in the first 6 months of age. It is a life-threatening process, and surviving animals remain weak, showing a high susceptibility to other infections. Rotavirus, *Clostridium perfringens, Salmonella, Strongyloides westeri* and *Cryptosporidium* are amongst the most frequent enteropathogens causing diarrhoea in foals.
Cryptosporidium infection can reach prevalences close to 25% in foals (Netherwood T et al, 1996, Epidemiol Infect 117: 375-383; Veronesi F et al, 2010, Zoonoses Public Health 57:510-517), although it is not always related to the presence of diarrhoea. Molecular studies of *Cryptosporidium* in equines are limited, but demonstrate the existence of human-pathogenic *Cryptosporidium* species and genotypes in that host (Chalmers RM et al, 2005, Vet Rec 156:49-50; Grinberg A et al, 2008, J Clin Microbiol 46:2396-2398; Burton AJ et al, 2010, Vet Parasitol 174:139–144; Veronesi F et al, 2010, Zoonoses Public Health 57:510-517). The main goal of this work is to investigate the presence of zoonotic cryptosporidiosis in a foal with neonatal diarrhoea.

MATERIALS AND METHODS: A 5 day old quarter horse female foal from Pergola (Italy) arrived to the Veterinary Clinical Pathology Service of the University of Bologna presenting yellowish and foul-smelling diarrhoea. A faecal specimen were collected and examined for the presence of enteropathogens. Examination of a modified *Ziehl-Neelsen* stained smear revealed profuse numbers of *Cryptosporidium* oocysts. No other pathogens were detected. For molecular analysis, total DNA was extracted directly from faeces using a stool extraction kit (QIAgen Gmbh, Hilden, Germany) according to the manufacturer s instructions, preceded by three cycles of freezing and thawing. To determine the *Cryptosporidium* species, a nested PCR of a small-subunit (SSU) rRNA gene fragment (≈840 bp) was used followed by RFLP analysis of the PCR products using the endonucleases *Ssp*I and *Vsp*I. An approximately 850-bp long fragment of the GP60 gene was amplified by a two-step nested PCR and subsequently sequenced. The terminology proposed by Sulaiman et al. (Sulaiman IM et al, 2005, J Clin Microbiol, 43: 2805-2809) was used in naming *C. parvum* subtypes.

RESULTS: PCR-RFLP of the (SSU) rRNA showed a banding pattern indicative of *C. parvum.* Several molecular studies reported the zoonotic *C. parvum* as the most common species affecting equines, although the *Cryptosporidium* horse genotype was also identified.
Sequence analysis of the glycoprotein (GP60) gene revealed that the isolate belonged to the subtype IIaA15G2R1. This subtype is especially common in calves worldwide and it is the dominant subtype in most areas studied, Italy included (Duranti A et al, 2009, Zoonoses Public Health, 56:176-82). It is also the predominant *C. parvum* subtype in humans from several European countries.

CONCLUSIONS: Our findings suggest that equines, especially foals, may be important reservoirs of zoonotic *Cryptosporidium* species and subtypes. For this reason, it is essential to carry out a suitable diagnosis of foal neonatal diarrhoea to apply the appropriate control measures and minimize zoonotic risk.

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Molecular characterization of *Cryptosporidium* isolates from calves in Sardinia (Italy)

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AIM: *Cryptosporidium* is a protozoan parasite infecting the gastrointestinal tract of a wide range of vertebrate hosts, including humans. Ruminants could be infected by several *Cryptosporidium* species, *Cryptosporidium parvum* being the most common causing diarrhoeal disease. This species has also been implicated in human cryptosporidiosis outbreaks. Since *C. parvum* is mostly found in pre-weaned calves, they therefore play an important role as reservoirs for human infection (Smith RP et al, 2010, Prev Vet Med 94:9–17; Xiao L, 2010, Exp Parasitol 124:80–89).
In order to provide data on the occurrence and zoonotic potential of *Cryptosporidium* isolates in calves from Sardinia, faecal samples from pre-weaned calves aged up to 45 days were analysed by means of microscopic and molecular techniques.

MATERIALS AND METHODS: 147 faecal samples were collected in 22 cattle farms located in the centre (18) and the north (4) of Sardinia. Samples were examined for the presence of *Cryptosporidium* oocysts by microscopy of stained Ziehl-Neelsen smears. A selection of positive samples were analysed using molecular techniques. *Cryptosporidium* species were determined by a nested PCR of a small-subunit (SSU) rRNA gene fragment (≈840 bp) and restriction fragment length polymorphism (RFLP) analysis with the endonucleases *Ssp*I, *Vsp*I and *Mbo*II. Subtyping of *C. parvum* isolates was performed by DNA sequencing of the 60 kDa glycoprotein gene (GP60; ≈850 bp). Subtypes were named according to nomenclature described by Sulaiman et al. (Sulaiman IM et al, 2005, J Clin Microbiol, 43: 2805-2809).

RESULTS: The individual prevalence was 47.6%, whereas the 77.2% of cattle farms harboured *Cryptosporidium* infected animals. A subset of 38 microscopy positive samples were selected for molecular characterization. PCR products of the SSU rRNA locus were obtained from the 74% (28/38) of the cattle isolates. *C. parvum* was identified in 26 isolates, whereas the remaining 2 isolates yielded a banding pattern indicative of *C. bovis*, a host-specific species of no importance in transmitting cryptosporidiosis to human.
Sequencing of GP60-PCR products was successful for 22 of the 26 *C. parvum* isolates, and 4 different subtypes were identified. Most of the isolates belonged to the allele IIaA15G2R1 (19/22), one of the major subtypes responsible for zoonotic cryptosporidiosis and one of the most prevalent *C. parvum* subtype in calves in Europe and North America. One isolate showed the allele IIaA16G3R1 (1/22), which is not common in cattle and seems to play a minor zoonotic role, since only one case of human infection has been recorded. Finally, two alleles within the family IId, IIdA20G1 (1/22) and the novel IIdA20 (1/22), were identified in a single farm remote from the other studied farms and located in the Northwest of the island. IId subtype is occasionally found in calves in European countries, but is common in lambs and kids. Nevertheless, it is noteworthy that IId subtype family is also considered to be zoonotic cryptosporidia.

CONCLUSIONS: The current study has revealed that *Cryptosporidium* spp is a prevalent parasite in cattle farms in Sardinia. Our data indicate that most isolates from calves in this geographical area have zoonotic potential. For that reason, persons handling pre-weaned calves (farmers, veterinarians, children, etc.) should take the suitable measures to avoid the risk of zoonotic infection.

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Emission of coccidian oocysts by two goat breeds during one year survey

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AIM: Eimerian infections can cause severe disease in livestock. A lot of parasitological studies in goats showed a high prevalence of coccidian infections in different geographic areas. In Italy a study found a prevalence of 91.94% (Di Cerbo AR et al, 2010, Small Ruminant Res, 88(2-3): 102-112) similar to 91.2% in 2011 in Brazil (Cavalcante A et al, 2011, Vet Parasitol, 183(3-4): 356-358). High prevalences in farms can be caused by several factors, one of the most important is probably the short time needed for oocyst sporulation that depends on *Eimeria* species but generally vary from 1 to 6 days. Further, goats could be infected by nine *Eimeria* species that are characterized by different pathogenicity. All *Eimeria* species are host specific except *E. caprovina* that can be transmitted between goats and sheep. Clinical signs and symptoms, including diarrhoea, hemorrhagic diarrhoea, weight loss and death, are generally more severe in young animals. Adult goats have an important role in maintaining a high level of environmental contamination in farm. Aim of the study was to investigate any variation in oocyst emission in adult goats belonging to Alpine and Nera di Verzasca breeds throughout a year.

MATERIALS AND METHODS: The study was carried out from January to December 2010 in a farm located in Lombardy where the two breeds are reared together in an exstensive system. Faecal samples were collected monthly from the rectum of 20 adult goats (10 Alpine and 10 Nera di Verzasca), pellets were prepared and were stored at 4°C until examination. Quantitative oocysts determinations were made by using FLOTAC DOUBLE technique (flotation solution MgSO4, s.g. – 1.280).

RESULTS: Coccidian oocysts had a prevalence of 100% almost all over the year. Out of 223 samples, there were 2 negative specimen, one in October and one in November. The average value for Verzasca were 889.26 opg (min-max= 0-7724; sd=1131.169) and for Alpine were 1396.69 opg (min-max= 0-12060; sd=1716.34). All year round oocysts count were higher for Alpine than Verzasca except in March and April. A low oocysts number was registered from June until the end of the year whereas the highest oocyst count was obtained in May. The ln transformed opg values were tested by Anova and significant differences were observed considering sampling (p=<0.001), breed, age and birth number (p=<0.005).

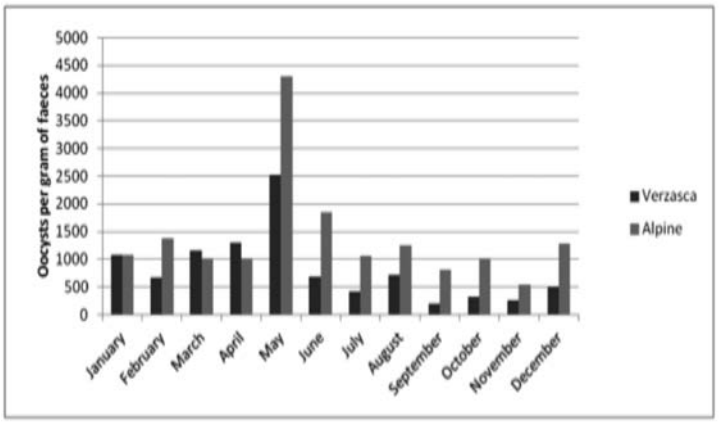

Fig. 1. Emission of oocysts by goat throughout the year.

CONCLUSIONS: The oocysts emission seems to be affected by two factors. First of all, the observed variation in opg could depend by metereological weather. Opg values increased as the temperatures raised, in fact in May were registered the highest temperatures. Decreasing of oocysts excretion during the second part of the year could be related both to the increased time spent by goats on outdoor pasture (due to more hours of light in spring) and to the better feeding with fresh grass, that could improve body condition score and immune system. Further, the autochthonous breed Nera di Verzasca goats showed a lower oocyst emission than the Alpine one. This difference in oocysts emission suggest that the Alpine breed could be less resistant to these protozoa. Our results show that coccidian emission by adult goats is relevant, therefore farm management strategy could be important in order to avoid kids infection.

Gastrointestinal nematode infection in sheep in Galicia (Northwest of Spain) after a decade of systematic anthelmintic treatment

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AIM: In Galicia (NW Spain) there are around 24,000 sheep flocks, with a mean of 11.5 animals/flock, contributing to the economic and environmental sustainability of the whole farm. Gastrointestinal nematode (GI) infections result in low productivity, due to stunted growth, poor weight gain and poor feed utilization. OVICA, Galician association of ovine and caprine breeders created in 1994, introduced veterinary support in 2002. From that moment onwards, commercial ovine farms included a systematic single dose treatment in spring and/or autumn intended to control GI infections. Pedreira *et al*. (Pedreira *et al,* 2006, Prev Vet Med, 75: 59-62), in a coprological survey carried out during 2001-2002, found 100% prevalence at flock and individual level in Galicia. The main objective of this study was to establish the present GI prevalence in Galicia and evaluate its evolution after a decade of systematic anthelmintic treatment designed to control these parasites.

MATERIALS AND METHODS: A total of 1,914 sheep from 74 commercial meat ovine flocks were sampled to evaluate the presence of GI infection. Faecal samples were collected directly from the rectum with plastic gloves and kept at 4ºC until analysed. Five grams of each sample were processed by flotation technique, with a sensitivity of 20 eggs per gram of faeces (epg). A flock was considered positive when a simple positive animal was detected. Age (categorized as 0-12, 13-48 and >48 months), treatment pattern (single dose in spring, autumn or in both of them) and drugs applied (macrocyclic lactones -ML-, ML+benzimidazoles -BZ- or only BZ) where used as factors for statistical analysis with GI prevalence. The association between GI infection and these factors were studied individually with a Chi-squared test (chisq.test() function) in R statistical package (R v. 2.15.0, R Development Core Team, 2012).

RESULTS: 1,233 out of 1,914 faecal samples examined were positive for GI infection (64.4%; C.I. 95% 62.22-66.56), whereas farm prevalence was 100%. The Chi-squared test showed that age (χ^2=0.114; P= 0.944) and treatment pattern (χ^2=0.163; P= 0.686) were not related to GI prevalence. However, drug applied was a determinant factor over GI infection (χ^2=48.227; P< 0.001); sheep treated with ML or ML+BZ presented lower prevalence (55.6%; C.I. 52.14-59.05) than those treated only with BZ (71.0%; C.I. 68.18-73.64).

CONCLUSIONS: The prevalence detected in this study was lower than that observed a decade ago by Pedreira *et al*. (2006). Our results revealed that systematic treatment with anthelmintics in all of the ovine farms for a decade has provoked a reduction of the individual prevalence; however, the farm prevalence is still 100%. For this reason, other control strategies, as pasture rotation, avoid introducing animals from other farms and separate goats from sheep, should be introduced in sheep flocks to achieve a real GI control. Finally, we can also conclude that the use of ML, alone or with BZ, was more efficient against GI nematodes than a treatment with only BZ.

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Toxoplasma gondii DNA in goats: a preliminary report

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AIM: This report aims to evaluate the seroprevalence of *Toxoplasma gondii* infection in goats in Tuscany as well as to detect and genotype parasite DNA in their blood and milk samples.

MATERIALS AND METHODS: Sixty serum samples from adult (1-3 year old) Alpine goats, randomly selected from 3 herds in Tuscany, were investigated by a modified agglutination test (MAT) for *T. gondii* antibodies, starting to a dilution 1/20. Goats from herds 1 and 3 were lactating, so a PCR assay was performed on 30 blood samples as well as on milk samples from PCR blood positive goats. PCR was performed as described by Jones et al (2000 Invest Ophthalmol Vis Sci. 41:634-44), with slight modifications. Determination of genotypes was carried out according to Su et al. (2010 Parasitology 137:1-11). Differences in seroprevalence among the different herds were evaluated by means of chi square test.

RESULTS: Thirty one sera scored positive for anti *T.gondii* antibodies, with an overall seroprevalence of 51.6%. Seroprevalence at individual herds was 93.3%, 33.3% and 46.6%, respectively. Three animals out of 30 yielded PCR positive blood samples, 1 milk specimen from them scored PCR positive too. Serological and molecular results are summarized in table. Genotyping of DNA showed hints for genotype III. PCR positive samples were obtained from goats belonging to herd 1, which showed also a very significantly higher seroprevalence values with respect to herds 2 and 3.

Antibody titer	1/20	1/40	1/80	1/60	1/640	1/2560	1/5120	1/10240	1/163840
Positive goats	7	2	10	1	1	3	1	5	1
Herd Number	1,3	3	1,2,3	2	1	1	2	1,2	1
N. blood/milk PCR positive goats						1/0		1/1; 1/0	

The overall seroprevalence agrees with a recent survey performed by the same serological technique (Dubey et al, 2011 Int J Parasitol.,41:827-833). The occurrence of protozoan DNA in goat milk could be suggestive of viable parasites presence, but to evaluate the effective risk a mouse bio assay will be recommended. However, the finding of 1 PCR positive milk sample out of 3 PCR positive blood specimens from the herd with the highest seroprevalence, appears a very interesting feature, underlining the importance of milk pasteurization before any processing or ingestion.

CONCLUSIONS: Food-borne toxoplasmosis in humans may result from the ingestion of tissue cysts or tachyzoites contained in meat, primary offal, or meat-derived products of many different animals (Tenter, 2009 Mem Inst Oswaldo Cruz, 104: 364-369) and consumption of unpasteurized milk (Santos et al., 2009 Vet Parasitol, 161: 324-326). The risk of acquiring an infection with *T. gondii* by drinking cow's milk, if any, is minimal (Jackson & Hutchison 1989 Adv Parasitol, 28:55-105), however, it cannot be excluded that any type of milk is a potential source of infection, if consumed raw. A study assessing risk factors associated with primary *T. gondii* infections in women of childbearing age suggested that drinking milk may be a potential risk factor for horizontal transmission to humans (Paul,1998, Przegl Epidemiol 52:447-454). Thus far, clinical toxoplasmosis in humans has been associated with consumption of unpasteurized goat's milk (Riemann et al., 1975, Sacks et al. 1982, De Andrade et al., 1984, Skinner et al., 1990 cited by Tenter, 2009). Even if raw goats' milk is a proven vehicle for pathogen transmission raw dairy products are frequently considered healthier than pasteurized ones (Basnet et al., 2010 Pediatrics 125:973-977). Furthermore goat's milk is believed to be better to use in fresher cheese. There are only few data about the resistance of different *Toxoplasma* stages (Pettersen, 1984 Acta Pathol Microbiol Immunol Scand B 92:175-176), during the production process and storage of fresh cheese (Hiramoto et al., 2001 Rev Saude Publica 35:113-118) demonstrating that untreated milk and dairy products could be an important source of *T. gondii* in human infection.

Eimeria infections in imported beef-calves

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AIM: Eimeriosis is considered one of the most common and important disease of cattle world-wide (Rahmeto A et al, 2008, Intern J Appl Res Vet Med, 6:24-30). All age groups of cattle are susceptible to infection, but clinical eimeriosis is most common in young animals (Daugschies A, Najdrowsk M, 2005, J Vet Med B, 52:417-427). A number of epidemiological factors influence the pattern of the infection: stress factors like weaning, change of diet, environment, poor nutrition and sanitation and overcrowding can increase level of infection and incidence of the disease due to stress-induce immunosuppression. More than 13 species of *Eimeria* have been described to infect cattle: *E. bovis* is considered one of the most pathogenic species (Ernst JV et al, 1984, Vet Parasitol, 15:213-221). Very few scientific information is available on the occurrence of coccidiosis in imported beef-calves in Italy. The aim of the survey was to investigate on *Eimeria* infections in a stock of beef-calves imported from France during the first month of housing.

MATERIALS AND METHODS: The study was performed in a fattening unit in northern Italy on 96 Limousin beef-calves coming from France. The animals (4 months old about) were raised at pasture and at the age of weaning were grouped, transported in Italy and housed in a farm. Both during the transporting phase and the survey period no anticoccidial drugs were given to the ruminants. Each animal was tested three times by coprological methods: at the time of his arrival in farm (T0), after 15 days (T15) and after 30 days (T30). Individual fresh fecal samples were examined for coccidian oocysts by qualitative (sedimentation-flotation in salt sugar solution 1.3 density) techniques. Coccidian oocysts were cultured at 24-26°C in a humid chamber with 2.5% aqueous solution of potassium dichromate and the differentiation of the *Eimeria* species was done on the basis of their measures and morphological characteristics as described by Levine (Levine ND, 1985, Veterinary protozoology, Iowa State University Press, Ames, IA). The statistical package SPSS was used for analyses of data and a value of $P \leq 0.05$ was considered significant.

RESULTS: The 60.4% (64.32-56.49 CI 95%) of the animals resulted positive for *Eimeria* spp. at T0, the 27.1% (29.09-25.11 CI 95%) at T15 and the 26% (30.5-21.5 CI 95%) at T30. The most widespread specie of *Eimeria* at each stage of the coprological controls was *E. bovis* followed by *E. alabamensis*. A spontaneous and statistically significant decrease of the prevalence has been highlighted during the entire period of investigation both for coccidia group and each single species of *Eimeria*. The prevalence reduction were more marked for *E. bovis* and *E. alabamensis*. Totally were isolated 7 species of *Eimeria*. The maximum number of species found at T0 in a single calf was five: the value decrease during time. At T15 and T30 the maximum number of mixed species was two. At each step of survey animals were mainly infected by one or two species at the same time. The differences calculated between prevalence at T0, T15 and T30 were statistically significant.

CONCLUSIONS: In the present study 7 species of *Eimeria* out of the 13 reported in literature were found. According to the literature mixed infections of a single calf were commonly saw; no case of infection caused by one single species of *Eimeria* was observed (Rahmeto A et al, 2008, Intern J Appl Res Vet Med, 6:24-30). The study showed that *E. bovis* was the most prevalent species at each step of the investigation: this species is the most frequently reported coccidian in outbreaks of clinical coccidiosis throughout the world (Faber JE et al, 2002, Vet Parasitol, 104:1-17). None of the calves had clinical signs of infection. The spontaneous and gradual decrease of prevalence values noticed during the month was linked to the reduction of stress in calves and to the improvement of the sanitary conditions in association with the litter inadequacy at oocysts maturation.

PREVALENCE	Coccidia	*E.alabamensis*	*E.auburnensis*	*E.bovis*	*E.bukidnonensis*	*E.canadensis*	*E.cylindrica*	*E.zuernii*
T0	60.4%	36.2%	17.2%	58.9%	17.2%	15.9%	8.7%	13.8%
T15	27.1%	4%	12%	30.4%	1.4%	5.3%	1.7%	5.3%
T30	26%	1.4%	0%	12%	0%	5.2%	0%	4.3%

Gastrointestinal helminths infection in beef-calves imported from central district of france

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AIM: Despite imports of beef-calves from foreign countries, especially from France, is in Italy a longstanding practice (particularly in Northern regions) data available on gastrointestinal helminths of these animals are scarce (Battelli G, Poglayen G, Martini M, 1989, Estr. Il Nuovo Progr. Vet., 2:3-5; Diaferia M, Pinaffo D, Piergili Fioretti, 2003, Atti Sisvet, 57:205-206; Tampieri MP, Galuppi R, Colautti C, Bonoli C, et al, 2004, Parassitologia, 46(Supp.1):69; Stancampiano L, Corradini D, Bulgarelli M, Micagni G, Battelli G, 2007, Parassitologia, 40:101-106). In intensive italian beef production systems animals are routinely treated using broad-spectrum antiparasitic drugs. The aim of this study was to investigate on gastrointestinal nematodes infections trends in a stock of imported beef-calves during their first month in the fattening farm.

MATERIALS AND METHODS: In 2011, ninety-six Limousin calves from Central District of France, raised on pasture until weaning (at about 4 months of age), were examined in a fattening unit in Piemonte region. Calves were checked at three time: at their arriving in farm (T0), after 15 days (T15) and after 30 days (T30). No animal received any antiparasitic drug during the survey. The detection of gastrointestinal (GI) nematodes was performed on individual fecal samples by sedimentation-flotation in a high specific weight saline solution and by quantitative methods using Mc Master chamber. Prevalence was calculated as the proportion of animals found positive to the qualitative coprological test; abundance was calculated as the arithmetical means of EPG (eggs per gram of faeces) of every sample (positive or negative).

RESULTS: At each time (T0, T15 and T30), 92 out of 96 animal examined were positive for GI helminths with a prevalence of 95.8% (101.23-90.37 CI 95%). GI strongyles infection was the most common during the period (94.8% - 100.23-89.37 CI 95%). This result didn't include data referred to *Nematodirus* spp. and *Strongyloides papillosus*: because their eggs are morphologically identifiable they were considered individually. For *Nematodirus* spp. and *Strongyloides papillosus* it has been observed a decline of prevalence (T0-T30) less marked than whipworms. The highest abundance was observed in the period for GI strongyles; the values of abundance for *Nematodirus* spp., *Strongyloides papillosus* and whipworms were lowest than GI strongyles ones. For *Nematodirus* spp. and whipworms, abundances values decreased less importantly than *Strongyloides papillosus* ones. All differences between T0 and T30 both for prevalence and abundances values were statistically significant ($P \leq 0.05$).

PREVALENCE	GI strongyles	*Nematodirus* spp.	*Strongyloides papillosus*	Whipworms
T0	94.8%	32.3%	15.6%	16.7%
T15	94.8%	30.2%	9.4%	7.3%
T30	94.8%	28.1%	9.4%	3.1%
ABUNDANCE	GI strongyles	*Nematodirus* spp.	*Strongyloides papillosus*	Whipworms
T0	183.0	4.4	13.3	3
T15	143.7	3.7	11.9	1.5
T30	117.0	1.5	3.7	0.7

CONCLUSIONS: Data obtained confirm the presence of a diversified parasitic fauna in calves imported from foreign countries, as reported by Tampieri, (2004; *L.C.*). This is related to grazing origin of animals: on pasture the pressure of infection for GI helminths is high, particularly for GI strongyles. Prevalence observed for GI strongyles were higher than those reported in previously italian work on the same topic by Battelli, (1989, *L.C.*), Diaferia, (2003, *L.C.*), Tampieri, (2004, *L.C.*) Stancampiano, (2007; *L.C.*). The constant positivity for GI strongyles and the differences in prevalence reduction observed during the period for *Nematodirus* spp., *Strongyloides papillosus* and whipworms could be related to the phases of their biological cycle. The decrease of abundance during the study period indicates a spontaneous reduction of the parasitic infections. In order to better understand the parasitic trend and to make decisions about antiparasitic treatments, it is necessary a longer period of control linked also to zootechnical and economic data.

Epidemiological study of bovine cysticercosis in a traditionally endemic area in Piedmont

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AIM: To identify potential risk factors for bovine cysticercosis at herd level in Piedmont, Italy, and to assess the most likely route of introduction of the eggs of *Taenia saginata* in the affected farms.

MATERIALS AND METHODS: The study was conducted in an area of 147 km² in the southern part of the province of Turin (ASL TO3, District of Vigone). In this area, there are approximately 26.000 cattle raised in 324 farms (65 dairy and 259 beef herds). Of the 13.500 cattle which are slaughtered annually, approximately 3.000 belong to dairy breeds (mainly Fresian) and 10.500 to beef breeds (2.500 Piemontese and 8.000 French breeds). Data on farms and individual animals affected by bovine cysticercosis between 2005 and 2011 were retrieved from official sources (Anagrafe Bovina – Banca Dati Nazionale; ASL TO3 - Registro dei casi di cisticercosi bovina e idatidosi). A questionnaire based case-control study on farm level was set up, in which the case group (N=32) consisted of farms with infected animals identified during the fore mentioned six years, and the control group (N=131) comprised farms with confirmed absence of cysticercosis during the same period. All interviews were conducted face to face by the same operator. Several risk factors dealing with management and location of the farm, water supply for the animals, feed, pastures and personnel were investigated. To identify significant ($P \leq 0.05$) associations between a potential risk factor and the status of infected farm, we used Pearson's chi-square and calculated the odds ratio (OR) with its 95% confidence interval (CI).

RESULTS: Between 2005 and 2011, 128 animals from 46 farms (17 dairy and 29 beef herds) were positive at inspection for the presence of viable or degenerated *T. saginata* metacestodes, hence prevalence was 0.16 and 14.2% at the individual and farm level, respectively. Of the affected farms, 3 were home of "cysticercosis storms", with 10, 21 and 40 individuals affected in few months; 13 had between 2 and 7 cases in separate episodes; 31 had a single case. Of the positive individuals, 61 (47.6 %) belonged to the Piemontese breed (40 individuals in the same problem farm), while 47 (36.7 %) were French breeds (31 in two problem farms) and 20 were Fresian (15.7 %). Cysticercosis was significantly less prevalent in French breeds, provided exclusion from the database of the positive cattle raised in "stormy" farms ($P \leq 0.001$). In the case-control study, the single most important risk factor was the location of the farm downstream to small municipal wastewater treatment plants (OR = 2.99, CI 1.24-7.21; P=0.011). In addition, positively associated with the occurrence of bovine cysticercosis were: a) mixed vs exclusively internal replacement (OR = 0.39, CI 0.16-0.94; P=0.030); b) mixed vs exclusively indoor housing (OR = 0.23, CI 0.05-0.86; P=0.025); c) use of multi-source vs single source hay (OR = 0.19, CI 0.06-0.59; P=0.002); d) use of multi-source vs single-source fresh forage (OR = 0.13, CI 0.06-1.06; P=0.042). In positive farms, cases of taeniasis amongst operators were more frequently "confessed" to the interviewer (in 9.4 vs 2.3 % of the farms) with a difference close to significance ($P = 0.09$). Remarkably, all cases of taeniasis in the positive farms (N=3) corresponded to a "cysticercosis storm" episode.

CONCLUSIONS: In the study area, 71 of 128 cases of bovine cysticercosis (55.5 %) could be traced back to a case of teaniasis in a farm operator. In a control perspective, education of this particular category would be justified and paying strategy. At the farm level, bovine cysticercosis was positively associated with a range of relatively minor risk factors implying enhanced opportunities of feed contamination with wastewater on occasion of intentional or unintentional floods. There was no evidence that importation of French beef for fattening represented a risk factor, and the same was true for the type of farm operators (if family members only or family members plus contracted personnel).

Toxoplasma gondii: epidemiological survey on sheep dairy herds in Central Italy

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AIM: *Toxoplasma gondii* is a worldwide protozoan parasite, agent of a food and water borne zoonosis (Dubey JP, 2009, Vet Parasitol, 163 (1-2): 1-14; Cenci Goga BT et al, 2011, Foodborne Pathog Dis., 8(7):751-762). Among meat animals, sheep not only are considered to be one of the most important source of human infection but are particularly sensitive to pathogenic effect of the parasites (miscarriages and stillbirths). In despite of several studies conducted in different countries on the seroprevalence of *T. gondii* in sheep, very scarce result the bibliographic data in Italy.
Aim of the present work was to conduct a seroepidemiological study on *Toxoplasma* infection in sheep raised in Tuscany herds and to analyse the risk factors for parasitic transmission.

MATERIALS AND METHODS: A total of 630 adult Sarda sheep from 33 dairy herds of the Grosseto district (Tuscany region) were randomly selected. The sample size was calculated on the total amount of herds (730) and sheep (122.000) present in the district, on the expected prevalence for *Toxoplasma* at individual animal level (30%, standard error 3.5%) and at herd level (90%, standard error 10%), assuming an interval of confidence (IC) of 95%. The average number of animals reared within each tested herd was 368.33 (± 183.48) and among these a mean number of 19.09 (± 8.99) sheep were selected for the study.
Blood samples were collected from sheep between May and June 2011 and, at sampling time, an audit form was completed with the breeders. Questions included the following information: rearing system, water source, separate water troughs and separate feeding troughs for young and adult animals, purchase of spare breeding animals, presence of cats in property, access of cats to stored feed and to water given to animals. Sera were tested for IgG antibodies to *T. gondii* using an IFAT (Immuno Fluorescent Antibody Test). Commercial *T. gondii* tachyzoites (Mega Cor Diagnostik, Horbranz, Osterreich) were used as antigens; rabbit anti-sheep IgG fluorescein isothiocyanate conjugate (Sigma Immunochemicals, St Luis, MO, USA) was used at 1/100 dilution. Sera were screened at dilutions of 1/64 (cut off) and those tested positive were serial diluted. True prevalence (TP) was calculated with sensibility (97.3%) and specificity (96%) of IFA assay claimed by the manufacturer and on the apparent prevalence value (AP) obtained from the results. To identify risk factors associated with *T. gondii* seropositivity, an univariate analysis of the interest variables with the Pearson's chi-square test or Fisher's exact test, when necessary, was conducted with GraphPad InStat 3 for Mac OS X.

RESULTS: 32 herds (96.97%) were found positive for *T. gondii* infection (at least one *Toxoplasma* positive animal). At individual level, seroprevalence was 33.97% (214 positive samples out of 630; 95% CI: 28.11-35.38%). Among the 214 reactive samples, 42 (19.63%) had an antibody titre of 1:64, 36 (16.82%) of 1:128, 39 (18.22%) of 1:256 and 97 (45.33%) of ≥ 1/512. The TP for *T. gondii* was calculated to be 32.12% (95% CI: 28.47-35.77%). In the univariate statistical analysis *T. gondii* seropositivity at animal level was significantly associated to rearing system ($p < 0.05$) [extensive rearing OD 1.76 (95% CI: 1.04-3.00)], herd size ($p < 0.0001$) [large herds OD 0.37 (95% CI: 0.25-0.54)], water source ($p < 0.05$) [farms with still water OD 2.80 (95% CI: 1.36-5.77)] and access of cats to water given to animals ($p < 0.0001$) [possibility of the access 2.12 (95% CI: 1.48-3.05)].

CONCLUSIONS: The results obtained in the present study demonstrated the high presence of *Toxoplasma* infection in the herds (96.97%) in Tuscany region and confirmed the prevalence value (32.12%) in sheep populations reported in Italy by some Authors (Masala G et al, 2003, Vet Parasitol, 117: 15-21; Fusco G et al, 2007, Vet Parasitol, 149: 271-274; Zedda MT et al, 2010, Zoonoses Public Health, 57: 102-108). Nevertheless, others AA reported higher values of prevalence (Gaffuri A et al, 2006, J Wildl Dis, 42(3): 685-690; Vesco G et al, 2007, Vet Parasitol, 146: 3-8; Natale A et al, 2007, Parassitologia, 49: 235-238). The variability in the prevalence rates may be attributed to different climatic factors, age, management and serological methods used (Piergili Fioretti D, 2004, Parassitologia, 46: 177-181).
The results may be useful to improve management practices for a better control of the disease.

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A new model to estimate the posterior probability of spatial distribution of helminth infections in sheep farms

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AIM: Helminth infections remain one of the main constraint on health and productivity in sheep farms worldwide. The advantage of mapping the location of the farms and of studying the spatial distribution of infections is clear since it enables focused intervention strategy and could address important scientific clues. Model-based geostatistics and Bayesian approaches are useful in the context of Veterinary Epidemiology when point data have been collected by appropriate study design (Schur N et al, 2011, Plos Negl Trop Dis, 5: e1194; Magalhães RJ et al, 2011, Adv Parasitol, 74: 267-296). In this work we moved from explanation of spatial distribution to prediction of probability of infection for all the geo-referenced farms of the Campania region starting from a sample of observed data. Little work has been done so far on predicting infection probabilities both in human and veterinary medicine to tailor epidemiological surveillance.

MATERIALS AND METHODS: Parasitological data came from a cross-sectional study carried out in the Campania region, southern Italy (Musella V et al, 2011, Prev Vet Med, 99(2-4): 69-77). All the sheep farms of the region were geo-referenced. A grid of 10 x 10 km was overlaid on the region for a total of 135 equal cells. For each cell the farm closest to the centroid was selected. Out of the total 135 quadrants, 121 were investigated. Faecal samples were collected and the *FLOTAC dual technique* (Cringoli G et al, 2010, Nature Protocol, 5: 503-515) was employed for coprological examinations of 23 different helminths. A Bernoulli likelihood on the presence/absence of parasitic infection in the 121 investigated farms was assumed. A Bayesian Gaussian spatial exponential model prior was specified on random terms in the linear predictor of a probit function of the probability of infection. The hyper-parameters of the correlation matrix have been chosen in such a way that the correlation between points be 0.95 at minimum distance and 0.013 at maximum distance. A Bayesian Kriging was performed to predict the probability of infection in 1500 unknown points which represent the centroid of the cells of a regular grid of 3 3 Km on the region. Furthermore, for each helminth, the probability of infection was calculated for each geo-referenced farm in the Campania region. A Markov chain Monte Carlo (MCMC) algorithm was used to obtain posterior estimate for probability of infection using WinBugs software (Lunn DJ et al, 2000, Stat Comput, 10: 325-337).

RESULTS: Each helminth infection was modelled separately. The spatial distributions of each helminth infection were very different and the distribution of the posterior predicted probabilities very heterogeneous. In particular, we report the geographical distribution and the posterior predicted probability obtained from the Bayesian geostatistical model only for two selected parasites which are very different in terms of prevalence: *Fasciola hepatica* (prevalence = 12.4%) and *Dicrocoelium dendriticum* (prevalence = 66.9%). The predicted posterior probabilities for *F. hepatica* ranged between 1.6% and 89.5%. The median was 7.47% and the 75% of the predicted data had a probability of infection below 19.3%. The range of estimated probabilities for *D. dendriticum* ranged between 2.7% and 99.8% and only 25% of the predicted probabilities had a value below 46.8%.

CONCLUSIONS: We proposed a probit Bayesian kriging model to obtain the map of posterior probability of infections for each one of the georeferenced farm of the region. No previous work has been done on this kind of representation in Veterinary Parasitology so far. We aim to introduce a multivariate spatial component, even if spatially shared components may be not the case in such application. We also aim to extend the model in order to integrate routine data to update posterior probabilites. The adopted approach represents a useful tool to communicate with field researchers and to address targeting of infection control treatments in the region.

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Dermatophytosis due to *Trichophyton mentagrophytes* in a sheep flock in Italy

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AIM: Dermatophytosis in sheep is generally regarded as an uncommon disease. The infection may be under diagnosed or not reported and lambs are considered more susceptible than adults. Its clinical importance is usually mild. Otherwise main consequences regard the long persistence of active lesions, the environmental spreading of fungal elements, the significant economic loss, the difficulties in managing and controlling ringworm as well as the impact on public health when a zoonotic mycotic species is involved. *Trichophyton verrucosum* represents the mainly involved fungal species (Scott, 1975 Onderst J Vet Res 42**,** 49-52.; Sargison and others, 2002 Vet Rec 150, 755-756). *Microsporum canis* was reported in some outbreaks of ovine ringworm (Roberts and Kepp, 1965 Sabouraudia 4**,** 96-97; Sharp and others, 1993 Vet Rec 132**,** 388) and to a lesser extent *Microsporum gypseum* (Hullinger and others, 1999 Vet Dermatol 10, 73-76). Infections caused by *Trichophyton mentagrophytes* have been less frequently reported and, being the records obtained from epidemiological surveys only (Pier and others, 1994 J Med Vet Mycol 32, 133-150; Khosravi and Mahmoudi, 2003 Mycoses 6, 222-225) clinical signs have not been described. In this report the occurrence and the clinical resolution of ringworm associated with *T. mentagrophytes,* in a sheep flock in Italy are described.

MATERIALS AND METHODS: The outbreak occurred in a Zerasca sheep flock, consisting of about 200 animals, maintained in Northern Tuscany. Eighteen ewes from a group of 60 adults exhibited several circular facial lesions, with a diameter up to 6 cm. The lesions were alopecic and erythematous, sometimes covered by thick crusts. Skin scrapings were collected from affected sheep to detect *Sarcoptes scabiei.* The hair specimens were seeded onto Sabouraud Dextrose agar added with 0.05% cicloheximide and plates were incubated at 25°C for ten days. The plates were checked for mycotic growth from the day 4 post-inoculation.

RESULTS: All cultured samples yielded a pure growth of *T. mentagrophytes* var. *mentagrophytes*, identifiable by macro- and microscopic features. Skin scraping failed to detect mites. Considered that antimycotic drugs licensed for food producing sheep are not present within the European Community, an unconventional treatment by using herbal remedies was selected. Essential oils (EOs) from *Origanum vulgare* and *Thymus serpillum* were selected for their strong antimycotic activity (Janssen and others 1988, Pharm Week Sci Ed 10, 277-280) due to the high amount of thymol and carvacrol, respectively. *Rosmarinus officinalis* was added considering that, even if its antifungal action is lower, it has marked antiinflammatory properties. The chemical composition of employed EOs is described elsewhere (Pistelli and others, Open Mycol J in press). The animals were topically administered a mixture composed by *O. vulgare* (5%), *R. officinalis* (5%) and *T. serpillum* (2%) in sweet almond (*Prunus dulcis*) oil as previously reported (Pisseri and others, 2009 Phytomedicine 16, 1056-1058) twice a day for 15 days directly on affected areas. After treatment, skin lesions gradually regressed and hair re-growth started. Adverse effects were never noticed. Control cultures performed two weeks after the end of treatment were all negative, and the recovery was definitive.

CONCLUSIONS: Overcrowded housing, bad conditions of bedding and poor ventilation of shelters where sheep congregated during the winter season could represent predisposing factors. The zoonotic character of disease, the wide spread of infection to other animals and the risk for environmental contamination make the treatment strongly advisable. At the best of our knowledge, this is the first clinical description of ringworm associated with *T. mentagrophytes* in sheep. The early identification of the causative agent and the treatment with EOs were useful to prevent the spread of infection from the face to other parts of the body, and to achieve a successful outcome.

Clinical *Dictyocaulus* infection in cattle grazing in Belluno province (Eastern Alps – Italy)

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Cattle infection by the lungworm *Dictyocaulus viviparus*, typical in areas with high environmental humidity as Northern Europe, is described as present, albeit sporadically, also in the grazing pastures of Italian Alps and Apennines (Urquhart G.M. et al., Veterinary Parasitology, 1998, Italian edition by C. Genchi, UTET, Torino, I). Notwithstanding, in Italian autochthonous cattle, it is considered as extremely rare, and generally cryptic. It could be argued that dictyocaulosis represents a forgotten disease, *de facto* unknown for most veterinary practitioners and farmers.

AIM: In the present work, we report two cases of fatal bovine dictyocaulosis, occurred in September 2011 in two mountain pastures (named A and B) of south-western Belluno province (Veneto region, Italy), which have suddenly refocused the attention on this disease.

MATERIALS AND METHODS: Cases on pasture A: a 10-month-old heifer, born in Belluno province, in her first grazing season, led on a pasture during summer 2011 together with beef cattle from other various farms (75 overall), died in September 2011, just 5 days after returning from the pasture. Cattle had never been treated by anthelmintic drugs during the grazing period, while they were treated by ivermectin once back from the pasture. At the end of the pasture period, symptoms such as coughing were observed in the herd, and the lungs of the heifer, dead from acute respiratory syndrome, were delivered to the IZSVe Belluno lab for necropsy. Cases on pasture B: 7 heifers 9-11 months old, all autochthonous of Belluno province and in their first grazing season, showed acute fever and severe respiratory symptoms in late summer (September 2011), after sharing the pasture with about 60 other dairy cattle (some of which were imported from Austria) from four different farms. One heifer died after 5 days and, after field necropsy evidencing dictyocaulosis, the others received anthelmintic therapy and completely recovered in about ten days. Even in this case, no anthelmintic treatment was scheduled for the herd before and during the grazing period, and eprinomectin was given only to the lactating dairy cattle just after returning from the pastures. In both A and B cases, verminous pneumonia was not immediately suspected, and the clinical outbreak was initially referred to viral and/or bacterial agents, driving first to antibiotic therapy.

RESULTS: In fatal cases, the lungs appeared hyperaemic, and presented on their surface solid areas of reddening, referable to pneumonia. At necropsy, the lesions appeared clearly referable to verminous pneumonia, since in both trachea and bronchi thousands of adult nematodes were evidenced, in association with acute catarrhal bronchitis. The trachea and bronchi appeared in some tracts almost obstructed by hanks of adult nematodes, mixed with abundant foamy catarrhal exudate. The parasite identification as well as the clinical symptoms and the observed lesions led to the diagnosis of acute dictyocaulosis, typical of cattle after their first grazing season.

CONCLUSIONS: The cases described above have refocused the attention on bovine dictyocaulosis, mainly due to its possible dramatic impact on young grazing cattle, resulting in severe and fatal clinical cases. The described cases could be due to co-grazing of autochthonous cattle with imported ones, or they could be the expression of an actual endemic cycle of *D. viviparus*, amplified in 2011 by the frequent summer rainfall and environmental humidity conditions. In any case, since at the moment we cannot exclude both these hypotheses, and lacking in this specific situation of reliable early diagnostic tests and of a larval vaccine, an anthelmintic treatment should be scheduled for the next few years in cattle grazing in the interested pastures, to break a possible lungworm pneumonia outbreak series (Ploeger HW and Holzhauer M., 2011, Vet Parasitol, doi: 10.1016/j.vetpar.2011.10.026). A metaphylactic treatment could also be focused at least on cattle during their first grazing season, being the most exposed to severe and fatal verminous pneumonia. Moreover, any imported cattle should be treated before introduction in the grazing herd.

Occurrence of *Giardia duodenalis* sub-assemblages in horses

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Giardia duodenalis is a flagellate intestinal protozoan infecting a wide range of animals and humans. High significant genetic heterogeneity has been found among *G. duodenalis* isolates, which has led to a classification in different assemblages with a varying degree of host specificity. Data available on the natural occurrence of this protozoan in horses are lacking, even though reports on the genetic make-up of equine isolates of *G. duodenalis* have revealed the presence of zoonotic assemblage A (AI), the possible host-specific sub-assemblage BIV, or the assemblage E (Traub RJ et al, 2005, Vet Parasitol, 130: 317-321; Veronesi F et al, 2010, Zoonoses Public Health, 57: 510-517).

AIM: The aims of the present study were to investigate the occurrence of *G. duodenalis* in Italian horses and to genotype the isolates retrieved.

MATERIALS AND METHODS: Nineteen horse properties located in the Marche and Abruzzo regions (Central Italy) were studied. Individual fecal samples were collected from 7 to 49 horses on each farm, for a total of 431 horses. Each sample was subjected to a PCR specific for a fragment internal to the gene encoding for the SSU-rRNA of *G. duodenalis* assemblages (Read C et al, 2002, Int J Parasitol, 32: 229-231). The identification of sub-assemblages was performed by another PCR, specific for a fragment internal to the gene encoding for the -giardin protein (Cacciò SM et al, 2002, Int J Parasitol, 32: 1023-1030; Lalle M et al, 2005, Int J Parasitol, 35: 207-213). Amplicons were sequenced and sequences generated were subjected to a phylogenetic analysis.

RESULTS: Thirty-seven (8.6%) animals from 11 properties scored positive for *G. duodenalis*. Different assemblages were detected at the PCR for SSU-rRNA gene: 16 samples were positive for assemblage A, 11 for assemblages B and 10 for assemblage E. Out of these 37 samples, 16 yielded amplicons also upon the PCR for the -giardin gene. In particular, 10 isolates identified as assemblages B at the SSU-rRNA analysis showed 99.6 to 100% homology with the sub-assemblages described as B1-2 and B1-6, 3 assemblage A showed 99.8% homology with sub-assemblage A1, while 1 assemblage E displayed 98.8% homology with sub-assemblage E3. One isolate characterized as assemblage A at the SSU-rRNA showed 99.6% homology with the sub-assemblage B1-2 and 1 characterized as E was 100% identical with sub-assemblages B1-6. The phylogenetic analysis of the *G. duodenalis* sequence dataset herein produced and sequences available in GenBank™ were concordant in revealing the existence of three main clades for SSU-rRNA genes and -giardin. Consistency in the topology of the tree inferred by the MP and NJ methods for both target genes was demonstrated.

CONCLUSIONS: The present results show that *G. duodenalis* in horses living in the study area occur with a non-negligible prevalence. The genetic analysis of the isolates *via* the analysis of two genes was consistent, with few exceptions. The characterization has confirmed that also horses, as domestic ruminants and pigs, can harbor assemblage E (Veronesi F et al, 2010, Zoonoses Public Health, 57: 510-517). Worthy of note is the finding in twelve horses of sub-assemblages B1-2 and B1-6, so far reported only in primates and in diarrheic humans (Cacciò SM et al, 2008, Int J Parasitol, 38: 1522-1531; Geurden T et al, 2009, Parasitology, 136: 1161-1168). Epidemiological and biological significance of these isolates is still to be established and warrant further investigations. Additionally, the study confirmed the presence in horses of the sub-assemblage A1 (Traub RJ, 2005, Vet Parasitol, 130: 317-320), which displays the greatest zoonotic risk to humans. Further studies are necessary to explore the correlation existing between the occurrence of this infection in horses and risk factors, in association with *G. duodenalis* sub-assemblages of human origin infecting horses.

Evidence of *T. equiperdum* infection in a natural dourine outbreak

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Dourine is a sexually transmitted parasitic disease of equids. It is caused by *Trypanosoma equiperdum* a flagellate protozoa.
Dourine is the only trypanosomosis transmitted sexually rather than by insect vectors. The pathogenicity of the described strains is variable, and no strains have been isolated since 1982.
The clinical diagnosis of dourine can be complex as clinical signs and anatomic lesions are not always present. Clinical signs are in many ways similar to those of surra, caused by *T. evansi.* The two parasites are genetically and antigenically similar. Direct laboratory diagnosis is also problematic, given the few parasites normally present in infected tissues and the low and short-lasting parasitemia.
Dourine itself is endemic in many areas of Asia, Africa, Russia, parts of the Middle East, South America and south-eastern Europe. In Italy it was originally eradicated in the 1940s and the country remained free until a serious epidemic in the mid-70s arisen, following which it was eradicated once again (Caporale V. et al. 1980, In Expert consultation on research on trypanosomiasis, Food Agriculture Organization, Rome: 16-18). In May 2011 a stallion, undergoing routine testing in Italy for stud purposes, tested positive for dourine. Since this first confirmed case, the epidemiological investigation, , revealed four other outbreaks epidemiologically linked.

AIM: This work describes the epidemiological, clinical and laboratory data enabling confirmation of the suspicion of dourine in Italy in the 2011 epidemic.

MATERIALS AND METHODS: Two stallions and four mares showing clinical signs and originating from the different outbreaks were transferred to Istituto G. Caporale, Teramo (ICT) in order to monitor the evolution of the disease and carry out further diagnostic tests. Clinical monitoring and necropsy of euthanised animals have been performed and samples of organs for histopathology have been taken.
Serum samples from the infected horses were examined by complement fixation test (CFT) and those testing positive then underwent IFAT (OIE, 2008). Samples were also examined with the CFT test used during the 1970s italian dourine epidemic. Cerebrospinal fluid samples taken after the euthanasia of the four mares were tested for anti-*T. equiperdum* antibodies.
Tissues taken from animals diagnosed with dourine were tested using a specific RealTime PCR method for the *Trypanozoon* subgenus to which *T. equiperdum*, *T. evansi* and *T. brucei* all belong.

RESULTS: The main signs observed in the infected horses were as follows: sudden weight loss, labial ptosis, swollen joint, urticarial plaque-like skin lesions, udder, scrotal and ventral edema, congestion of the genital mucosa.The lymphatic organs presented secondary reactive hyperplasia by histology. The urticarial plaques showed a characteristic picture of pustular dermatitis, neuritis of facial and lingual nerves.
CFT carried on sera gave high titer positive results that have been confirmed by IFAT, in the meanwhile anti *T.equiperdum* antibodies have been detected by CFT in CSF of euthanised animals.
Samples testing positive on RealTime PCR, were as follows: mammary tissue, secretions and draining lymph nodes; urticarial plaque-like skin lesion; genital tissues and intra-articular fluid. Live *T. equiperdum* were found in the mammary secretion of a naturally infected mare.

CONCLUSIONS: The clinical symptoms of the examined horses and the direct and indirect laboratory tests confirmed the diagnosis of trypanosomiasis. Although there are no diagnostic methods able to unequivocally discriminate between *T. equiperdum* and *T. evansi,* however in the outbreaks discussed here the epidemiological findings supported the diagnosis of dourine. It thus seems clear that the infection is being spread by sexual transmission: this is the only recognized route for dourine, but has not been reported for surra.

Gastrointestinal parasites in two pig herds of central Italy

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AIM: Limited recent data are available on gastrointestinal parasites of pigs in Italy (Scala A et al, 2008,Vet Parasitol, 26:362-5; Gualdi V et al, 2003, Parasitol Res, 90: 63-5; Canestri Trotti G et al, 1984, Parassitologia, 26: 299-304).The aim of this pilot study was to acquire new data on the occurrence of gastrointestinal parasitic infections in pigs of different age and breed and reared in different management systems.

MATERIALS AND METHODS: During the period from January 2011 to March 2012, 104 animals from two different pig herds located in the province of Pisa were examined for gastrointestinal parasites. The first (A) is an intensive farm with a consistency of about 5,800 industrial cross breed pigs, while the second is an outdoor herd (B) of about 300 Cinta Senese breed pigs. Animals of both herds were treated with anthelmintic drugs. In particular, animals of herd A are fed with a commercial food containing ivermectin, while animals of herd B are treated only on the basis of results of regular parasitological analysis. Faecal samples were collected from four different age groups of animals of each herd, i.e. sows, piglets less than 1 month, weaner pigs 8–50 kg and fattening pigs (>50 Kg). Samples were qualitatively examined by low (s.g. 1.2) or high (s.g. 1.4) density solution flotation test to evaluate the presence of intestinal worm eggs and/or protozoan (oo)cysts. A commercial rapid immunoassay (RIDAQUICK® *Cryptosporidium*/*Giardia* Combi cassettes, R-Biopharm Italia srl) was used to detect *Giardia* and *Cryptosporidium* faecal antigens. In addition, samples from animals resulted infected by coccidia were dissolved in 2% potassium dichromate ($K_2Cr_2O_7$) solution and maintained at 20°C until the sporulation of the oocysts for their identification at the species level.

RESULTS: An overall prevalence of 44% and 80% resulted for herd A and herd B, respectively. Among isolated protozoa, *Giardia duodenalis* (2.2%) and *Isospora suis* (2.2%) were found in piglets from herd A, *Cryptosporidium* spp. was found in piglets, sows and weaners from herd A (14.6%) and in piglets from herd B (15.7%), *Balantidium coli* was found in sows from herd A (13.5%), while *Eimeria debliecki* and *Eimeria suis* were found in sows and weaners from herd B (60%). Among helminths, *Ascaris suum* (13.3%) was isolated in sows and fattening pigs from herd A, while gastrointestinal strongyles (6.6%) were found only in sows from herd B.

CONCLUSIONS: In the examined pig herds, protozoan and zoonotic or potentially zoonotic gastrointestinal infections resulted widespread. Suitable control measures are required in order to assess and minimize the risks for animal and public health as well as for production of farm animals. Among isolated parasites, only *Cryptosporidium* was present in both herds and resulted prevalent in piglets. Further studies are in progress to determine the genotypes and sub-genotypes of *Giardia* and *Cryptosporidium* isolated in this study in order to assess their zoonotic potential.

Serological, histopathological and biomolecular updates on rabbit encephalitozoonosis in Sardinia

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Encephalitozoonosis by *Encephalitozoon cuniculi* is an infection which can affect a wide range of hosts primarily rodents and lagomorphs but also could be an opportunistic zoonosis.

AIM: To update the epidemiological situation of encephalitozoonosis in Sardinia rabbit farms two years after the first investigation.

MATERIALS AND METHODS: In 2011, 363 slaughtered rabbits coming from six intensive farms (100% of Sardinian rabbit farms) were examined by serological, histopathological and biomolecular approaches. At slaughterhouse, individual blood samples from 283 young meat rabbits (MR) and 80 end career "old breeder" (OB) were collected for serological examination with the Carbon Immunoassay Test (CIA, Medicago, Uppsala, Sweden). All samples were centrifuged at 1500/rpm for 10 minutes, and sera stored at -18°C until CIA test. All the kidneys of slaughtered animals (363) were examined for macroscopic lesions classified according to a rating score in 5 classes, from 0 (no lesions) to 4 (wrinkles kidney). Kidney samples with macroscopic lesions (three samples for each class of score for a total of 15 samples) were used for histopathological studies. Samples previously fixed with 10% formalin, were stained with haematoxylin-eosin (HE), Azan Mallory and Ziehl Neelsen techniques. Twenty-seven kidney samples were selected for Dna extraction and PCR investigation carried out according to the protocol described by De Nadai et al. (2011, Atti XIII Congresso Nazionale S.I.Di.L.V: 196-197).

RESULTS: All monitored farms were serologically positive to *Encephalitozoon cuniculi*. An overall prevalence of 29.8% was found in examined animals (108/363). Prevalence was of 21.5% (61/283) for MR and 58.7% for OB (47/80) (χ^2= 41.28; p<0.00001).
The 78.1% (283/363) of kidneys didn't show macroscopic lesions (class 0) while the prevalences found for each class of lesions were 8.8%, 4.7%, 6.3% and 1.1% for class 1, 2, 3 and 4 respectively.
Histopathological examination confirmed kidneys belonging to class 0 didn't have microscopic lesions, while in kidneys with score 1 and 2 mild and focal interstitial lympho-plasmacellular nephritis associated with none or mild interstitial fibrosis was observed. Kidneys with score 3 and 4 showed mild to severe multifocal interstitial lympho-plasmacellular nephritis associated with mild to moderate interstitial fibrosis. Ziehl Neelsen staining has allowed to recover the protozoan in kidneys with score 2, 3 and 4 while no evidence of parasite was found in kidneys with score 0 and 1. None of the examined samples resulted positive with PCR, even those with score 4.

CONCLUSIONS: The results of this survey confirmed that *Encephalitozoon* is a widespread parasite in Sardinian rabbit farms (percentage of infected farm= 100%) while in the 2010 only 66.7% of the examined breedings were infected (Pipia AP et al, 2010, LXIV ANNUAL MEETING OF SISVET: 56). In particular the present investigation showed a higher prevalence (29.8%) compared with 2010 (prevalence=17.9%; χ^2= 14,14; p<0.001). Regarding the two categories of surveyed animals (MR and OB), differences statistically significant for MR examined in this survey (P=21.5%) versus 2010 (P=4.9%) (χ^2= 31,73; p<0.00001) were found. Moreover, the prevalences of infected rabbits in OB groups sampled in 2010 (P=47%) and in this survey (P=58.7%) did not show any significant difference (χ^2= 2.62; p=0>0.05). As regards the histhopatological results, the Ziehl Neelsen staining allowed us to identify the parasite in animals with score 2, 3 and 4. On the other hand PCR did not show any positive results also in high seropositive subjects and with macroscopic lesions: this could be due to the fact that in chronic lesions the parasite is not present or in very low amount in the kidney tissue.

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Surveillance of cystic echinococcosis and molecular characterization of Echinococcus granulosus strain in slaughtered sheep in Sardinia

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AIM: Cystic echinococcosis (CE) is an important zoonotic, parasitic infection causing morbidity and mortality in humans as well as significant economic losses in livestock (Budke CM, 2006.Emerg Infect Dis. 12, 296–303).The parasite lifecycle includes dogs and other canids as definitive hosts, whilst sheep and numerous ungulates are intermediate hosts harbouring the hydatid cysts (Craig PS 2007 Lancet Infect Dis.7, 385-394). Sheep is the key reservoir for *E. granulosus* (Scala A, 2004, Parassitol. 46, 443-444). In this study it was conducted an assessment of the prevalence of CE in slaughtered sheep in Sardinia and therefore evaluated on fertility and vitalilty of collected cysts and strain identification.

MATERIALS AND METHODS: The studied area census was the region of Sardinia island that has an area of 24,000 square kilometres and is divided into 377 municipalities. The Sardinian sheep farms are 16.119 with 3.317.432 herds (Banca Dati Nazionale data).The survey was conducted from June 2008 to September 2010 in 11 abattoirs distributed in the territory of the island that slaughtered high number of sheep. Sheep slaughtered in different Sardinians slaughterhouses were examined for the presence of hydatid cysts. First was collected, information about farm of origin, the age of the sheep was, evaluated by teeth observation, were collected, than during the post mortem inspection the offals were examined and the positive organs found positive were processed in laboratory. Hydatid cysts observed were counted and vitality of protoscoleces were evaluated. Cysts of 74 samples were dissected, and the germinal layer and protoscoleces were collected. It was conducted a PCR on mitochondrial NADH dehydrogenase1 and RFLP analysis to strain identification. The data collected was elaborated with statistical analysis. For each age group, starting from 2 up to and greater than 7 years, the probability of finding cysts in the liver and lung with relative confidence intervals of 95% have been estimated by the method of Wilson. The trend of the probability of infesctation relative to the age was estimated using a generalized linear regression (logistic regression as in Scala A.,2006 Vet.Parasitol.135,33-38). All elaborations were performed using the software R.

RESULTS: We analysed 1851 sheep from 127 Sardinian's breeding of 41 municipalities, it was relevated the prevalence of 52,45% (1024 positive sheep). 2867 cystis out of all 8158 (35,1%) cysts analysed wasere found fertile. All 74 samples analysed by PCR-RLFP method showed that sheep harbored the G1 genotype (common sheep strain). The study shows a positive association between age and probability of infection, which increases by factor of 1.17 per year: time coefficient (t) in the logistic curve is 0.15896 (symmetric range confidence: 95% 0.1043265 – 0.21393) with a standard error of 0.02795; of Wald test is 5.688 with p-value $1.28x10^{(-8)}$ (statistical significance 99.9%): odds ratio exp (0.15896) = 1.172 (symmetric range confidence: 95% 1.1099628-1.2385369). The logistic curve interpolates the experimental data except the last class, for which the data are rare.

CONCLUSIONS: In Sardinia it's confirmed the highest prevalence of CE in sheep was confirmed; moreover it is demonstrated the linear correlation between age of sheep and presence of cysts. The principal strain observed was "sheep strain". The need for more relevant information underline the important of implementation of official data flow on CE for slaughtered sheep that will provide useful epidemiological information. Then in a integrated approach for CE control the culling of older sheep could be an important tool as well as use of praziquantel in dogs, sanitary education and vaccination of sheep.

Detection of bovine *Paramphistomidae* infections in two agricultural regions in NW Uruguay and NW Spain

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AIM: To analyze the infection by *Paramphistomidae* trematodes in cattle from two agricultural regions of Uruguay and Spain.

MATERIALS AND METHODS: Faecal and blood samples were collected from 374 bovine in Salto (Uruguay, South America) and 429 from Galicia (Spain, SW Europe), and tested by using a copromicroscopic probe and an ELISA with excretory/secretory antigens collected from adult *Calicophoron daubneyi* (*Paramphistomidae*) specimens.

RESULTS: In the Uruguay, the percentage of cattle passing *Paramphistomidae*-eggs by faeces was 7% (95% Confidence Interval 5, 10). A significantly higher prevalence of paramphistomosis in the Hereford x Angus cattle (OR= 3.5) was recorded, as observed for the oldest ruminants (>3.5 yr). An overall seroprevalence of 29% (95% CI: 25, 34) was obtained by ELISA, with the highest values in the Friesians (OR= 3) and the youngest bovines (<2.5 yr).

Twenty-six percent (95% CI: 22, 30) of the cattle from Spain passed eggs by faeces, and cattle aged 2.5-7 yr reached significant highest prevalences. By means of the ELISA, a percentage of fifty-five cattle (95% CI: 50, 59) had antibodies against the gastric fluke, and the highest seroprevalence was observed among the bovines under 6 yr.

The percentages of cattle harboring adult *Paramphistomidae* flukes were significantly higher in Spain than in Uruguay. The prevalence of sensitization against these trematodes was also higher among the cattle from Spain.

CONCLUSIONS: There is a need for reducing the risk of infection by *Paramhistomidae* spp in cattle from Uruguay, and special attention should be provided to their accurate management for avoiding their exposure to the gastric trematode. Further studies are in progress for identifying the genera and species of *Paramphistomidae* affecting ruminants in Uruguay. Paramphistomosis seem to be on the increase in cattle from NW Spain, partly due to the lack of an effective treatment against the trematode.

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Dicroceliosis in cattle: epidemiological updates in Sardinia and anthelmintic field trial

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AIM: To give a contribute for the control of bovine dicroceliosis we report the results of a survey carried out in two steps: 1) updates the prevalence of *Dicrocoelium* infection in Sardinia by direct parasitological investigation of the liver at the slaughterhouse and copromicroscopical examinations; 2) field test to evaluate the effectiveness of albendazole against *Dicrocoelium dendriticum*.

MATERIALS AND METHODS: In 2011, two faecal pools (each one of almost five animals) from cattle of various breeds (Sarda, Brown, Limousine, Charolais and their crosses) and belonged to 54 farms under extensive methods in Sardinia were sampled; fecal samples were examined by sedimentation and flotation technique with Zn sulphate solution (PS 1,350) after centrifugation (2000rpm x 10 minutesFurther, direct parasitological examination of 271 cattle livers bred with extensive methods (from 63 farms) was carried out in various abattoirs in northern Sardinia, in order to detect adult specimens of *D. dendriticum*.
For the field trial, 12 cattle of Brown/Sarda breed ranging from 9 months to 10 years of age, naturally infected with *D. dendriticum* and raised with extensive methods were selected. Cattle were treated at D0 with 15 mg/kg of body weigth of albendazole (equal to 15ml/100kg of Valbazen 10% ® - Pfizer). Copromicroscopic investigations were carried out with the Flotac® Double technique with a Zn sulphate solution (ds 1,350). The efficacy of anthelmintic treatment was tested using a Faecal Egg Count Reduction test [FECR = (EPG D0 – EPG DX)/ EPG D0] comparing at each sampling date the mean of the number of eggs per gram (EPG) at Day 0 (D0) with the mean EPG at D14, D21, D45 and D75.

RESULTS: Eggs of *D. dendriticum* were detected in fecal pools in 11.1% of monitored farms (6/54), while specimens of *D. dendriticum* were found in 4.1% of examined animals (11/271) which came from 10 different farms, equal to 15.9% of the examined farms.
The mean levels of EPG of *D. dendriticum* detected in field trial were: 8 EPG at D0; 3,3 EPG at D14; 0,2 EPG at D21; 2,2 EPG at D45; 10,5 EPG at D75.
The Mann-Whitney test performed on EPG means between D0 and D14, D21 and D45 of the monitoring has detected highly significant differences ($P < 0.01$). The levels of efficacy of albendazole were: 60.9% at D14; 97.8% at D21; 79% at D45; no level of effectiveness was found at D75, period in which the values of EPG were higher than those of D0. The maximum rate of copro-negativity of animals was recorded at D21 with 83.3% of animals negative for eggs of *D. dendriticum*.

CONCLUSIONS: The prevalence rate of 11.1% for *D. dendriticum* detected with copromicroscopic exams shows a wide spread of the fluke in cattle bred with extensive methods in Sardinia, although the slaughterhouse data show a significant reduction compared to the period 1994-95 (31.1 vs. 4.1% - $\chi^2 = 62.2$, $P < 0.0000$) (Scala A et al, 2007, Praxis Veterinaria, 18 (3): 10-13).
The occurrence of this fluke in the liver, considering the low pathogen action of this parasite in cattle, is still an economic loss of income due to the seizure of the organ at the time of the post-slaughtering visit in this important livestock area of Sardinia. To limit the damage caused by this disease appears useful the implementation, in the positive farms, of an anthelmintic treatment based on albendazole carried out according to the above mentioned protocol, maybe 21 days (offtime minimum period for meat consumption) before slaughtering, when an efficiency of 97.8% could be achieved and over 80% of the treated animals could be negative to the copromicroscopic exam.

Horse intestinal parasitoses at two military operations in Italy

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Various species of helminths cause intestinal infections which seriously compromise health and welfare of horses (Brady HA, Nichols WT, 2009, J Vet Eq Sci, 29: 285-295). The most diffused are nematodes belonging to the Strongylinae and Cyathostominae subfamilies and *Parascaris equorum* (von Samson-Himmelstjerna G, 2012, Vet Parasitol, 185: 2-8). Larvae of large and small strongyles cause severe damages to the intestinal tract migrating through the blood vessels or emerging from the gut wall, respectively, while their adult stages cause decreased rates of performance, debilitation and aspecific intestinal distress; the roundworm *P. equorum* has an important pathogenic role, particularly in foals, which often show diarrhea, ill thrift and ileal impaction (von Samson-Himmelstjerna G, 2012, Vet Parasitol, 185: 2-8). Among tapeworms, *Anoplocephala perfoliata* is the most diffused and pathogenic, as it may cause colics by ileo-caecal junction impaction (Proudman CJ, Trees AJ, 1999, Parasitol Today, 15: 156-159). Hence, knowledge of the diffusion of these helminths is crucial in horse operations for reliable worm control programs. In Italy, the Military Riding Operation of Montelibretti and the Army's Veterinary Operation of Grosseto are two major horse stables in Italy, where a high number of horses is kept. These stables are recognized to be of crucial relevance both from a sporting and a reproductive standpoints.

AIM: The aim of the present trial was to evaluate the presence of common horse endoparasites in these aforementioned Military stables, by using three different copromicroscopic techniques. Moreover, the ability of qualitative tests in detecting parasite eggs was compared each other.

MATERIALS AND METHODS: From April to July 2011, faecal samples from 238 horses kept in the Military Riding Operation of Montelibretti (131 horses; site A) and the Army's Veterinary Operation of Grosseto (107 horses; site B) were collected. For each horse a questionnaire on clinical and parasitic history and farm management was filled. All faecal samples were examined by a flotation using a $NaNO_3$ solution with a specific gravity of 1.350 (Taylor MH et al, 2007, Veterinary Parasitology, Blackwell Publishing, UK), by the Proudman's test (Proudman CJ, Edwards GB, 1992, Vet Rec, 131: 71-72) and by a modified McMaster technique with a 50 EPG sensitivity (Sloss MW et al, 1994, Veterinary Clinical Parasitology**,** Iowa State University Press, Ames, Iowa, USA). Data were statistically analysed by using chi-square test for 2 proportions while for groups with $n<20$ they were analysed by Fisher's exact test (level of significance, $P<0.05$).

RESULTS: Out of 131 horses at site A 92 (70%) were positive for endoparasites. In particular, 80 samples were positive for Strongylidae (S) (87%), 2 for *A. perfoliata* (A) (2.1%), 6 for S and A (6.5%), 1 for S and *P. equorum* (P) (1.1%), 1 for S and *Oxyuris equi* (Ox) (1.1%), 1 for S, A and P (1.1%), and 1 for S, P and Ox (1.1%). In Site B 49 (46%) of 107 horses were positive for endoparasites. Specifically, 31 samples were positive for S (63.3%), 8 for A (16.3%), 5 for S and A (10.2%), 4 for S and P (8.2%), and 1 for S, A and P (2%). Only samples positive for S were positive at the quantitative examinations with the McMaster method, with ranges of 50-2150 EPG and 50-600 EPG in sites A and B respectively. The comparison of the 2 qualitative diagnostic tests showed a significant difference in detecting cestode eggs between flotation and Proudman's test, in that the latter was much more sensitive ($P<0.0001$). All other comparisons were not statistically significative.

CONCLUSIONS: The present work showed a high presence of Strongylid infections, while other parasites were found with low infection rates and faecal egg shedding. However, all major horse helminths were present in the stables, thus potentially causing relevant diseases. These results confirm the necessity to evaluate the occurrence of different horse parasites both by standard flotation and the Proudman's assay, which has a higher ability in detecting cestode eggs. Finally, the importance of a regular parasite's screening instrumental to appropriate effective worm control programs is underlined. Such an approach would improve health, welfare and performances of horses kept in these relevant Military Operations and in horse stables in general.

Epidemiological and biomolecular updates on Cystic Echinococcosis in pigs of Sardinia

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AIM: To report the results of an epidemiological and biomolecular update on cystic echinococcosis (CE) in familiar pig breedings of Sardinia, in order to define better the role of these ungulates in the transmission dynamics of the infection.

MATERIALS AND METHODS: From March 2008 to March 2012, a total of 299 pigs home slaughtered were examined during post mortem official veterinary inspection visit. In every pig slaughtered found positive to CE infection, fertility, location and morphotype of hydatids were evaluated. From each cyst, laminar layers and protoscoleces were removed and stored at -20°C. DNA was extracted from 28 samples of hydatid material using a commercial kit (Roche DNA template extraction kit).
PCR screening according to Dinkel et al. (2004, Int J Parasitol, 34: 645-653) was performed on all DNA isolates, in order to discriminate the G1 strain of *E. granulosus* from the G5 and G6/7 strains, with 4 different PCR reactions. Then sequencing reactions were undertaken on PCR products as described by Bowles J, McManus DP (1993, Int J Parasitol, 23: 969-972) for NADH and COI mitochondrial genes. Nucleotide sequence analysis was undertaken using the National Center for Biotechnology Information BLAST programs and databases. Multiple sequence alignments were made with the ClustalW method with Bioedit software and compared with GenBank sequences.

RESULTS: An overall prevalence of 33.8% (101/299) was detected, with a fertility rate of 24.4% (73/299) equal to 72.2% of positive subjects (73/101). A prevalence of 29.1% (87/299) was found in the lungs while in the liver this value was a little bit lower, 26.4% (79/299) (χ^2= 0.53, p=0.465). Fertility rates were 19.3% and 18.1% (χ^2= 0.17, p=0.675) for lungs and liver respectively. Mixed infection (lungs + liver) was 21.7%, while exclusive infection in lungs and liver was respectively of 7.3% and 4.7% (χ^2= 1.89, p=0.169). Myocardial, skeletal muscles, spleen and kidney infection was observed with a prevalence respectively of 1%, 1%, 0.6% and 0.3%. Of the 28 DNA isolates from pigs PCR screened and then sequenced, 100% (36) belonged to G1 strain (*E. granulosus sensu stricto*).

CONCLUSIONS: The results of the present survey have confirmed the presence of CE in the familiar swine breedings of Sardinia, as previously found by Varcasia et al. (2008, Actualities in animals breeding and pathology, Timisoara, Romania). What is surprising is that the trend of CE infection is growing up as well the percentages of fertile hydatids: prevalence (33.8%) was almost three times higher than those found in 2008 (11.1%) (χ^2= 48.26, p<0.00001), and fertility (24.4%) was almost on the same trend when compared to 2008 (7.6%) (χ^2= 34.52, p<0.00001). This scenario highlight as the pig, when not reared in intensive breeding, could play in Sardinia as very good intermediate host of CE, and also when infected with no specific genetic variants like G1 (*E.g. sensu stricto*) that could grow up in this ungulate with a successful rate of fertility. In the present survey was not possible to find the pig strain of the parasite, as done for the first time in Italy in 2006 (Varcasia et al., 2006 Parasitol Res, 99:622-626). The coprophagy and the possibility to graze uncontrolled (strictly prohibited by law, but unfortunately still practiced) make this ungulate very dangerous for the public health, not only as good host for the persistence of CE, but also for the diffusion of swine pests and trichinellosis.

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Case report of *Coenurus cerebralis* in a Limousine bull in Sardinia, Italy

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AIM: To report a case of coenurosis in cattle approximately 22 years after the first report in this host in Sardinia where this metacestodosis is an important health problem in sheep. Further we report the results of genetic analysis of cysts isolated from the infected animal.

MATERIALS AND METHODS: A two years old Limousine bull was euthanized in the Bolotana (NU) municipality (Central Sardinia). The animal was removed and a necroscopy was carried out in the lab in order to understand death causes. The remote anamnesis achieved from the farmer reporting that the bull showed neurological symptoms from one year of age previously classified as nutritional problems. The farmer also says that the bull have by self produced the skull fracture by hitting a gaff in the farm. The farmer also breed in the same place a flock of 500 sheep and report 5-6 cases per year of Coenurosis in these animals, and that 10 years ago he have to kill of the replacement sheep (more than 100 animals) for massive infection with Coenurosis. At necroscopy any lesions except for the fracture of the bones of the skull and of the right horn were observed,. The skull was opened with a saw, the brain removed and carefully examined. Two cysts both in the right emisphere were found. Aliquots of the parasitary material were removed and DNA extracted with a commercial kit and then sequenced after PCR for NADH and COI mitochondrial genes, as described by Bowles J and McManus DP (1993, Int J Parasitol, 23: 969-972; 1993, Acta Trop, 53: 291-305). Nucleotide sequence analysis was undertaken using the National Center for Biotechnology Information BLAST programs and databases. Multiple sequence alignments were made with the ClustalW method with Bioedit software and compared with GenBank sequences.

RESULTS: The direct parasitological exam and stereo & light microscopy showed that the two cysts were typical Coenuri. The two cysts sized respectively 49 and 23 cm^3 and they showed a laminar layer with crystalline liquid and white spots inside. They correspond to multiple clusters (32 and 25 respectively) of protoscoleces in the inner part of the membrane. Protoscoleces measured 1991 m in length and from 1556 m wide, and show a scolex with a rostrellum sized 381 m and four suckers of 320 m diameter. Thirty-two to 34 hooks were arranged in two circles on the rostrellum with 17 large hooks and 16 small ones. Hooks length was of 172 m and of 129 m respectively for large and small ones. Morphologically, the cysts and the scoleces examined in our investigation showed the same features of *C. cerebralis* by *Taenia multiceps*. Biomolecular investigations through mt-DNA sequencing have confirmed the morphological diagnosis, and strain typing have shown that the bull was infected with Tm1 strain of *T. multiceps*, according to Varcasia A et al. (2006, Parasitol Res, 99: 622-626).

CONCLUSIONS: Coenurosis from *Taenia multiceps* is quite common in Sardinia where several survey were carried out on epidemiology and biomolecular characterization from the same authors. In Sardinia, the last record in cattle was pointed out 22 years ago by Cubeddu et al (1990, Atti Soc. It. Buiatria,). Recently Bovine Coenurosis was described in Greece by Giadinis (2009, Vet Record 164: 505-506) and in 2011 by Avcioglu et al. (2011, Vet Parasitol, 176: 59-64) have found *T. multiceps* bovine cerebral isolates from Turkey and the strain typing have shown that they were quite different from Mediterranean isolates (Tm1-Tm3) and Goat Emirates isolates (Tm4), showing that maybe cattle could be infected from different strains of the parasites. The present isolation in the limousine bull was probably to refer to a bigger sensivity due to the breed and also to the young age of the animal, as well to the grazing in area polluted by eggs of *T. multiceps* eliminated from infected sheepdogs that live togheter with sheep flocks where this metacestodosis is often diagnosed.

Seroprevalence of *Babesia caballi* and *Theileria equi* in donkeys from Campania (southern Italy)

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AIM: Equine piroplasmosis (EP) is a tickborne disease of equids (including horses, donkeys, mules and zebras), which is caused by infection with two intra-erythrocytic protozoan parasites, *Babesia caballi* and *Theileria equi*. The EP has a wide geographical range according to the distribution of tick vectors and is endemic in Africa, South and Central America, the Middle East, Asia and Southern Europe (Kouman et al, 2010, Vet Parasitol, 169: 273-278). In some European countries, including Italy, there was an increasing interest on donkeys due to their use as pets, for onotherapy and for the rediscovery of donkey milk as food source for children affected with cow milk allergy (Veneziano et al, 2011, Vet J, 190: 414-415). Despite this, there are few data regarding the donkeys parasitic diseases especially those with a protozoal etiology. Because information on EP in donkeys in Italy is very limited, the aim of this study was to determine the seroprevalence of *B. caballi* and *T. equi* infections and the associated risk factors, in donkeys, born and raised in Campania region - Southern Italy.

MATERIALS AND METHODS: The survey was conducted on 21 donkey farms from 16 municipalities. Blood samples were collected from 203 donkeys in autumn 2010. The sample size was calculated as proposed by Thrusfield M, 1995, Veterinary epidemiology, Blackwell Science, UK. Blood samples were collected in 21 donkey farms from 16 municipalities. General data, including gender, age, breed, use, period of grazing during year, presence of horses, dogs and ruminants in the farms were obtained through a questionnaire completed during sample collection. The IFAT was performed for the detection and quantitative determination of specific IgG antibody against *B. caballi* and *T. equi* (IFA IgG Kit, Fuller Laboratories). The cut-off value of 1:80 was used according to the manufacturer's instructions. The positive sera were then titrated in two-fold dilutions to determine the end-point titres. In order to evaluate possible risk factor associated to the seroprevalence, epidemiological data (gender, age, breed, use, lactation, grazing, presence of horse, dogs, ruminants) were offered to binary logistic models (SPSS for Windows, 13.0). Further, a complete clinical exam was done on each donkey.

RESULTS: The results of IFAT are shown in the table.

Antibody titer	n° positive /n° tested	Prevalence (%)
B. caballi	26/203	12.8
T. equi	44/203	21.7
Co-infected	46/203	22.7
Total	116/203	57.1

The antibody titers ranged from a dilution of 1:80 to 1:360 for *B. caballi* and 1:80 to 1:1280 for *T. equi*.

No statistically significant correlations were found between serological positivity and donkey gender, age, breed, use and grazing. *B. caballi* seropositivity were found higher in donkeys breed together with horses (OD=3.07; P<0.05). However, *T. equi* seropositivity were found higher in lactation animals (OD=4.34; P<0.05) and lower in the donkeys breed with dogs and ruminants in the farms (OD=0.4; P< 0.01). For both parasites the co-infection status resulted as a significant risk factor (P<0.01). The clinical examination of the most seropositive animals failed to show any evidence of abnormalities. Only one donkey, with a titer of 1:640 against *T. equi*, showed the acute symptoms of disease and, particularly lethargy, fever, anaemia, hemoglobinuria and limphnodes enlargement.

CONCLUSIONS: The preliminary data showed that *B. caballi* and *T. equi* are widespread among donkeys in Campania region. Although, almost all of the sampled animals were asymptomatic, some of these donkeys may act as parasite carriers; further studies will be performed by using molecular methods to detect the presence of parasites DNA from seropositive animals. Therefore, improved surveillance of EP is recommended for donkeys.

Detection of a cluster of cystic echinococcosis in dairy cattle in Veneto Region using retrospective and spatial analyses

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AIM: Cystic echinococcosis is a parasitic zoonotic disease, which remains an important public health problem with heavy economic impact in Mediterranean Region (Garippa G, Manfredi MT, 2009, Vet Res Commun, 33: S35-S39). This study is part of a research project funded by Veneto Region which involves the Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), the Local Health Units (ULSS) of Veneto, the Epidemiological Centre of Veneto Region (CREV) and the area of Parasitology of Padova University. The main objective of the program is to monitor the presence and control the spread of cystic echinococcosis and cysticercosis in bovines in Veneto Region, with particular reference to the identification and management of any indigenous transmission cycles.
In this study the presence of possible cluster of autochthonous cases of cystic echinococcosis among bovine population of the Region was investigated.

MATERIALS AND METHODS: The veterinary services of the 21 ULSS of Veneto Region were contacted to obtain data about positive cases of echinococcosis in cattle originating from farms in their territories and slaughtered from January 2006 to December 2010. Besides, further data were provided by two large slaughterhouses for the period January 2006-December 2008.
Animal identification tag was used to identify provenance and movements of positive animals. Positive animals were considered autochtonous cases of bovine cystic echinococcosis, if they never moved from Veneto Region. Only farms where one or more autochthonous cases spent all their life or, at least, up to 6 months before being slaughtered were considered positive farms.
The spatial aggregation of positive farms was investigated through a spatial scan statistic, using the Bernoulli probability model. The statistical significance of the clusters was established using Monte Carlo hypothesis testing, assuming that the infected farms were randomly distributed across the population. The size of the scanning window in the spatial scan statistic was set to include up to 1% of the total population (8,173 farms).

RESULTS: Out of the total 21 ULSS contacted, 19 provided the requested data. From 2006 to 2010, 269 positive animals were reported by ULSS, 98% (264/269) occurring in dairy cattle farms.

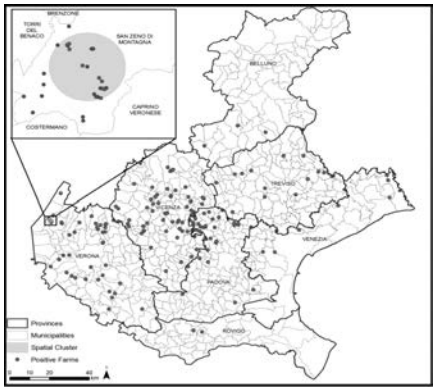

Fig. 1. The single significant cluster identified in the Province of Verona

Including data provided directly by slaughterhouses, the total number of cystic echinococcosis reported in Veneto Region from 2006 to 2008 was 395. Out of the 395 positive bovines, 279 (70.6%) were autochthonous cases and for 244 animals it was possible to trace the positive farm (173 farms). The scan statistic was performed including both the positive farms and the remaining population of dairy cattle farms. One single significant cluster was identified in the Province of Verona (Fig. 1), including 9 positive farms within a radius of 1,333 m.

CONCLUSIONS: This study highlights the importance of slaughterhouses as epidemiological monitoring centre. The spatial analysis identified significant aggregation of positive farms in a limited rural and mountainous area of Verona Province. An epidemiological investigation is ongoing in order to better describe this cluster and generally in order to assess the most reliable risk factors (e.g. environmental contamination of pasture by shepherd dogs, first of all) and to characterize genotypically the isolates from the Region.

SESSIONE 10

EPIDEMIOLOGIA DELLE MALATTIE PARASSITARIE DEGLI ANIMALI D'AFFEZIONE

Helmintic infections in north Sardinia kennel dogs

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AIM: The parasitic infections are one of the most important problems in kennel dogs and their regular monitoring is decisive for control programs. The aim of the present survey is to evaluate the diffusion of helmintic infections in kennel dogs of north Sardinia (ASLs of Sassari and Olbia-Tempio)

MATERIALS AND METODS: Between February and April 2012, all the kennels in the territory of the ASLs of Sassari and Olbia Tempio (n. 14) were investigated for helmintic infections. Twenty boxes for each kennel were examined. Fresh faecal sample were collected from the ground of each box, pooled, and preserved in 5% formalin. The pools were examined with the *FLOTAC dual technique* using two flotation solutions: Sodium Chloride solution (FS2 eg 1.2) and zinc sulfate solution (FS7 eg 1.35) (Cringoli et al, 2010, Nature Protocols, 5: 503-515).

RESULTS: All the kennels were positive to parasites, the prevalence of the six helminth species/taxa found are reported in the table in order of importance.

Helminth	N. positive kennels (%)
Trichuris vulpis	14 (100%)
Ancylostomidae	13 (92.8%)
Toxocara canis	12 (85.7%)
Toxascaris leonina	8 (57.1%)
Dipylidium caninum	3 (21.4%)
Eucoleus aerophilus	1 (7.1%)

CONCLUSIONS: The results showed high prevalences of helminth infections, particularly of *T. vulpis*, Ancylostomidae and ascarids. This is mainly due to the survival of eggs in the environment, but also to the inadequacy of facilities, with problems of overcrowding and of the hygiene control, but also to an uncorrected environmental prophylaxis and treatment strategies. Taking into account the importance of kennels to the animal welfare, the present findings suggest the need to improve the standards of hygiene, the parasitological surveillance and the specific and regular use of anthelmintics.

Occurrence of feline and canine lungworms in central and southern Italy in 2009-2011

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Eucoleus aerophilus syn. *Capillaria aerophila* (affecting dogs and cats), *Aelurostrongylus abstrusus* (affecting cats) and *Angiostrongylus vasorum* (affecting dogs), are emerging lungworms of pets in several countries and spreading into previously non endemic regions. Different drivers, e.g. climate changes, modifications of host phenology, animal trading and movements, may concur in changing their epidemiological patterns (Morgan E, Shaw S, 2010, J Small Anim Pract, 51: 616-621; Traversa D et al, 2010, Parasit Vectors 3: 62). Indeed, these nematodes are emerging also in Italy, where they have been repeatedly reported in the past few years (Traversa D et al, 2010, Parasit Vectors 3: 62; Di Cesare A et al, 2011, Parasitol Res 109: S87-S96). Their clinical importance is relevant and the infections may present with a wide range of symptoms and degrees of severity, mainly depending upon parasite burden and host-related factors (i.e. age, immune response and presence of concomitant diseases) (Conboy G, 2009, Vet Clin North Am Small Anim Pract 39: 1109-1126; Traversa D et al, 2010, Parasit Vectors 3: 62).

AIM: The present work investigated the occurrence of major lungworms infecting pets in selected regions of central and southern Italy from January 2009 to December 2011.

MATERIALS AND METHODS: Investigated sites were Marche (Site A), Lazio (Site B), Abruzzo (Site C), and Apulia (Site D) regions. Individual faecal sample were taken from 543 dogs (D) and 553 cats (C) from Site A (57 D and 51 C), B (100 D), C (375 D and 432 C) and D (11 D and 70 C). Additionally, environmental pools, each consisting of 2 to 9 individual stool samples, were taken from kennels and catteries. Specifically, 301 and 6 pools were collected from 5 (Site C), and 1 (Site D) kennels, while 16, 18 and 5 pools were collected from 2 catteries in each of Sites B, C and D.
All samples from Sites A, B, C and D were examined by floatations using a sugar solution with a 1.200 specific gravity (s.g) and a zinc sulphate solution with a 1.350 s.g., and, when possible, also with the Baermann technique. The only exception was represented by dog individual samples from Site B, which were examined with the Baermann technique and only some of them with the floatation procedures.
Eggs of *C. aerophila* and first-stage larvae (L1s) of *A. abstrusus* and *A. vasorum* were identified by key morphological and morphometric features (Sloss MW et al, 1994, Veterinary Clinical Parasitology**,** Iowa State University Press, Ames, Iowa, USA; Traversa D et al, 2010, Parasit Vectors, 3: 62).

RESULTS: *Capillaria aerophila* in dogs: 2.63% (1/38) and 7.73% (29/375) of individual canine samples were positive in Sites B and C, respectively; 3.32% (10/301) of the environmental samples from Site C were also positive; *Capillaria aerophila* in cats: 1.96% (1/51) (Site A), 3.70% (16/432) (Site C) and 14.28% (10/70) (Site D) individual samples were positive.
Aelurostrongylus abstrusus: 5.13% (2/39) (Site A) and 13.86% (47/339) (Site C) individual samples were positive for L1s. Larvae were also found in 5.56% (1/18) of environmental samples from Site C.
Angiostrongylus vasorum: 2.0% (2/100) and 4.13% (15/363) individual samples were positive from Site B and C respectively. L1s were also found in 4.98% (15/301) of pools from Site C.

CONCLUSIONS: These results indicate that lungworms are not uncommon and circulate in central and southern regions of Italy. Therefore, given the impact that these infections have for animal health and welfare and the zoonotic potential of *E. aerophilus*, it is advisable to routinely include these parasitoses in the differential diagnosis of canine and feline cardio-respiratory diseases for appropriate control measures and therapeutic interventions. Furthermore, given the lack of epidemiological data available in Italy especially for *E. aerophilus* and *A. vasorum* in the North, larger surveys are necessary to obtain more information on the diffusion of these parasites and on their impact on health and welfare of companion animals.

Mixed trichuroid infection in a dog from Italy

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Capillaria aerophila (syn. *Eucoleus aerophilus*), *Capillaria bohemi* and *Trichuris vulpis* are worldwide diffused trichuroid nematodes affecting wild and companion animals. The canine intestinal whipworm *T. vulpis* is the most common in veterinary practice while the respiratory *C. aerophila* and *C. bohemi* are poorly known and they are seldom reported in pets. A correct diagnosis of the infections caused by these worms is crucial. Indeed *T. vulpis* is responsible of intestinal distresses of varying severity and its zoonotic potential is under debate (Traversa D, 2011, Parasit Vectors, 8: 32); *C. aerophila* and *C. bohemi* induce damages to the respiratory system of the affected host and, the former, may cause lung capillariosis in people (Lalosević D, 2008, Am J Trop Med Hyg, 78: 14-16; Conboy GA, 2009, Vet Clin North Am Small Anim Pract, 39: 1109-1126; Traversa D et al, 2010, Parasit Vectors, 3: 62). A reliable diagnosis of canine trichuroid infections is based on the identification of typical eggs, which, however, present close morphometric and morphological features. Such similarities represent serious drawbacks in diagnosing single and mixed infections.

AIM: The present work presents a case of a natural mixed trichuroid infection, and highlights diagnostic difficulties in the differentiation of their eggs when found simultaneously.

MATERIALS AND METHODS: In December 2011 a dog living in Lazio region (Latina Municipality, Central Italy) was clinically examined and evaluated for the presence of endoparasites. A faecal sample collected from the rectum of the dog was subjected to a standard flotation with a zinc sulphate solution of 1.350 specific gravity (Sloss MW et al, 1994, Veterinary Clinical Parasitology, Iowa State University Press, Ames, Iowa, USA). The slide (coverslip of 24x50 mm) was examined using a light microscope at 200X and 400X magnifications and, given that trichuroid eggs with similar shape and appearance were detected, all parasitic elements were examined morphologically and morphometrically for being identified at the species level (Traversa D, 2011, Parasit Vectors, 8: 32; Traversa D et al, 2011, Parasitol Res, 109: S97-S104).

RESULTS: Nematode ova present in the sample showed a typical trichuroid lemon/barrel-like appearance with two plugs at the extremities. The careful appraisal of all detected eggs showed the presence of three different trichuroid nematodes, i.e. *T. vulpis*, *C. aerophila* and *C. bohemi*. Forty-six trichuroid eggs were detected in the examined slide, specifically 27 eggs were identified as *T. vulpis*, while 12 and 7 were recognized to belong to *C. aerophila* and *C. bohemi*, respectively. Those of *T. vulpis* were symmetrical, with ring-like thickening at the plug bases, a yellowish-brown and smooth surface and a size ranging from 83.50 ± 3.00 m length and 36.00 ± 3.50 m width. *Capillaria aerophila* eggs showed inferior lengths of major and minor axes, i.e. 64.45 ± 1.50 m and 34.95 ± 3.40 m, respectively. Also, they showed asymmetrical bipolar plugs and a network of anastomosing ridges and bridges on their walls. Ova of *C. bohemi* were morphologically kindred to those of *C. aerophila*, being smaller than those of *T. vulpis* and with asymmetrical plugs. However, they were smaller also than eggs of *C. aerophila* (size of 55.30 ± 1.30 m and 32.40 ± 2.60 m), and presented a surface with tiny pits and a space between the embryo and the wall.

CONCLUSIONS: The present report demonstrates that when barrel/lemon-shaped eggs are found at the copromicroscopic examination, their size, plug aspects and wall surface pattern must be carefully examined. In fact, given that *Capillaria* eggs are often not taken into account during faecal examinations, such findings are often misdiagnosed with whipworm ova. This is even more true in presence of mixed infections in which one species is more prevalent over the other/s, as in the case presented herein, where *T. vulpis* eggs were in a number greater than those of *Capillaria* spp.. Indeed, knowledge on the diffusion of *Capillaria* genus is fragmentary, most likely for frequent misdiagnosis occurring at faecal examinations (Traversa D, 2011, Parasit Vectors, 8: 32), and, as in the present case, the absence of intestinal/respiratory symptoms may lead to the underestimation of the occurrence and possible spreading of trichuroid infections. In conclusion, the attention *versus* these three species of trichuroids infecting dogs should be kept high and they should be always included into the differential diagnosis of intestinal and respiratory parasitoses of companion dogs.

Giardia duodenalis and other intestinal parasites in kennel-dog populations in North-eastern Italy

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Dogs can be parasitized by several intestinal helminths and protozoa. Among these, *Giardia duodenalis* is recognized as a re-emerging pathogen in children and immunocompromised individuals (Luján HD, Swärd S, 2011, Giardia. A model organism, Springer-Verlag, Wien, Austria). Although genetic studies have shown that *G. duodenalis* is classified into seven genetically distinct assemblages (A-G), the majority of isolates from human beings and some species of domestic animals falls into assemblages A and B, suggesting the possibility of interspecies transmission (Thompson RCA et al, 2000, Parasitol Today, 16: 210-213).

AIM: The objectives of this study were to evaluate the prevalence of *Giardia* and other intestinal parasites in kennel-dog populations in Veneto and Friuli Venezia Giulia (FVG) Regions (North-eastern Italy), to identify potential risk factors, and to compare different techniques employed in the diagnosis of giardiosis.

MATERIALS AND METHODS: Individual faecal samples were collected in 5 kennels, 4 in Veneto (from Feb to Jun 2011) and 1 in FVG Regions (from Nov 2008 to Jun 2009). Samples were initially screened by copromicroscopic techniques (M), and then analysed for *Giardia* presence by direct immunofluorescent assay (IFA-Merifluor®, Meridian Bioscience s.r.l., Italy) and nested-PCR procedure, amplifying a region of the SSU-rDNA using the primers RH11 and RH4 (Hopkins RM et al, 1977, J Parasitol, 83: 44-51), and the primers GiarF and GiarR for the secondary PCR (Read et al, 2002, Int J Parasitol, 32: 229–231). PCR products were sequenced and compared with the *Giardia* sequences available in GenBank™. Differences in parasite prevalence according to potential risk factors (kennel, breed, age, sex, and data on anthelmintic treatments within the two months before sampling) were evaluated by Pearson's chi squared Test (significance level $p<0.05$). Parasites with an overall prevalence below 10% were excluded from the statistical analysis. Concerning *Giardia* infection, agreement between the results of the different techniques was measured by K statistics. The software used was SPSS for Windows, version 16.0.

RESULTS: A total of 230 faecal samples were examined by M and IFA, while PCR was carried out on 212 samples. Information was acquired on sex (n=178), age (n=154) and anthelmintic treatments (n=136). Overall, 129 (56.1%) dogs were positive for helminths and/or protozoa. Among helminths, the highest prevalence values were detected for *T. vulpis* (33.5%), *T. canis* (12.6%), and *A. caninum* (10.9%) (Table). The kennel was detected as a significant risk factor ($p<0.001$) for each parasites included in the statistical analysis. The prevalence of *T. vulpis* was significantly lower ($p<0.001$) in treated animals (8.8% *vs.* 57.8%). Breed, sex and age were not identified as risk factors. The overall prevalence of giardiosis was 12.6% when detected by M, and increased to 18.3% (42/230) and 38.2 % (81/212) by IFA and PCR, respectively. Among different diagnostic tests, K statistics provided fair (K=0.294; M *vs.* PCR), moderate (K=0.483; IFA *vs.* PCR) and good (K=0.619; M *vs.* IFA) strengths of agreement. The sequencing analyses were carried out on 60/81 PCR positive samples, and *G. duodenalis* assemblages C (accession number DQ385548), D (DQ385549) and B1 (FJ668859) were detected in 41, 18 and 1 dogs, respectively.

Table 1. Prevalence of intestinal parasites detected by microscopy in five different Kennels (a-e) of North-eastern Italy

Parasite	Veneto				FVG	Total
	Number of positive dogs (prevalence %)					
	a (n=26)	b (n=47)	c (n=52)	d (n=17)	e (n=88)	(n=230)
Trichuris vulpis	7 (26.9)	18 (38.3)	37 (71.2)	1 (5.9)	14 (15.9)	77 (33.5)
Toxocara canis	4 (15.4)	13 (27.7)	0	0	12 (13.6)	29 (12.6)
Ancylostoma caninum	0	3 (6.4)	20 (38.5)	1 (5.9)	1 (1.1)	25 (10.9)
Eucoleus aerophilus	0	0	0	0	7 (8.0)	7 (3.0)
Dipylidium caninum	0	0	2 (3.8)	0	2 (2.3)	4 (1.7)
Giardia species	13 (50.0)	13 (27.7)	2 (3.8)	0	1 (1.1)	29 (12.6)
Coccidia *(Isospora)*	0	0	2 (3.8)	1 (5.9)	9 (10.2)	12 (5.2)

CONCLUSIONS: The findings of this study confirm that confinement of many animals in a limited area results in a high presence of helminths and protozoa parasites, and significant variations in their prevalence probably occurs in relation to the hygienic measures adopted in a kennel. The high prevalence of *Giardia* (up to 38.2% by PCR) is in agreement with previous studies (Capelli G et al, 2006, Vet Rec, 159, 422-424; Paoletti B et al, 2008, Ann. N.Y. Acad Sci, 1149: 371-374). Although further molecular evidence are needed, this study seems to confirm dog as a possible host for *Giardia* assemblage B, cluster B1, also reported in human beings (Lalle M et al, 2005, Int J Parasitol, 35: 207-213). Effective programs for controlling *Giardia* infection are seldom performed, and prevalence of infection is very probably underestimated using a single copromicroscopical test.

Investigation on intestinal helminths in owned-dog populations in Rome and Padua: preliminary results

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Intestinal nematodes affecting dogs represent a major threat for animal health and welfare. The most important are ascarids (i.e. *Toxocara canis* and *Toxascaris leonina*), ancylostomatids (*Ancylostoma caninum, Uncinaria stenocephala*) and whipworms (*Trichuris vulpis*). Some of them, e.g. *T. canis* and *A. caninum*, may play a relevant zoonotic role. Hence, these intestinal nematodes can be considered the most important parasites affecting dogs worldwide in terms of diffusion and risk for animal and human health. Indeed, dogs are exposed to these parasites throughout their life, although parasitic burdens, egg output and infection rates are higher in puppies. However, it is nowadays established that patent intestinal infections occur in dogs of all ages. Also, all categories of dogs, from the cossetted pets to the stray animals, can be infected by intestinal nematodes (rev. in Traversa, 2012, Parasites & Vectors, in press). Hence, there is a significant merit in a continuing update on their distribution, occurrence and spreading.

AIMS: The present work describes the first results of a study presently ongoing in three municipalities of Italy (Padua, Rome and Teramo) aiming to evaluate the prevalence of canine intestinal nematodes in privately owned and kenneled dogs, along with the extent of canine faecal contamination in green public grounds. The herein presented results regard the occurrence of canine nematodes in companion dogs from Padua and Rome municipalities.

MATERIALS AND METHODS: Sampling on companion dogs started in February 2012. Samples were collected *random* by veterinary practitioners working in Rome and Padua cities. The minimum sample size (n=206 in each city) was calculated considering the population size (infinite), expected prevalence (16%), confidence interval (95%) and maximum error desired (5%). Dogs that received an anthelmintic treatment within the previous three months were excluded from sampling. For each dog, a questionnaire was completed to give information on potential risk factors as follows: provenance (Rome or Padua), breed, gender, age, presence of enteric symptoms, home environment (apartment or gardening house), cohabitation with other dogs, attending of green public areas, and number of annual anthelmintic treatments planned by veterinarians (from 1 to 4). Faecal samples were screened by qualitative copromicroscopical floatation test using a sodium nitrate solution (density 1.3). Differences in parasite prevalence according to potential risk factors were evaluated by Pearson's chi squared Test (significance level $p<0.05$). The software used was SPSS for Windows, version 16.0.

RESULTS- A total of 367 faecal samples (193 and 174 from Rome and Padua, respectively) were collected till April 2012. The information acquired were: breed (n=363), gender (n=358), age (n=339), enteric symptoms (n=365), home environment (n=355), cohabitation with other dogs (n=358), attending of green public areas (n=357), and annual anthelmintic treatments planned by veterinarians (n=218). Overall, 37 (10.1%) dogs were positive for at least one helminth. The statistical analysis was performed for *T. vulpis* and *T. canis*, which were those parasites that showed the highest prevalence values (5.7% and 4.2%, respectively). Other recorded parasites were *A. caninum* and *U. stenocephala* (in two different dogs from Rome), and *Eucoleus aerophilus* (in one dog from Padua). No significant differences in prevalence ($p>0.05$) were found between dogs from Rome and Padua, both for *T. vulpis* and *T. canis* (5.2% *vs.* 6.3% and 3.6% *vs.* 4.6%, respectively). The prevalence of *T. canis* was significantly higher ($p<0.05$) in dogs ageing less than 2 years of age (7.4% *vs.* 2.3%), and cohabitating with other dogs (7.1% *vs.* 1.3%). No other risk factors were detected by statistical analysis. Annual or twice yearly anthelmintic treatments resulted to be planned by the majority of veterinarians (211/218).

CONCLUSIONS: The preliminary results of this study confirm that cosseted dogs may be infected by intestinal helminths as *T. vulpis* and *T. canis*, with prevalence values in agreement with previous surveys (Capelli et al, 2006, Vet Rec, 159: 422-424). The

presence of these parasites in owned dog may be a consequence of wrong approaches in diagnosis and/or anthelmintic treatment planning. These first findings endorse that the risk of environmental contamination of green public areas (parks, gardens, children playgrounds) with infective stages of these parasites is concrete. The ongoing study will provide further information on this regard.

Ecology and biology of *Acanthocheilonema reconditum* (Grassi, 1889)

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AIM: The aim of the study was to investigate the prevalence and incidence of *Acanthocheilonema reconditum* in a confined population of dogs living in an endemic area of Sicily. In addition, to investigate the circadian rhythm of this filarioid, the periodicity of microfilaraemia was monitored in highly microfilaraemic dogs. The competence of fleas and ticks as vectors of *A. reconditum* was also investigated by dissecting arthropods collected from the same dog population.

MATERIALS AND METHODS: In March 2011 and in March 2012, blood samples from 152 dogs from Messina shelter were screened for *A. reconditum* microfilariae by modified Knott's technique. The periodicity of microfilaraemia was investigated by bleeding 2 highly microfilaraemic dogs (d1 and d2) twice a day for 10 days and, later on, every 2 weeks for 1 year. A third animal (d3) was blood sampled every 3 h for 96 h. Fleas (n = 78) and ticks (n = 272) infesting dogs were collected and dissected for the detection of *A. reconditum* developing larvae.

RESULTS: *A. reconditum* microfilariae were detected in 11.2% (17/152) and 13.3% (16/120) animals at the first sampling and one year apart, resulting in a cumulative year incidence of 5.9%. Positive animals had a mean number of blood microfilariae (mfs) of 184.5 mfs/ml (± 392.4). At the 1-year follow-up, 16 (13.3%) out of the remaining 120 dogs positive for *A. reconditum* presented a mean number of 199.1 mfs/ml (± 639.5). D1 and d2 showed a higher number of microfilariae in most of the morning samples but not d3. *A. reconditum* developing forms were detected in 5.1% (4/78) of dissected fleas, but not in any of the 272 *Rhipicephalus sanguineus* collected from the same dogs.

CONCLUSIONS: Our data confirm the endemicity of *A. reconditum* infection in the study area and provide, for the first time, information on the cumulative incidence of this filarioid. Interestingly, new infestations were recorded in dogs sharing the same kennels with at least 1 dog microfilaraemic in the previous year as a likely consequence of passage of adult fleas between and among co-housed animals. This ecological aspect makes the epidemiology of *A. reconditum* very different from that of other filarioids transmitted by mosquitoes or ticks. The mean number of *A. reconditum* mfs recorded in our study is one of the highest reported either in natural (Pennington NE, Phelps CA, 1969, J Med Entomol, 6: 59-671969; Cringoli et al,. 2001, Vet Parasitol, 13: 243-2522001) or experimental infestations (Lindemann BA, and McCall JW, 1984, J Parasitol, 70:167-168). Although in the first experiment a higher number of mfs was recorded during the morning in 8 out of 10 days of study, the absence of any circadian rhythm was observed in d3 indicating that there is no defined periodicity of *A. reconditum* microfilaraemia in dogs. This is in line with the short period of flea blood feedings (about 10 min) and the absence of a defined circadian rhythm. The rate of *A. reconditum* infestation in fleas (5.1%) is much lower than that reported before (Pennington NE, Phelps CA, 1969, J Med Entomol, 6: 59-67Pennington and Phelps, 1969). Finally, this study provided definitive evidence of that *R. sanguineus*, does not act as a vector of this filarioid species, as erroneously reported in some reports ((Pennington NE, Phelps CA, 1969, J Med Entomol, 6: 59-67; Korkejian and Edeson, 1978, Ann Trop Med Parasitol, 72:65-78; Cringoli et al, 2001, Vet Parasitol, 13: 243-252)Pennington and Phelps, 1969; Korkejian and Edeson, 1978; Cringoli *et al.* 2001).

Dog and cat dermathophytoses: eleven years of diagnostic activity at the Laboratory of Mycology

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AIM: This paper presents a survey, limited to dogs and cats, on the results obtained from the epidemiological analysis of 11 years data (January 2000/December 2010) of routine dermatophytoses diagnostic activities, developed at the Laboratory of Mycology of the Department of Veterinary Medical Science (Ozzano Emilia-Bologna). The aim was to observe the possible evolution over time of an important pathology for aesthetic, clinical, economic and, not least, social impact, because of ringworm of dogs and cats can be zoonotic infections.

MATERIALS AND METHODS: A total of 3778 animals (1518 dogs and 2260 cats) with lesions or asymptomatic were examined. The samples were collected by skin scarification or brush's technique and sent to our laboratory from vets or collected directly at the facilities of the Department. Diagnosis of ringworm was made by culture on Mycosel agar (BBL), incubated at least 10 days at 26 ° C. The identification of dermatophytes was performed on the basis of macro- and microscopic characteristics (Rebell and Taplin, 1974, Dermatophytes, their recognition and identification, University of Miami Press). Records for individual subjects were collected in a database and the data obtained were analyzed: ^{2} test was used to compare the presence/absence of *M. canis* with different host characteristics (breed, sex, age, origin, clinical state, i.e. presence/absence of lesions), and year.

RESULTS: Cats were significantly ($p<0.01$) more infected with dermatophytes (32%) than dogs (7.5%). *Microsporum canis* was more frequently isolated (30.6% cats and 4.5% dogs). Other dermatophytes were isolates, alone or in some cases in association with *M. canis*: these were mainly *M.gypseum* (1.7% dog and 0.7% cats) and *T. mentagrophytes* (1.2% dogs and 0.2% cats); occasionally also *T. terrestre* and *M. cookei* were found. For *M.canis*, the number of asymptomatic carriers were significantly ($p <0.01$) higher in cats (69%) than in dogs (2.8%). Persian cats were significantly related to a higher prevalence of *M. canis* than other cats breed, while as regards to the dogs, there was impossible to obtain this data due to the high variability of the examined breeds. The prevalence of *M. canis* was significantly greater in young animals (≤12 months) than in older individuals and in females cats than males cats ($p <0.01$), whereas no sex differences were found in dogs. Feral cats were significantly more positive ($p <0.01$) compared to owned cats. The owners of cats positive for *M. canis* had more frequently ringworm (21.5%) that the owner of dogs positive (13.2%), but the difference was not significant.

CONCLUSIONS: Overall, in the period 2000/2010 the average number of cats (205) and dogs (138) per year examined for dermatophytes in our laboratory of Mycology, decreased if compared with previous survey: 613 cat and 266 dog/year in the triennium 1989-1991 (Tampieri et al, 1994, Micologia dermatologica, 8,15-24); 286 cat and 208 dog/year in the five-year period 1995-1999 (Galuppi et al, 2002, O&DV, 9, 51-56), even if a year variability was present. This could be explain initially by the move of the laboratory in a decentralized base in 1993, and the subsequently diffusion of diagnostic kit for veterinary practitioner. A slight decrease in the prevalence of dermatophytes in both animals, was observed, more evident in dog. Nevertheless, the epidemiological features of dermatophytoses in cats don't seem to be changed, even if it is difficult explain the higher frequency of infection in female than in male. A significant decrease of the infection due to *M.canis*, with an increase of that due to other dermatophytes (especially *M. gypseum* and *T. mentagrophytes*) were instead observed in dog. A decrease in the report of ringworm in owner of dogs and cats with dermatophytoses was observed compared to the triennium 1989-1991 (21.5 vs 36.1% in cat' owners and 13.2% vs 20.9% in dog' owners), but it is difficult to determine if this data is real, due to an increased attention by veterinarians and owners against this zoonosis, or only the consequence of the lack of anamestic data. *M. canis,* the most frequent agent of dermatophytoses in cat and dog, is also a frequent agent of humans' *tinea corporis* and often *tinea capitis* in children. Prevention and care of dermatophytes infections in companion animals are important for well-being of animals and for public health.

Endoparasitosis in Sicilian pets: a retrospective analysis of fecal sample examinations from 2001 to 2011

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AIM: A retrospective analysis on the results of parasitological examinations preformed by our Unit in the period 2001-2011 was conducted.

MATERIALS AND METHODS: Only parasitological exams performed on fecal samples of pets (dogs and cats) and reptiles (snakes and tortoises) were included in the analyses. To avoid biases, exams performed for research activities were excluded and only fecal samples referred by local practitioners or veterinary clinics were included in the analyses. Data were transferred on digital spreadsheet and analyzed using a descriptive statistical analysis. Fecal samples were tested using sodium nitrate flotation (specific gravity 1.3) for helminths eggs, zinc sulfate flotation (specific gravity 1.2) for *Giardia intestinalis* cysts and Baermann technique for the detection of metastrongyloid larvae.

RESULTS: A total of 2,788 fecal samples were examined for parasites in the period 2001-2011. In particular, 1,841 samples were from dogs, 753 samples from cats and 194 samples from reptiles. The presence of at least one species of endoparasites was diagnosed in 24.3% of dogs, 25.0% of cats and 73.2% of reptiles, respectively. The most frequent diagnosed parasitic agents in dogs were *G. intestinalis* (29.5%), followed by ascarids (10.8 %), *Isospora canis* (5.9 %), *Trichuris vulpis* (3.6 %), *Ancylostoma caninum* (3.3 %) and tapeworms (1.1 %). Regarding cats frequencies of endoparasites were the following: metastrongyloid larvae (23.9 %), *Isospora felis* (10.8 %), ascarids (10.8 %) and, less frequently, *G. intestinalis* (17.4 %) and tapeworms (4.9%) were observed. About reptiles 75.5% of samples tested positive for endoparasites. In this latter group, the most frequently diagnosed parasites were oxyuris (70.8%), ascarids (19.2%) and tapeworms (18.2%).

CONCLUSIONS: The analyzed data, in this work, could be considered as an epidemiological snapshot of the endoparasites in domestic animals and reptiles (snakes and tortoises) in Sicily. It is important to note that the number of request of fecal exams per year increased during the observation period (i.e. 58 exams in 2001 and 540 exams in 2011); although, the overall prevalence of endoparasites showed a significant decrease (32.7% in 2001 and 19.2% in 2011). This trend could be explained by the increased awareness on parasites and parasitic diseases registered in both pet owners and practitioners. The most frequently diagnosed endoparasites in dogs was *Giardia* whilst in cats were metastrongyloids. These data are partially in contrast with those reported in a similar study made in Germany between 2003 and 2010 (Barutzki D and Shaper R, 2001, Parasitol Res 109: 45-60) in which the most frequently diagnosed parasites were *Giardia* in both dogs and cats. Moreover, the metastrongyloids prevalence was 0.2% in cats whilst in the present work was 23.9%. This finding could be explained assessing that the Baermann technique is usually required when, based on clinical symptoms, a firm suspect of lungworm infection already exists. Concerning reptiles, the overall prevalence was really high and could be explained by the scant knowledge on parasitic diseases and their treatment in these unusual pets. That constitutes a serious risk for Public Health because these exotic animals could be illegally imported and drive their parasites from a country to another one. Interestingly, results reported for reptiles in this study are similar, except for protozoan infections, to those described in a similar survey conducted on reptiles maintained as pet in Sicily (Napoli E et al, 2011, Veterinaria, 25: 53-56). According to results presented in this study and their epidemiological relevance is possible to stress that a stable communication between veterinary facilities and public health offices should remain to update the epidemiological status picture about parasitic and spread and frequencies among animal populations. This could improve the efficacy of campaign against these pathogen agents still dangerously present.

Acknowledgments: This work is dedicated to the memory of Biagio Calì, our colleague, who got the idea to locate a register at Parasitology Laboratory, browsing the register our mind goes to him.

Updating on Coccidia in pet rabbits

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AIM: Although rabbit represents the third most popular house pet after dog and cat, little is known about the most common parasitic diseases found in the clinical practice. With regard to coccidia, scarce data are available on prevalence and characteristics of the infection in pet rabbits, whereas a lot is known about production animals. The current study examined the prevalence of *Eimeria* infections in pet rabbits of different origin in Bologna province.

MATERIALS AND METHODS: A total of 100 pet rabbits have been examined, from April 2011 to January 2012, for coccidian infection. The animals were kept under different management conditions: private owners, pet rabbit farms and a shelter for abandoned ones. A fecal sample was collected from each animal and anamnestic and clinical data were recorded. Each fecal specimen was examined using a qualitative coprological analysis and individual coprocoltures were performed to obtain the coccidian oocysts sporulation in order to allow the identification of the *Eimeria* species(Eckert J, Braun R, Shirley MW, Coudert P, 1995, Guidelines on techniques in coccidiosis research, European Commission, Directorate-General XII, Science Research and Development, Agriculture Biotechnology, Luxemburg). Overall data were statistically analyzed by SPSS v. 14.0.

RESULTS: Thirty-seven out of 100 rabbits (37%) resulted positive for coccidia. The morphological analysis (shape, presence or absence of micropyle and oocyst residuum) and the measurements (length and width) of the intact sporulated oocysts allowed to recognize 10 *Eimeria* species, shared in positive samples as follows: *Eimeria piriformis* 62.2 %; *E. vejdovsky* 48.6%;*E. flavescens* 27 %; *E. coecicola, E. perforans, E.magna* 21.6%; *E. media, E. stiedai* 8.1%; *E. exigua* 5.4%; *E. intestinalis* 2.7%. Mixed infection with two different species occurred most frequently (29.73%). Age was not found to be a variable statistically influencing the positivity for coccidia ($p>0.05$); it was neither found an association between the protozoan infection and the presence of clinical signs ($p >0.05$). With regard to management conditions, the prevalence of infection was high in rabbits living in shelter (92.3%) and in those belonging to pet farms (44%), whereas only the 4.08%of the house rabbits resulted positive for coccidia. These differences resulted statistically significant ($p < 0.05$).

CONCLUSIONS: In the present study 10 species of *Eimeria* out of the 11 reported in literature were found, most commonly occurring with mixed infection. It has not found an association between the infection and the presence of clinical signs; it is known that the majority of the rabbits infected with coccidian are asymptomatic carriers, developing a host-parasite equilibrium (Pellérdy LP, 1965, Coccidia and coccidiosis, Akadémiai Kiadò, Budapest.). Otherwise, age did not influencing the positivity to coccidia was an unexpected result: young rabbits are largely reported to develop coccidiosis more easily than older ones, due to a lower resistance or less immunity to the infection (Pakandl M. et al. 2008: Parasitol Res 103: 1265-1271).The environment turned out to be the real factor influencing the positivity for these protozoa; on the one hand, only the 4.08% of the house rabbits was positive, showing that the infection is generally prevented by a domestic management. On the other hand, the infection was highly promoted by both the shelter's (92.3%) and the farm's (44%) environments, where the free-living, the overcrowding, the humidity and the stress are, respectively, predisposing condition to the maintenance of the infection (Pellérdy LP, 1965, Coccidia and coccidiosis, Akadémiai Kiadò, Budapest).

Toxoplasmosis in Saharawi camps: seroprevalence in cats

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AIM: Sahrawi, literally 'people from the desert', are nomadic and pastoral tribes who inhabited the Western Sahara." Under the project *"Soutien à l'élevage de bétail dans le camps de réfugiés Sahraoui"*, financed by the UE and supported by the ONG "Africa '70, 198 Saharawi people have been serologically investigated (Castagna B et al., present congress) for *T.gondii* specific antibodies, showing a 32% of seropositivity for IgG and 12.5% for IgM, signal of a recent or active infection. The aim of this study was to investigate the *T. gondii* antibodies prevalence in a group of cats circulating in Saharawi refugee camps, in order to suggest the origin of the human infection.

MATERIALS AND METHODS: During autumn 2010, a total of 47 blood samples have been collected from cats of different ages and gender. Serum, obtained by centrifugation (1500g/10min) was stocked at -20°C. All the sera were examined for *T. gondii* specific antibodies (IgM and IgG) by an Indirect Fluorescent Antibody Test (IFAT) using a commercial antigen (Mega Cor Diagnostik) and anti-feline-specific IgG labeled with fluorescein isothiocyanate as conjugates (Sigma Immunochemicals, St Luis, MO, USA). The serum samples were screened at starting dilutions of 1/10 and 1/20 for IgM and IgG respectively (Macrì G et al, 2009, Parasitol Res, 105:35-40). The samples were also tested for the IgG detection by an indirect Enzyme Linked Immunosorbent Assay (ELISA) kit and by an "home-made" ELISA. The first was a commercial kit in multispecies indirect ELISA "ID Screen ® toxoplasmosis indirect" (IDVET, Montpellier-France), using as antigenic substrate a purified peptide of the main P30 *T. gondii* protein and as conjugates a multi-species peroxidase (po), according with manufacturer's instruction. The second was an ELISA "home- made" developed in the Laboratory of Serology of Istituto Zooprofilattico Sperimentale of Umbria e Marche and was performed following the protocol described by Mangili PM et al. (2008, Large Animal Review,14: 193). Furthermore to evaluate the impact of immunosuppressive retroviruses, all serum samples were also tested for Felv antigen and FIV antibodies using a commercial rapid assay kit (SNAP FIV Antibody/Felv Antigen Combo Test: IDEXX). The overall data were statistically analyzed.

RESULTS: The overall seroprevalence recovered by IFAT was 40.42% (19/47, IC 95%: 26.3-54.45) for IgG and 34.04% (16/47; IC 95%: 20.5-47.58) for IgM. All the animals tested IgM positive were also positive to IgG, whereas 6.38% (3/47; IC 95%:-0.6-13.37) showed only detectable IgG. Nineteen samples tested positive by IFAT showed positivity also by the commercial ELISA. Sixteen on 47 animals (34.04%, IC 95%: 20.5-47.58) were positive by the "home-made" ELISA whereas 3 samples (6.38 %, IC 95%:-0.6-13.37) exhibited ambiguous results. IFAT showed a perfect agreement with p-30 ELISA (K=1); however lower, but "almost perfect" agreement (K= 0.86; IC 95%: 0.76-0.95) was observed between IFAT and "home-made" ELISA. In 2/47 cats (4.25%; IC 95%: -1.5-10.1) were detected FIV antibodies, whereas Felv antigens were not detected in any cat. Age and sex were not found to be variables influencing statistically the serological status of the cats ($p>0.05$).

CONCLUSIONS: In our study the 34.04% of cats tested positive both for IgG and IgM. This finding could be attributed to a recent or active infection as well as to a parasitic reactivation linked to immunosuppressive condition i.e, retroviral infection (2/47; 4.25%) (Lappin MR et al., 2010, Top Companion Anim Med, 25(3):136-41). The results obtained applying different serological techniques showed an overall concordance of 97.93%, suggesting that all the tests screened might be useful for toxoplasmosis serological screening in cats. With regard to risk factors for *T. gondii* infection for Saharawi population, the climatic condition of the desert are not compatible with the consumption of raw or undercooked meat and vegetables, important sources of infection for human. It comes that the infection could be acquired mostly from the environment, via oocysts, that may survive for months in soil or water (Dabritz HA, 2009, Zoonoses Public Health, 57(1):34-52; Dubey JP, 1995, J Parasitol, 81:410-15); in fact several epidemiological studies have identified soil contact and soil-related occupation as important sources of infection (Chacin-Bonilla L et al, 2001, Am J Trop Med Hyg,65:131-135; Jones JL et al, 2001, Am J Epidemiol,154:357-365).

Giardia in stray dogs in the city of Naples

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AIM: *Giardia* has been reported in dogs worldwide; the prevalence of *Giardia* tends to vary considerably between studies and is often influenced by different factors related to the dog characteristics and the diagnostic test used (Thompson RC et al, 2008, Vet J, 177: 18-25). In order to add data to the epidemiological scenario of canine giardiosis in Italy, the aim of the present study was to investigate the prevalence of *Giardia* in stray dogs living in the city of Naples, Campania region, southern Italy.

MATERIALS AND METHODS: Between March and June 2011, faecal samples were collected from 204 stray dogs living in Naples and transported to the veterinary hospital "*Frullone*" of the city. Each faecal sample was tested for the presence of copro-antigens of *Giardia* using the IDEXX SNAP® test. Samples which resulted positive using this test were further analysed by the following techniques: the Xpect® *Giardia/Cryptosporidium* snap test, the Meri-Fluor direct immunofluorescence assay (IFA), and the *FLOTAC-400 double technique* (FDT) (Cringoli G et al, 2010, Nat Protoc, 5: 503-515) which employed zinc sulphate (density = 1.350) as flotation solution and had an analytic sensitivity of 2 cysts per gram (CPG) of faeces. When using IFA the number of *Giardia* cysts found were ranked into three levels. Statistical analysis was performed using Windows SPSS® (version 17.0). Cross tables and Chi-square tests were used to calculate possible correlations between positivity to *Giardia* and the following dog variables: age, sex, size and faecal consistency (normal, pastry or diarrheal). Spearman's Rho correlation was used to evaluate the relationship between the *Giardia* CPG detected by FDT and the level of infection detected by IFA (ranked as 1, 2 or 3). For all the statistical analyses, significance was assessed at $P < 0.05$.

RESULTS: Out of the 204 samples examined, 29 (14.2%; 95% Confidence Interval = 9.9-19.9%) resulted positive to *Giardia* using the IDEXX SNAP® test. The results of the statistical analysis did not show any association between the positivity to *Giardia* and the independent variables (dog characteristics) taken into consideration. Regarding the comparison of the different diagnostic techniques used, all (100%) the samples resulted positive using the IDEXX SNAP® test, were also positive with IFA and FDT, whereas the Xpect® produced 1 false negative result. A good correlation (Spearman's Rho = 0.7; $P < 0.05$) was found between IFA (considered the gold standard) and FDT concerning the quantitative results. The *Giardia* CPG detected by the FDT ranged between 2 and 1028 CPG.

CONCLUSIONS: In conclusion, the findings of the present study revealed a *Giardia* prevalence of 14.2% in faecal samples from stray dogs living in Naples. These results are in line with the recent findings of *Giardia* in dog faecal samples from urban environment of Naples (Rinaldi L et al, 2008, Res Vet Sci, 84: 413-415) and also with the prevalence values (16.1%) recently reported in stray dogs from north-east and central Italy (Capelli G et al, 2006, Vet Rec, 23: 422-424). The results also confirm the accuracy of FLOTAC-400 for the diagnosis of *Giardia* and other intestinal protozoa in dogs as already demonstrated for protozoa infections in humans (Becker SL et al, 2011, J Clin Microbiol, 49: 2183-2190; Gualdieri L et al, 2011, Acta Trop 117: 196-201). However, the zoonotic potential of these findings was not assessed due to the lack of information on assemblages detected.

Kennel dogs and helminth infections in the Campania region

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AIM: Kennel dogs can serve as sentinels and/or reservoirs of parasitic infections of veterinary and zoonotic interest (Cabezón O et al, 2010, Parasitol Res 107:1505-1508). The guidelines from the European Scientific Counsel Companion Animal Parasites recommend that careful consideration should be given to helminth control programmes for dogs in kennels and that faecal monitoring should be conducted regularly to identify the parasitic species present and the effectiveness of control programmes (http://www.esccap.org/). The aim of the present cross-sectional copromicroscopic survey was to evaluate the extent of helminth infections in kennel dogs from the Campania region of southern Italy.

MATERIALS AND METHODS: Between June 2011 and March 2012, a cross-sectional survey was conducted in 49 kennels distributed on the whole territory of the Campania region. A minimum of 10 boxes were examined in each kennel; fresh faecal samples were collected from the ground of each box (composite sample) and preserved in formalin 5%. Each faecal sample was then examined using the *FLOTAC dual technique*, using two flotation solutions on the same faecal composite, namely a sodium chloride based solution (FS2; density = 1.200), and a zinc sulphate based solution (FS7; density = 1.350).

RESULTS: Helminth infections were found in the 100% of the 49 studied kennels as follows:

Helminth	No. positive kennels	Prevalence (%)	95% Confidence interval
Trichuris vulpis	47	95.9	84.9-99.3
Ancylostoma caninum	36	73.5	58.7-84.6
Toxocara canis	36	73.5	58.7-84.6
Toxascaris leonina	12	24.5	13.8-39.2
Crenosoma vulpis	9	18.4	9.2-32.5
Angiostrongylus vasorum	6	12.2	5.1-25.5
Oslerus osleri	2	4.1	0.7-15.1
Dipylidium caninum	33	67.4	52.3-79.6

CONCLUSIONS: THE findings of the present survey show a high prevalence of helminths (including many zoonotic agents) in kennel dogs from southern Italy despite the regular use of anthelmintic treatments. This situation has important consequences on different issues concerning animal welfare, treatment and control, and public health. Because of failures in individual (use of anthelminthics) and collective (reduction of environmental contamination) preventive measures currently in place for kennel dogs, regular parasitological surveillance, appropriate treatment strategies and high quality standard of hygiene are strongly needed to guarantee the health and welfare of pets, and to enhance the safety of people.

First report of *Angiostrongylus vasorum* in a dog of Sardinia: parasitological and clinical findings

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AIM: *Angiostrongylus vasorum* (Nematoda, Metastrongylidae) is reported in dogs in central and southern Italy by Della Santa D et al (2002, Veterinaria 2:9–14), Traversa D et al (2008, Parasitology, 152:162–166), Sasanelli M et al (J Small Anim Pract, 2008, 49:417-420), Tieri E et al (Veterinaria italiana, 2011, 47(1):77-78) and Brianti E (2012, personal communication). The aim of this paper is to report the first identification of *A. vasorum* in a dog in Sardinia and its main parasitological and clinical characteristics.

MATERIALS AND METHODS: This report describes a clinical case relative to a five years-old, female, half italian hound dog of guested in a public kennel in Sassari (Sardinia, Italy). In September 2011 after a diagnosis of Leishmaniasis, the dog was treated with Miltefosine and Allopurinol. In December of the same year the dog was submitted to a clinical visit at the Department of Veterinary Medicine in Sassari where blood and faecal samples were taken for total blood cell count, biochemical analyses and serum protein electrophoresis and parasitological examination. These last were performed with sedimentation and flotation in Zinc Sulphate (sd 1,200) and centrifugation (10 minutes/2000 rpm) and examination with Baermann technique.
The larvae isolated were identified using the morphometric keys reported by Euzeby (1981, Diagnostic Experimental des helminthoses animales, livre 1, Edition Information Techniques des Services Veterinaires, Paris) and McGarry J W, Morgan ER (2009,Veterinary Record, 165: 258-261).
In a second step, diagnosed the presence of larvae of *A. vasorum*, additional diagnostic tools like chest radiograph images, echocardiography (2D, M-Mode e Doppler), saline contrast echocardiography (microbubbles obtained with agitation of a NaCl 0.9%) (Matos JM et al, 2012, Journal of Veterinary Cardiology, in press), and an ophthalmologic examination were performed.

RESULTS: Coprological exams revealed the presence of larvae unidentifiable in the flotation technique, which were subsequently identified based on their morphometric characteristics as larvae of *A. vasorum*, thanks to their isolation with Baermann technique. The general and particular clinical examination of the respiratory system was normal. Blood tests revealed no significant alteration of the monitored parameters, except for a hypergammaglobulinemia. Radiography showed radiological signs typical of generalized interstitial pattern, in agreement with what reported in other papers (Jenny R et al, 2010, Journal Veterinary Emergency and Critical Care, 20(1): 98–109). No heart abnormalities were observed on conventional 2D,M-mode and Doppler echocardiography and no signs of pulmonary hypertension were found. However saline contrast microbubbles were identified in left cardiac chambers 3 cardiac cycles after they had arrived in right cardiac chambers. In healthy heart can be explained by assuming the individual recruitment of pulmonary AV shunts, as recently reported by Matos JM et al (2012, Journal of Veterinary Cardiology, in press) on some dogs experimentally infected. It was not detected any vascular hemorrhagic injury at retinal or scleral level.

CONCLUSIONS: This study represents the first report of *A. vasorum* in a dog of Sardinia. Although any symptoms of the presence of the parasite were manifested, the patient revealed pulmonary lesions observable only with instrumental tools. These lesions could be hazardous for pulmonary and respiratory functions. The absence of clinical signs could be due to the mild physical activity of the dog that permitted a good compensation of pulmonary functions.
The hypergammaglobulinaemia detected with the progress of anti-Leishmania therapy show a recovery to normality, a clear sign that it was caused by the protozoan.
Unlike other reports our case did not present vascular lesions or bleeding at the scleral or retinal levels (Helm J et al, 2009, Journal of Small Animal Practice, 50: 255-259).
The finding of *A. vasorum* in dogs also in Sardinia, suggests to include in the diagnostic iter of respiratory diseases of the dog also parasitological tools for the identification of larvae of this nematode in the feces.

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Case report: identification of *Cryptosporidium felis* in a symptomatic cat co-infected with *Giardia duodenalis*

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AIM: *Cryptosporidium spp*. is a protozoan parasite that infects a large number of vertebrate species, including humans, cats and dogs. The parasite has a worldwide distribution and is ubiquitous in the environment. To date, at least 20 species and over 40 genotypes of *Cryptosporidium* have been identified, including *Cryptosporidium felis* that infects cats as major host (Xiao L and Fayer R, 2008, Int J Parasitol, 38: 1239-1255). Epidemiological surveys conducted worldwide have reported a prevalence in cats ranging from 0 to 29% (Lucio-Forster A et al, 2010, Trends Parasitol, 26: 174-179). In Italy, studies on cat cryptosporidiosis are scarce; the prevalence of *Cryptosporidium spp.* determined by fecal examination was reported to be about 24% (Rambozzi L et al, 2007, J Feline Med Surg, 9(5): 392-396). However, a survey of 108 cats of central Italy using IFAT revealed no positives (Paoletti B et al, 2011, Res Vet Sci, 91(3): 397-9). In the present work, we report a case of symptomatic cryptosporidiosis in a young cat that was co-infected with another parasite, *Giardia*.

MATERIALS AND METHODS: In December 2010, a 7-months old male cat was presented to the veterinary clinic with a persistent and recurrent diarrhoea. No other clinical signs were noticed. The faecal sample was tested using a direct immunofluorescent test (MERIFLUOR® *Cryptosporidium/Giardia*, Meridian, Bioscience Inc.), which allows a semi-quantification of cysts/oocysts. Molecular analysis for *Giardia* included a nested-PCR targeting the 16S gene (Hopkins RM et al, 1997, J Parasitol 83: 44-51). PCR for *Cryptosporidum* targeted the oocyst wall protein (COWP) gene (Traversa D et al, 2004, Appl Environ Microbiol, 70(7): 4367–4370). Amplicons were sequenced and compared with sequences available in the GenBank database.

RESULTS: The immunofluorescent test revealed the presence of *Giardia* (9000 cysts/g) and *Cryptosporidium spp.* (100 oocysts/g). The positivity for *Giardia* was confirmed by PCR (genotyping in progress), whereas the sequence of the PCR product for *Cryptosporidium* revealed 100% homology with *Cryptosporidium felis.* While waiting for confirmation of *C. felis* by ISS, the cat was treated against *Giardia* with fenbendazole and recovered. The follow up was not possible, and further information about the cat were not available.

CONCLUSIONS: Cryptosporidiosis in cats and dogs is usually asymptomatic and clinical signs are rarely observed. Generally, in animals younger than 6-months or immunocompromised, clinical signs are associated with diarrhea due to the parasite invasion and development in the intestinal epithelium (Lucio-Forster A, 2010, Trends Parasitol, 26: 174-179). In the case reported here, diarrhea was likely due to *Giardia* infection, even though clinical signs may have been worsened by the co-infection with *Cryptosporidium.* To the best of our knowledge, this is the first molecular description of *C. felis* in a cat of Italy. The pathogenic role of *C. felis* in cats needs to be further assessed.

Occurrence of *Angiostrongylus vasorum* in dogs with compatible clinical pictures

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The adult stages of *Angiostrongylus vasorum* live in the hearth and pulmonary arteries of dogs, causing a plethora of respiratory and cardiac symptoms. Coagulopathies, neurological disorders, and sudden death may also occur. Sometimes larval stages disseminate systemically and cause parasitic emboli, granulomatous inflammation and organ damages (Morgan E, Shaw S, 2010, J Small Anim Pract, 51: 616-62; Lepri et al, 2011, Parasitol Res, 109:505-508). In the past few years the parasite has been increasingly reported in dogs even in previously free areas, Italy included (Morgan E, Shaw S, 2010, J Small Anim Pract, 51: 616-621; Traversa D et al, 2010, Parasit Vectors 3:62). Nonetheless, information on infection rates and occurrence of the parasite in animals with compatible clinical pictures in the country are scattered.

AIM: The aim of the present work was to evaluate the occurrence of *A. vasorum* in dogs referred with symptoms compatible with angiostrongylosis in selected regions of Italy.

MATERIALS AND METHODS: From January to April 2012 individual faecal samples were collected from 323 dogs (100 from Abruzzo, 100 from Lazio, 12 from Marche, 38 from Lombardy, 43 from Veneto and 30 from Apulia). Animals were selected when they showed at least one clinical sign compatible with angiostrongylosis. Samples were undertaken to the Baermann technique and larvae were morphologically and morphometrically identified (Traversa D et al, 2010, Parasit Vectors 3:62).

RESULTS: Twelve (3.7%) dogs resulted infected by *A. vasorum*. Prevalence in different regions was 9% in Abruzzo and 2% in Lazio (central Italy), and 2.6% in Lombardy (northern Italy), while no positive dogs were found in Marche, Veneto and Apulia. Infected dogs showed at the same time from 1 to 6 symptoms compatible with the infection. Symptoms were general respiratory distress (n. 11 dogs), cough (n. 7), exercise intolerance (n. 7), chronic cough (n. 4), dyspnoea (n. 3), wheezing (n. 2). Cardiopulmonary disorders and coagulopathies were also detected, i.e. pale mucous membranes (n. 1), coagulopathy (n. 1), melaena (n. 1) and shock (n. 1). Weight loss and depression were also reported in 3 and 4 dogs, respectively.

CONCLUSIONS: The present study, which will be completed in September 2012, is the first large scale survey on the presence of *A. vasorum* in naturally infected dogs from Italy and provides new data on its distribution in the country. Although in the last few years the parasite has been described in the country, data are mainly limited to central areas (prevalence from 0.96% to 8.9%) (Di Cesare A et al, 2011, Parasitol Res 109:87-96; Tieri et al, 2011, Vet Ital 47:77-88) and only single cases have been reported from other regions (Della Santa D et al, 2002, Veterinaria, 2: 9-14; Scaramozzino P et al., 2007, IV Congr Naz AIPVet: 112-116; Sasanelli M et al, 2008, J Small Anim Pract, 49: 417-420; Lepri E et al, 2011, Parasitol Res, 109: 505-508; Di Cesare A et al, 2011, Parasitol Res, 109: 87-96). Present data confirm the presence of *A. vasorum* in Italy and suggest a possible geographic expansion, as already hypothesized elsewhere (Morgan E, Shaw S, 2010, J Small Anim Pract, 51: 616-621; Traversa D et al, 2010, Parasit Vectors 3: 62). As expected, clinical signs of infected animals herein examined were consistent with angiostrongylosis but aspecific. In fact, this infection often poses important diagnostic challenges because clinical pictures overlap a series of other conditions of dogs and copromicroscopic techniques have inherent hindrances. Hence, here is the necessity of stimulating concern on this infection among vet practitioners, which should always include *A. vasorum* on the list of differential diagnoses when symptoms are consistent. This is of particular importance because, albeit the parasite has a high clinical impact and may be life-threatening, the anthelmintic treatment is simple, straightforward and most often successful.

SESSIONE 11

LEISHMANIOSI E FILARIOSI

High resolution melting analysis coupled to real time PCR for detection and discrimination of *Dirofilaria immitis* and *Dirofilaria repens* in canine peripheral blood

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Dirofilaria immitis and *D. repens* are the most common species of filarial nematodes described in dogs with increasing spread into new geographical areas. The diagnosis of canine dirofilariosis is usually based upon the microscopical detection of circulating microfilariae together with ELISA detection of serum circulating antigens or antibodies. In the recent years, different molecular protocols have been developed for detection and species-specific identification of circulating microfilariae. Most of the above protocols are designed with the use of two-step analysis, different primers pairs, and/or sequence-specific probes (Rishniw M et al, 2006, Vet. Parasitol. 135: 305–314).

AIM: High-resolution melting analysis (HRMA) is a molecular technique with increasing use in diagnostic microbiology and parasitology for species identification and genotyping. This technique offers a low-cost, closed-tube approach to amplicon analysis with the capacity for single-nucleotide discrimination and easy integration with real time PCR (Reed GH et al., 2007, Pharmacogenomics 8, 597–608; Areekit S et al, 2009, J Med Assoc Thai, 92, Suppl.3:S24-S28). In this work we describe a new molecular method based on real-time PCR coupled to HRMA to detect and differentiate *D. immitis* and *D. repens* on DNA extracted from canine peripheral blood. This method is particularly suitable for routine diagnostics as a quick and sensitive single-step duplex protocol with a unique primer pair and with no need for sequence-specific probes. This allows a shorter analysis time, a reduced cost, and a comparable amplification efficiency for both targets. The last is especially useful in case of simultaneous infection with the two *Dirofilaria* species.

MATERIALS AND METHODS: The analysis were performed starting from anticoagulated canine peripheral blood samples for routine *Dirofilaria* diagnostics. In total, 5 *D. immitis* positive blood samples, 8 *D. repens*-positive blood samples and 3 blood samples with mixed infection were selected for the analysis. Furthermore, in order to test the the range of sensivity of the method, microfilaraemic blood and negative blood were mixed to give reconstructed positive blood sample of different microfilarial load for each *Dirofilaria* species (4 mf/ml and 32250 mf/ml for *D. immitis*; 4 mf/ml and 100000 mf/ml for *D. repens*). Knott's test-negative blood samples were included as negative controls, and *D. immitis* and *D. repens* adult worms were used as reference samples. The DNA was extracted from selected samples as previously described (Gioia et al, 2010, Vet. Parasitol. 172: 160-163). The primer pair for real time PCR amplification was designed in a region of *Dirofilaria* mitochondrial gene for cytochrome oxidase, subunit I, where primer annealing sequences are conserved whereas the interposed sequences show no intraspecific variability but interspecies variability at different positions. The HRMA protocol included a real time PCR reaction, a subsequent DNA melting process, and a normalization step for data analysis. The analysis was performed on the Eco™ Real-Time PCR System (Illumina, Inc., San Diego, CA, USA).

RESULTS: each blood sample positive to microscopical evaluation was also positive to real time PCR-HRMA. The analysis of high resolution melting curves allowed clear discrimination of the two *Dirofilaria* species, and mixed infection, according to the different melting temperature of the corresponding amplicons. The assay tested positive also on blood samples reconstituted with the lowest mf number, confirming the high sensitivity of the assay.

CONCLUSIONS: This quick, sensitive and specific real time PCR-HRMA assay represents a new tool for epidemiological studies and routine disease assessment of the two most common filarial parasite in dogs, reducing the cost, the contamination risk and the time of analysis.

Mixed Infection with different *Leishmania donovani* complex strains in Sudanese patients

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AIM: Sudanese *Leishmania donovani complex* is considered a genetically homogenous group, however *L. donovani* isolates cause very different clinical forms. In our previous study, phylogenetic analysis of the gp63 gene of Sudanese isolates indicated the existence of two monophyletic clades: *L.major* and *L.donovani complex* (Babiker AM *et al.,* 2011, Italian J Trop Med, 16:65-73). In the same study we observed multi nucleotidic superposition at a definite fragment on the gp63 gene in many samples. In this study we cloned two of these "complicated" samples to analyse sequences obtained from clones and confirm the presence of different sequence patterns in the same patient.

MATERIALS AND METHODS: Two bone marrow samples (14HBM and 10HBM) obtained from two visceral leishmaniasis patients that presented multi nucleotidic superposition from position 810 to position 895 on the GP63 nucleotidic sequence, were amplified using the primers gp63-3/gp63-4 which generate 347bp (positions given according to the GenBank GP63 sequence accession n° GQ301544). The two amplificates were cloned separately, DNA of 30 colonies from each sample were extracted, amplified and sequenced. Phylogenetic analysis was conducted using neighbour Joining method with 1000 bootstraps replicate implemented in Mega 4 software.

RESULTS: Twenty-six and twelve sequences were obtained from samples 14HBM and 10HBM respectively. Phylogenetic analysis revealed 11 different sequence patterns, seven for sample 14HBM clustering into three major groups (1,2 and 3) and four for sample 10HBM, clustering into two groups (A and B) (Fig1). The genetic similarity of the cloning sequences of this study with *L.infantum* and *L.donovani* reference sequences is shown in table 1.
Interestingly, only two groups of cloned strains (group 1-14HBM and group B-10HBM) showed a high genetic similarity with *L.infantum* and *L.donovani* species, respectively. All other strains showed a genetic variation from the reference sequences ranging from 4.3 to 25.2% (Tab 1).

CONCLUSIONS: The preliminary results herein shown indicate that a visceral leishmaniasis patient can harbour seven different sequences of *Leishmania* parasite, some of which encoding for different proteins, this could explain differences in the clinical course of *Leishmaniasis* infections.

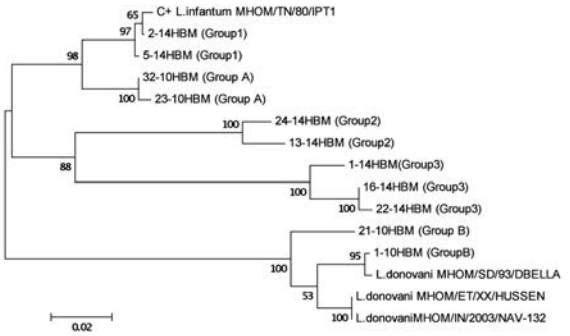

Fig. 1. Phylogenetic analysis of cloning sequences from samples 14HBM and 10HBM

Table 1. *L.infantum* and *L.donovani* reference sequences.

Cloning sequences	*L. infantum*	*L. donovani*
14 HBM		
Group1	99.2%-99.6%	78.4%
Group 2	78.7%-85.8%	83.1%-85.2%
Group 3	85.1%-83.0%	74.8%-79.5%
10 HBM		
Group A	95.7%-95.3%	82.5%-76.8%
Group B	82.2%-83.7%	94.5%-99.6%
L. infantum	0.0	16.8%

Group1 14HBM appeared closely similar to *L.infantum*, this finding is not concordant with previous studies that confirm *L.donovani* as the only single visceralizing species in Sudan and East Africa (Jamjoom MB et al; 2004. Parasitology). Findings of this study open discussions about the synergetic/ antagonistic interaction between these isolates both in mammalian and vector hosts, the route of transmission of these infections and the specificity of the isoenzyme-based identification for *Leishmania* parasite. However, these results need to be confirmed by a multilocus approach.

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Cercopithifilaria sp. and its intermediate host in dog populations from Mediterranean basin: a neglected, but widespread filarioid of dogs

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AIM: Besides the well-known filarial species presenting haematic microfilariae (e.g., *Dirofilaria immitis*, *D. repens*, *Acanthocheilonema reconditum* and *A. dracunculoides*) those with only dermal microfilariae (e.g., *Onchocerca lupi* and *Cercopithifilaria* spp.) have been little studied. Following the recent retrieval of dermal microfilariae identified as *Cercopithifilaria* sp. (from here referred to as *Cercopithifilaria* sp. I) in a dog from Sicily (Otranto D et al, 2011, Vet Parasitol, 182: 221-229), several studies investigated the geographical distribution of this filarioid among dog populations from Mediterranean basin (Otranto D et al, 2012, Parasit Vectors, 5: 1), its genetic make-up (Otranto D et al, 2012, Mol Cell, 26: 81-9), and assessed the role of *Rhipicephalus sanguineus* as vector of this filarioid (Brianti E et al, 2012, Vet Parasitol, 183: 330-337). Results of these studies are here reviewed and discussed.

MATERIALS AND METHODS: To assess the competence of *R. sanguineus* as intermediate host of *Cercopithifilaria* sp. I an experimental tick infestation was performed on an infested dog using 300 nymphs. To detect the presence and the development of filarial larvae in ticks, pools of nymphs were examined by both microscopic dissection and molecular analysis at pre-determined time points (i.e., the same day of tick collection from the initial infestation and 10, 20, 30 and 50 days after tick collection). *Cercopithifilaria* sp. I prevalence among dog populations and its intermediate hosts was investigated through a large epidemiological survey on skin samples (n=917) and ticks (n=890) collected from dogs at different time points in Italy (843), central Spain (51) and eastern Greece (23). Skin samples and ticks were examined for *Cercopithifilaria* sp. I by microscopy, molecular analysis, or both.

RESULTS: *R. sanguineus* proved to be a suitable intermediate host for *Cercopithifilaria* sp. I, as developing filarial larvae were detected in 10 (5%) out of 200 dissected nymphs that were previously fed on the infested dog. Third infective stage larvae were observed in 4 nymphs 30 days after their collection from the dog. Twelve out of 181 tick samples molecularly examined were positive for *Cercopithifilaria* sp. I, which corresponds to an overall infestation rate of 6.6%.

In the epidemiological survey the overall prevalence of *Cercopithifilaria* sp. I in sampled animals was 13.9% and 10.5% by microscopy of skin sediments and by PCR, respectively. The highest prevalence rate of infested animals was recorded in Spain either by microscopical examination of sediments (21.6%) or by molecular detection on skin samples (45.5%) whereas the lower positivity rate was in Greece (4.3%). In Italy, according to the region of sampling and to the diagnostic test employed the mean prevalence of *Cercopithifilaria* sp. I infestation in dogs varied from 10.9% in Apulia up to 19.5% in Sicily. Infestation rate as determined by tick dissection (from 5.2% to 16.7%) was higher than that detected by PCR (from 0% to 3.9%). Different developing stage larvae (i.e., L1, L2 and L3) were found in ticks with a maximum number of 1469 first stage larvae in a single tick.

CONCLUSIONS: Data here presented provide comprehensive evidence on the widespread distribution of *Cercopithifilaria* sp. I among dog populations in Mediterranean countries. *R. sanguineus* has been also identified as an intermediate host and putative vector of this filarioid confirming the strict association between the genus *Cercopithifilaria* and ixodid ticks. In addition to the most frequent species of filarioids known to infest dogs (i.e., *D. immitis*, *D. repens* and *A. reconditum*), *Cercopithifilaria* sp. I should also be considered especially in tick-exposed dogs. Further efforts should be undertaken to define the taxonomic identity of this filarioid and to elucidate its pathogenic role at local (dermal) and/or systemic level.

Myocardial damage in dogs affected by heartworm disease (*Dirofilaria immitis*): immunohistochemical study of cardiac myoglobin and troponin I in naturally-infected dogs

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AIM: It has recently been reported that dogs affected by canine heartworm disease (Dirofilaria immitis) can show an increase in plasma levels of myoglobin and Troponin I, two markers of muscle/myocardial injury. In order to determine if this increase is due to myocardial damage, the right ventricle of 24 naturally-infected dogs was examined by routine histology and immuno- histochemistry with anti-myoglobin and anti-Troponin I antibodies.

MATERIALS AND METHODS: Dogs from a local dog shelter in Las Palmas, Spain, were used in the study. The study was approved by the Ethics Committee of the Las Palmas de Gran Canaria University and was carried out in accordance to current European legislation on animal protection. Inclusion in the study was based on a positive result for circulating D. immitis antigens (PetChek1 HW PF, IDEXX Laboratories Inc, Westbrook, Maine, USA), elevated levels of plasma myoglobin and/or detectable plasma Troponin I levels, as measured according to Carreton et al. (2011 Vet. Parasitol. 176: 313-6). Recruited dogs were humanely euthanized. Hearts were examined for the presence of macroscopic lesions. Nematodes, when present, were counted and parasite loads were divided into "low" (≤ 10 worms) and "high" (≥ 10 worms). The myocardium was sampled in the same position for all dogs, i.e. along the midline of the right ventricle free wall. Samples were fixed in 10% buffered formalin until processing. Tissue sections were stained with haematoxylin/eosin for routine histological examination. For immuno-histochemistry, sections were incubated with an anti-human/canine myoglobin monoclonal antibody (product # M7773, Sigma-Aldrich, Madrid, SP) and an anti-human Troponin I polyclonal antibody (product #PA1-86820, Pierce Biotechnology, Rockford, IL., USA).

RESULTS: The main histological feature in myocardial tissue from the right ventricle was focal necrosis. Patches of hypereosinophilic, necrotic myocardium were frequently observed, even though they were not present in all tissue samples from all dogs. Occasionally, neutrophilic inflammatory infiltrates could also be observed. Where necrosis was present, there was a consistent loss of staining for myoglobin and Troponin I. There was no apparent association between worm burden and the presence of myocardial lesions.

CONCLUSIONS: The use of markers of myocardial injury is widely used in human medicine (Singh et al., 2010 Coron. Artery Dis. 21:244–256). Even though the presence of troponins in plasma is highly specific for injury to cardiac myocytes, myoglobin is less specific and can also indicate skeletal muscle damage. Carretòn et al. (2011 Vet. Parasitol. 176:313-6) reported that approximately 40% of dogs with naturally-acquired HWD showed plasma levels of circulating Troponin I, while approximately 20% had increased concentrations of circulating myoglobin. Here, we wanted to verify the cardiac origin of these two proteins in order to confirm their use during clinical workup in naturally-infected dogs. Interestingly, loss of staining for myoglobin and Troponin I was almost always associated with a necrotic lesion of the myocardium. This has also been reported by Fishbein et al (2003 Cardiovasc Pathol. 12:65-71) in a canine model of myocardial ischemia.

Acknowledgements: The results of the present study would confirm the use of this marker in the initial clinical evaluation and in the follow-up of dogs with CHWD.

Human leishmaniases in Liguria, northern Italy: parasitological features in the past 17 years

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AIM: Liguria is the only Region of northern Italy where elevated incidences of human and canine leishmaniasis are commonly recorded, being of similar magnitude of those from traditional foci of Tyrrhenian and Ionian southern regions. Disease clusters have long been reported from western Liguria (Gabutti G et al, 1998, Lancet 351: 1136) with seroprevalence in dogs ranging 22-30% (Zaffaroni E et al, 1999, Vet Parasitol, 81: 11-19). Because of its location at the France border and the historical tradition of international trade activities, Liguria is considered of particular interest for possible introduction of new species and/or genotypes of *Leishmania*. Hence, we have long undertaken collaborations with the main Departments of Infectious Diseases in the Region, aimed at case diagnosis and identification of agents responsible for human leishmaniases.

MATERIALS AND METHODS: Bone-marrow aspirate, peripheral blood or skin biopsy samples from patients referred to our Unit from 1995 to 2011 for diagnosis of suspected visceral (VL) or cutaneous leishmaniasis (CL), were evaluated. Diagnosis was confirmed by serological (IFAT), parasitological (Giemsa-stained smears and *in vitro* culture), and molecular methods. Further, parasites from positive cultures were identified by MLEE analysis of 13 isoenzymes (Gramiccia M, 2003, Ann Trop Med Parasitol, 97 (Suppl. 1): S65-S73) and by molecular methods. In particular, ribosomal ITS-1 nPCR-restriction fragment length polymorphism (RFLP) analysis was performed for *Leishmania* species identification (Schönian G et al, 2003, Diagn Microbiol Infect Dis, 47: 349–358). In addition, *Leishmania infantum* strains were genotyped by PCR-RFLP of different gene markers: kDNA minicircles (Laurent T et al, 2007, Infect Genet Evol 7: 206–212) and intragenic region of the *cpb* gene (Tintaja Q et al, 2004, J Inf Dis 189:1035–43).

RESULTS: In total, 133 patients were referred to the Unit of whom 80 were diagnosed as having leishmaniasis, 68 VL and 12 CL. There were 34 children aging 10 mo-15 yrs (42.5%) and 46 adults (17-75 yrs) (57.5%). VL occurred in 29 HIV co-infected adults, corresponding to 63% of all adult cases. On average, diagnosed cases were 5 per year, with a minimum of 1 case in 2004, and a maximum of 12 cases in 2009. Among 70 patients whose the residence was known, the majority were from the western provinces of Imperia (34 cases) and Savona (23 cases). Fifty-two *Leishmania* cultures (44 VL and 8 CL) were obtained and genotyped by ITS-1 nPCR-RFLP: 51 resulted to be *L. infantum* and one *L. major*. Eighteen strains were also typed by MLEE. Three zymodemes of *L. infantum* were recorded, MON-1 (the commonest *L. infantum* zymodeme in the Mediterranean) identified in 15 strains (14 VL and 1 CL), MON-34 (a dermotropic variant of MON-1 detected occasionally in Mediterranean) in one CL strain, and MON-72 (a viscerotropic variant detected so far only in Campania region) in one VL strain from a 3-yrs-old patient resident in Liguria region, for whom no travels in Campania were reported. The MLEE analysis of the *L. major* strain was consistent with the Tunisian origin of the imported CL case (MON-25). *L. infantum* strains genotyping patterns by *cpb* and kDNA PCR-RFLP are in course of analysis.

CONCLUSIONS: The leishmaniasis focus of Liguria seems to have been quite stable during the study period. The disease affected all age groups, but HIV co-infected adults and the paediatric population appears to be particularly vulnerable to the parasite. We investigated for possible introduction of new *Leishmania* species or genotypes in this area. MLEE findings did not indicate introductions of *L. infantum* zymodemes typical of neighbouring regions of France (e.g. MON-11 or MON-186). Molecular typing within the widespread MON-1 would confirm the autochthonous origin of cases. The detection of MON-72 in a child would suggest an introduction of this zymodeme in the region.

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New evidence on the distribution of *Dirofilaria* spp. in Southern Italy

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Due to their pathogenicity and zoonotic role, *Dirofilaria immitis* and *Dirofilaria repens* are the most important filarial species affecting dogs in Europe. *D. immitis* is mostly endemic in the north of Italy, where prevalence can be up to 80% in dogs and 20% in cats not subjected to preventive treatments (Genchi C et al, 1992, Proc. Heartworm Symposium, Batavia, IL, pp. 39–46.). Recent reports have suggested that the distribution of *D. immitis* throughout Italy is changing, due to several factors, including the progressive diffusion of *Aedes albopictus* from north to south (Otranto D et al, 2009, Parasit Vectors, 2: S2; Genchi C et al, 2011, Vet Parasitol, 176:295-299). For this reason, an update on the distribution of *Dirofilaria* spp. is pivotal to assessment of the risk of their spreading (Otranto D et al, 2009 o.c).

AIM: This survey aimed to acquire data on dirofilariosis in native privately owned dogs and cats living in Apulia region (province of Foggia) and on the role of *A. albopictus* as a vector of *Dirofilaria* spp. in the same area.

MATERIALS AND METHODS: From March 2011 to February 2012, individual blood samples were collected from 308 dogs and 12 cats referred to 15 different private veterinary practices. All samples were subjected to: a) Knott's modified method; b) detection of circulating *D. immitis* antigen; and c) duplex real-time PCR for the simultaneous detection of *D. immitis* and *D. repens*. Data regarding sex, age, size, breed, fur length, indoor/outdoor housing, use, day and night habitats, symptoms, and geographical origin were obtained and correlated with the prevalence of filariae by univariate statistical method (χ^2 test or Fisher's exact test). In addition, 175 specimens of *A. albopictus* were collected from the dogs' areas of origin; these specimens were then pooled (15 individuals/pool) and molecularly examined.

RESULTS: Seventeen dogs (5.52%) and one cat tested positive for *Dirofilaria* spp. in at least one diagnostic test. Specifically, *D. immitis* was detected in 11 dogs (3.57%), and *D. repens* in 6 (1.95%), while the cat scored positive for *D. immitis*. Two pools of *A. albopictus* tested positive for *D. immitis*.
It was found that guard-dogs were significantly more positive for *Dirofilaria* spp. (OR: 5.09, 95% C.I.: 1.28-17.24; χ^2:9.59, $p<0.05$), whereas dogs living in apartments were significantly less infected by *Dirofilaria* spp. (OR: 0.28, 95% C.I.: 0.07-0.95; $p<0.05$).
Evidence of possible cardio-pulmonary (i.e., cough, in the cat) and systemic signs of infection (i.e., weakness, weight loss, in 3 dogs) was found in subjects tested positive for *D. immitis*.

CONCLUSIONS: This is the first study carried out in Apulia region using both conventional and molecular assays to evaluate the occurrence of filariae affecting pet animals. The prevalence of *Dirofilaria* spp. detected in this study overlapped the values recorded in the same area by Puccini and Abbenante (1980, Atti SisVet, 34: 323), as well as a more recent survey in the other provinces of Apulia (Otranto D et al, 2009 o.c.). Living outdoors is an expected risk factor, being animals more exposed to mosquito bites. The detection of *D. immitis* in one cat and in *A. albopictus* is worth noting, as this is the first report in southern Italy. Although a wider survey is needed, these findings indicate that *D. immitis* has become endemic in southern Italy, likely favoured by the high density of *A. albopictus* in the investigated area, which has increased three times over the past 4 years (Giangaspero, unpublished data). Given the impact on animal and human health, veterinarians from southern Italy can no longer consider heartworm as an 'exotic' parasite, and this filarioid infestation should be included in routine diagnosis. Finally, it is essential to implement an effective surveillance system for *A. albopictus* throughout areas where it has previously been regarded as non-endemic.

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Differences between *Leishmania infantum* and *Leishmania* (*Viannia*) *brasiliensis* in three human clinical cases: a morphological approach by SEM and TEM

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AIM: Cutaneous leishmaniasis (CL) attends different therapeutic protocols by Old World leishmanias or New World leishmanias. Species diagnosis is very difficult and it is performed only in reference facilities. Studies about morphological patterns between Old World and New World leishmanias may be useful for a rapid and specific clinical approach.

MATERIALS AND METHODS: Promastigotes coming from three positive cultures of *Leishmania infantum* (one) and *L. (Viannia) brasiliensis* (two) were studied by SEM and TEM. Promastigotes were cultivated in NNN medium as for usual protocols. All these Leishmania strains were isolated by Italian human patients. All the promastigote suspensions were assayed by SEM and TEM by usual methods. For electron microscopy (EM), promastigotes were fixed in 2,5% glutaraldehyde and 0,1M cacodylate buffer ph7,2. For scanning EM, we used poly-L-lysine coverslips and dried samples were coated with gold-palladium in a AGAR-Autosputter Coater. For transmission EM, conventional methods were applied. The samples were observed using the LEICA S420 scanning electron microscope and ZEISS EM109 transmission electron microscope.

RESULTS: SEM observations reported specific differences in the outer shape of *L. infantum* and *L. (Viannia) brasiliensis* promastigotes (Fig 1, 2). *L. Viannia brasiliensis* had shorter body shape, with a length of 7 µm (range 5-8) and a width of 2 µm vs a length of 13 µm (range 10-16) and a width of 2 of *L. Leishmania infantum.* The flagellum of *Leishmania infantum* (11 µ, range 9 -16) was longer than *L. (Viannia) brasiliensis* (9 µ, range 6-11) but the flagellum/body odd of *L. (Viannia) brasiliensis* was larger than *L.Leishmania infantum* (1.3 vs 1.2). Ultrastructural examination (TEM) showed similar organelles and flagellar structures in *L. infantum* and *L. braziliensis* promastigotes.

CONCLUSIONS: These observations remark clear differences in the outer shape of the two species. The higher flagellum/body of *Viannia* induce a quicker movement more than Leishmania of New World. This characteristic is clearly detected at observation by Optical Microscopy (MO). All these observations may be useful for presumptive specie diagnosis of promastigotes in cultures of clinical specimens.

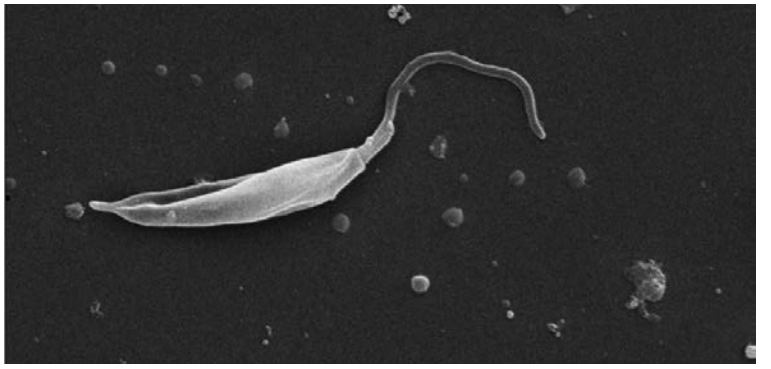

Fig. 1. *Leishmania infantum* promastigotes, 8000x

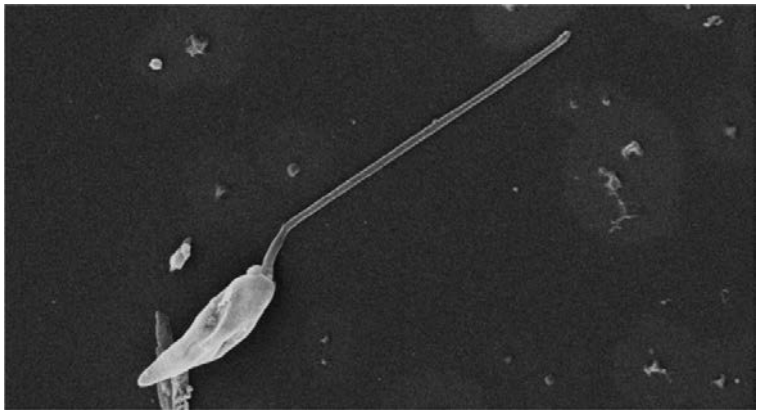

Fig. 2. *L. (Viannia) brasiliensis* promastigotes, 8000x

Cardiopulmonary parasites of red foxes and dogs from Liguria, north-western Italy

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AIM: Canine lungworms are often found in foxes throughout Europe and an increasing number of cases have been reported in dogs in the last decade (Taubert A et al, 2009, Vet Parasitol, 5: 175-180). The aim of our study was to provide epidemiological data on lungworms of foxes and dogs living in the same area, in order to investigate the potential transmission of pathogens between wild and domestic animals.

MATERIALS AND METHODS: From 2009 to 2012, we examined 130 foxes and 347 rural dogs from N-W Italy (Imperia and Savona districts). Sex, age, origin and dog attitude (hunting/kennel/pet) were registered. The cardiorespiratory system of foxes was examined by tracheal inspection, pulmonary smears and lungs and heart dissection; rectal faecal samples were subjected to centrifugal flotation (CF) with $ZnSO_4$ (s.g. 1.350). For each dog we collected faecal and blood samples: faecal samples were subjected to $ZnSO_4$ CF and to the Baermann technique (1-3 samples per dog). Larvae and eggs were identified by optical microscopy; furthermore some eggs of Trichuridae were examined by scanning electron microscopy (SEM). Blood samples were submitted to the Knott's modified test and to a serological exam for the detection of *Dirofilaria immitis* antigen (DiroCHEK®, Synbiotics). Microfilariae identification was confirmed by histochemical staining and PCR (Rishniw M et al, 2006, Vet Parasitol, 135: 303–314). Prevalence values with 95% confidence interval (CI) were calculated and, for adult worms found in foxes, also abundance, intensity and range.

RESULTS: Parasites found in foxes (with cardiopulmonary examination) and in dogs (with coprological and hematological analysis) and the corresponding epidemiological measures are shown in Table 1. Based on coprological analysis, *C. aerophila* was present in 27% of the foxes.

CONCLUSIONS: Our results show a high prevalence of lungworms in foxes and their presence in dogs in the study area. Regardless of the high prevalence of *A. vasorum* in foxes, only one case was found in dogs. This fact could be due to the low palatability of the intermediate hosts of *A. vasorum* for dogs and to diagnostic difficulties (Bolt G et al, 1994, Vet Rec 135: 447–452). On the contrary, capillariosis is diffused in both species, possibly because of the parasite direct cycle. Capillariosis is probably underestimated in pets, as eggs of Capillariid nematodes can be confused with eggs of *Trichuris vulpis* due to the similar morphology; by SEM we could observe the differences among *C. aerophila*, *C. boehmi* and *T. vulpis* egg shells. *D. immitis*, *D. repens* and *A. reconditum* were found in dogs in a region historically free from filariosis, with the exception of a few infections by not identified microfilariae (Pampiglione S, 1986, Parassitologia, 28, 297-300). The absence of filariosis in foxes confirms the hypothesis that foxes represent an epiphenomenon in the epidemiology of *D. immitis* (Stancampiano L et al, 1998, Parassitologia, 40: 171). Although lungworms diagnosis in dogs is uncommon, our data show they are present and should be considered as a differential diagnosis in animals with cardiopulmonary distress; furthermore their presence suggests the need for prophylaxis in dogs in the study area.

Table 1. Prevalence (P %) with 95% confidence interval (CI) of parasites from foxes and dogs living in the same area and, for adult worms from foxes, abundance (A), mean intensity (I) and range (R)

	Foxes					Dogs	
	P %	95% CI	A	I	R	P %	95% CI
Angiostrongylus vasorum	75	67-82	7,5	9.9	0-53	0.3	0-0.8
Capillaria aerophila	44	36-53	2,0	4.5	0-35	11	8-14
Crenosoma vulpis	13	7-18	0,7	5,6	0-55	0.3	0-0.8
Filaroides spp.	3	0-6	0,02	0,7	0-3	-	-
Capillaria boehmi	-	-	-	-	-	1.7	0.4-3
Dirofilaria immitis	-	-	-	-	-	1.7	0.4-3
Dirofilaria repens	-	-	-	-	-	1	0-2
Acentocheilonema reconditum	-	-	-	-	-	7	5-10

Molecular detection and differentiation between *Dirofilaria immitis* and *Dirofilaria repens* in mosquitoes using a duplex real-time PCR

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Dirofilaria immitis and *Dirofilaria repens* (Spirurida, Onchocercidae) are filarioids of animal and human concern (Genchi C et al, 2009, Vet Parasitol, 163: 286–292; Pampiglione S et al, 1995, Parassitologia, 37: 149–193; Otranto D, Eberhard ML, 2011, Parasit Vectors, 4: 41), transmitted by bloodsucking culicid mosquitoes belonging to the genera *Culex*, *Aedes*, *Anopheles* and *Mansonia* (Coluzzi M, 1964, Parassitologia, 4: 57-62; Sauerman DM Jr, 1985, Mosq News 43: 222-225; Cancrini G et al, 1995, Parassitologia, 37: 141-145; Cancrini G et al, 2003, Vet Parasitol, 118: 195–202). The detection of *Dirofilaria* spp. in mosquitoes by insect dissection is time-consuming and hardly to be applied in large epidemiological surveys (McCall JW et al, 2008, Adv Parasitol, 66: 193–285). Over the last decades, several PCR-based assays have been shown to provide rapid, sensitive, and species-specific methods for the detection and delineation of *D. immitis* and *D. repens* DNA in invertebrate hosts (Favia G et al, 1996, Parasitology, 113: 567–571; Cancrini G et al, 2007, J Med Entomol, 44: 1064–1066; Thanchomnang T et al, 2010, Vet Parasitol, 168: 255–260; Latrofa MS et al, 2012, Vet Parasitol,185: 181–185). Nevertheless, these methods were not used on large scale.

AIM: This study evaluated a recently developed duplex real-time PCR assay (Latrofa MS et al, 2012, Vet Parasitol,185: 181–185) for screening large number of mosquitoes for *D. immitis* and *D. repens.*

MATERIAL AND METHODS: From May to October 2010, 43 carbon dioxide-baited traps were placed in 20 sites allocated in six provinces of Veneto region (north-eastern Italy). All mosquito specimens collected (n = 40,892) were maintained refrigerated until being counted and identified using standard morphological keys (Severini F et al, 2009, Fragmenta Entomologica, 41: 213–372). Females were grouped in 955 pools (from minimum 1 to maximum 57) and subjected to molecular analysis by real-time PCR using two species-specific primer sets targeting on partial cytochrome *c* oxidase 1 (*cox*1) mitochondrial DNA and on second internal transcribed spacer (ITS-2) of nuclear ribosomal DNA (Latrofa MS et al, 2012, Vet Parasitol,185: 181–185). The specificity of the duplex real-time PCR assay was established by melting-curve analysis.

RESULTS: Mosquitoes were identified as *Culex pipiens* (n = 37,865; 92.6%), *Ochlerotatus caspius* (n = 2,264; 5.5%), *Aedes vexans* (n = 720; 1.8%) and *Culex modestus* (n = 43; 0.1%). Out of 955 pools, 23 (2.41%) were positive for *Dirofilaria* spp. of which, 21 (2.2%) for *D. immitis* and two (0.21%) for *D. repens*. An overall estimated rate of infection (ERI) of 0.06% was recorded being higher in *Och. caspius* and *Ae. vexans* (i.e., 0.18% and 0.14%, respectively). At least one mosquito pool was positive for *Dirofilaria* spp. in each province with the highest ERI being recorded in Vicenza and Padova provinces (i.e., 0.42 and 0.16%, respectively). Mosquitoes collected in all provinces were positive for *D. immitis* whereas only two (i.e., Padova and Rovigo provinces) resulted positive for *D. repens*. All mosquito species, but *Cx. modestus*, were positive for *D. immitis*, whereas *D. repens* was only found in *Cx. pipiens*.

CONCLUSIONS: The duplex real-time PCR assay herein used showed a high specificity for the detection and delineation between *D. immitis* and *D. repens* in naturally infested mosquitoes, representing an alternative to microscopic investigation, particularly for large-scale screenings and surveillance programmes. This assay might be proposed as a tool for the epidemiological surveillance of these *Dirofilaria* species, mainly in areas where these species are endemic and/or occur in sympatry (Cancrini G et al, 2003, Vet Parasitol, 118: 195–202; Otranto D, Dantas-Torres F, 2010, Parasit Vectors, 3: 2).

Leishmania infantum Iron Superoxide Dismutase purification and its use in the diagnosis of Canine Leishmaniasis in Andalucia (Spain) and Lombardia (Italy)

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AIM: *Leishmania* spp. are digenetic parasites whose progression into the host occurs inside resident professional macrophages of the immune system. Promastigotes released during an infected sandfly bite are phagocytosed by macrophages; similarly, amastigotes released by host macrophage are rapidly re-phagocytosed by new one. After macrophages activation several reactive oxygen species (ROS) are formed, including superoxide radicals ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2), and reactive nitrogen species (RNS), including nitric oxide (NO•) (Serarslan G, 2005, Clinic Exp Dermat, 30(3), 267-71). Superoxide dismutases (SODs) have an important role in the defence from superoxide radicals to protect normal cells as well as a number of pathogens from ROS (Fridovich I, 1989, J BiolChem;264:7761–4; McCord JM, Fridovich I.,1998, Free RadicBiol Med;5:363–9; Bannister JV, 1987, Crit Rev Biochem; 22:111–80). SODs have been classified into three types: copper-zinc (Cu-Zn-SOD), manganese SODs (Mn-SOD), both present in most prokaryotes and eukaryotes, and iron SOD (Fe-SOD), detected in some prokaryotes, protozoans and chloroplasts of plants and algae. $O_2^{\bullet-}$ is produced by macrophages NADPHox during phagocytosis. Trypanosomatids Fe-SOD dismute it in H_2O_2 and O_2 by both parasites forms, promastigotes and amastigotes. H_2O_2 is convert in H_2O by the parasites antioxidant defence: $T(SH)_2$ (Reductase Trypanothione), TXN (Trypanoredoxin), PRX (Peroxiredoxin) and APX (Ascorbate Peroxidase) (Van Assche, T, 2011, Free RadicBiol Med, 51(2), 337-51.; Piacenza, L, 2007, Biochem J, 403(2), 323-34). The parasites Fe-SOD play a very important role in the infection and establishing of the disease. Its high immunogenicity make it a useful molecular marker in diagnosing trypanosomatids infection (Marín C, 2004, Parasitology 129: 79-86; Mateo H, 2010, Clin Biochem. 43(15):1257-64). In this study we purified a Fe-SODe (excreted) by promastigotes of *Leishmania infantum* in stationary phase and applied to the diagnosis of canine Leishmaniasis.

MATERIALS AND METHODS: After incubating in the medium without FBS for 24h at 26° C the cells were harvested and the supernatant collected and precipitated with 35% and 85% Ammonium Sulphate. The pellet resulting from this precipitation is applied to a QAE-Sephadex A-50 (Ion Exchange Chromatography) and the Fe-SODe peak is recollected and applied to a QAE-Sephadex G-100 (Molecular Weight Chromatography). The Fe-SODe peak, in both chromatography, was identified using the Bradford protein determination method (Sigma Inmunochemical, St. Louis) and Fridovich method for the SOD activity determination (Beyer WT Jr and Fridovich I, 1987, 26:1251-1257). The pI and MW were determinated by a polyacrylamide PhastGel IEF 3-9 and by native gel electrophoresis in a PhastGel Homogeneous 12,5% geles stained with silver staining solution and the Fe-SODe activity was revealed as described above. The efficacy of Fe-SODe purified was testing in 155 dogs sera, 1/80 dilution, from Andalucía (Spain) and Lombardia (Italy), by ELISA and Western Blot techniques comparing with the whole soluble extract (H) of promastigotes and Fe-SODe not purified.

RESULTS: 155 dogs sera were tested by ELISA using as antigens H (9.67% positives), Fe-SODe not purified (27.74% positives), and Purified Fe-SODe (46.45%). Western blot was carried on with Fe-SODe not purified as antigens (22% positives) and with Fe-SODe purified (46.45% positives).

CONCLUSIONS: H is not a good molecular marker giving a 57 false negatives. Fe-SODe not purified is better molecular marker but is not 100% reliable giving in ELISA and Western blot techniques, respectively 29 and 38 false negatives/positives. Fe-SODe purified show the same results in both techniques, 72 positives sera. Many study had demonstrated that Fe-SODe is a very useful antigens for the detection of cutaneous, mucocutaneous and visceral Leishmania and no present cross reaction with other protozoa like *T. cruzi* (Longoni SS, 2011, Vector Borne Zoonotic Dis 11(7):815-21; Marín C, 2009, Am J Trop Med Hyg;80(1):55-60). We demonstrate that Fe-SODe purified appears to provide good sensitivity as molecular marker. In accordance with previous study, serological assay performed with Fe-SODe purified is the better choice for canine as well as human Leishmaniasis diagnostic.

Development and clinical trial of a DNA vaccine as immunotherapy during Leishmaniasis

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AIM: Visceral leishmaniasis (VL) is a zoonotic disease caused by *Leishmania infantum* in the Mediterranean area and by *Leishmania chagasi* in Latin America and China. VL is an opportunistic infection in immunocompromised and/or human immunodeficiency virus-positive patients. It is estimated that 350 million people are at risk in 88 countries, with a global incidence of 1–1.5 million cases of cutaneous and 500,000 cases of visceral leishmaniasis.
Dogs are the main reservoir of *Leishmania infantum* parasites. Disease management represents a serious problem, since anti-leishmania drugs have limited efficacy in both symptomatic and asymptomatic dogs, which are infective to phlebotomine vectors. In many tropical and sub-tropical countries the development of a safe and easily-available vaccine has high priority. DNA vaccines represent one of the most recent innovations in the field of immunization. A DNA vaccine typically consists of a foreign gene, encoding a protein antigen of interest, cloned into a bacterial plasmid that can be injected. This study aimed to evaluate the effect of a DNA vaccine based on two *Leishmania* antigens in leishmaniotic dogs.

MATERIALS AND METHODS: The coding sequences of the two *Leishmania* antigens were cloned into the pVAX-1 vector. To provide an enhanced immunological response, the proteins were linked together with a sequence encoding a glycine bridge. Twelve leishmaniotic dogs from a leishmaniasis-endemic area (Naples, Italy) received three consecutive injections of DNA vaccine at 15-days intervals (vaccine group). Another group of five leishmaniotic dogs received the same amount of pVAX-1 without the coding sequences of *Leishmania* antigens (control group). *Leishmania* DNA load, IFAT, INFγ, TNFα, IL-4 mRNA expression levels and clinical parameters were tested before and after the therapy, every 3 months for a period of 12 months.

RESULTS: Analysis of the data in the vaccinated dogs showed: i) a decrease *Leishmania* DNA load in lymph node samples, ii) an increase of INF mRNA expression levels in PBMC samples, iii) a decrease of the IFAT title. All vaccinated dogs also showed an improvement in the clinical symptoms. Approximately, 10 - 12 months after the first vaccination, three dogs showed the reappearance of clinical symptoms, and 6 months after the first vaccination six dogs showed a progressive increase of *Leishmania* DNA load in lymph node samples.

CONCLUSIONS: Our results show that the vaccine developed in this study may represent a useful tool in the treatment of leishmaniotic dogs. However, further trials are needed to evaluate the effectiveness of immunotherapy alone or in association with conventional therapy.

Dermal distribution pattern of *Cercopithifilaria* sp. microfilariae in dogs

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AIM: Among filarioids affecting dogs, those presenting dermal microfilariae (e.g., *Onchocerca lupi* and *Cercopithifilaria* sp.) have been neglected for long time also due to the difficulties in their diagnosis, which is achieved by recovering microfilariae from skin snip samples. These parasites are largely unknown to the scientific community and their relevance in veterinary medicine is considered minimal. Microfilariae of *Cercopithifilaria* sp. (from here referred to as *Cercopithifilaria* sp. I) have been recently described and molecular characterized in a dog from Sicily (Otranto D et al, 2011, Vet Parasitol, 182: 221-229) and, afterwards, reported to infest dogs from Spain, Greece and many southern Italian regions (Otranto D et al, 2012, Parasit Vectors, 5: 1). This large distribution has been associated with the occurrence of *Rhipicephalus sanguineus*, which is considered the main competent vector of this nematode (Brianti E et al, 2012, Vet Parasitol, 183: 330-337). However, little is known about the anatomical distribution of microfilariae of *Cercopithifilaria* sp. I in the host body as well as their localization at dermal level. Hence, the aim of this study was to investigate the anatomical distribution of microfilariae of *Cercopithifilaria* sp. I in dogs, instrumentally to strive its diagnosis on skin sample collected from specific body regions.

MATERIALS AND METHODS: Twenty dogs (previously tested for *Cercopithifilaria* sp. I) of different age, sex and weight were sampled from two regions (ten animals for each site) of southern Italy (i.e., Apulia and Sicily). Sampled dogs were naturally exposed to *R. sanguineus* infestation during the previous spring and summer. Skin samples were collected using individual 3mm diameter biopsy punches from eight anatomical sites (i.e., head right and left, inter scapular region, rump, armpit right and left, thigh right and left) and soaked in saline solution. Skin sediments were individually observed under light microscopy (i.e., two fields of 18x18 mm coverslip each) and microfilariae found were counted and morphologically identified. All skins samples were also molecularly processed for specific amplification targeting *Cercopithifilaria* sp. I.

RESULTS: One out of the 20 examined dog tested negative for *Cercopithifilaria* sp. I. At sediment examinations, microfilariae of *Cercopithifilaria* sp. I were retrieved in 16 (84.21%) out of the 19 positive dogs with a number of positive anatomical sites from 1 up to 7 and a mean of 3 sites per dog. Up to 48 microfilariae were counted in the sediment of a single anatomical site (i.e., head left) with an abundance per individual animal ranging from 1 to 95. Larval abundance in dogs was positively correlated with the number of positive anatomical sites ($R_s = 0.943$; $p < 0.0001$). Microfilariae were unevenly distributed on the body, with higher frequencies on inter scapular region (n=13; 68.4%) and on the head (n=9; 47.4%) and lower on rump, armpits and flat thigh. Based on the interpolation of frequencies observed and median values of microfilariae abundance in the eight anatomical sites, the chance for detecting microfilariae in skin samples was higher in and around inter scapular regions and head and lower in the other anatomical regions. Molecular analysis of skin samples showed lower frequencies of positive results when compared to the microscopic examination either by dogs or by anatomical sites, except for three dogs that scored positive only at the molecular diagnosis.

CONCLUSIONS: Larvae of *Cercopithifilaria* sp. I are distributed unevenly on the superficial dermal tissues of infected dogs, being mostly present on the head, ears and neck regions overlapping some of the same sites where *R. sanguineus* ticks feed most frequently. This might also account for by the fact that these regions are also those with a higher microfilariae abundance, more likely as a consequence of the longer time of tick feeding and, hence, opportunity for nematode transmission. Based on the results of microscopic examination of first and second sediments, the first assessment showed to be the most suitable for diagnosing dermal microfilariae. Results of this study add further information on the biology of this little known filarioid of dogs and provides useful information to enhance the diagnosis likelihood of *Cercopithifilaria* sp. I infection in dogs, by sampling specific body regions (i.e., head and inter scapular region).

Evidence of the efficacy of the mass use of preventive measures in the control of a recently established focus of Canine Leishmaniosis in north-eastern Italy

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AIM: The northward spreading of Canine Leishmaniosis (CanL) is well documented in Italy (Maroli et al, 2008, Med Vet Entomol, 15: 358-363), but field experiences aimed to control the newly established autochthonous foci are still scarcely documented.
In north-eastern Italy, many new foci of CanL were described since 1994. A small focus was first identified in 2005 and then described in Calaone locality (Baone Municipality), in the southern part of the Colli Euganei, an isolated hilly area in the central part of the Veneto Region. After the identification of the focus of CanL, local health authorities strongly invited dog owners to use preventive measures (deltamethrin-impregnated collars and imidacloprid 10% and permethrin 50% in spot-on formulation). The aim of the study is to describe the focus and to present the preliminary results of the evaluation of preventive measures in order to control and possibly eradicate the parasite.

MATERIALS AND METHODS: Dog owners of the southern part of Colli Euganei were invited to test their animal during three one-day sampling campaigns, organized on free basis in the late spring period. Totally 245 dogs were sampled in 2006 (44 in Calaone), 230 in 2007 (42 in Calaone) and 79 in 2010 (62 in Calaone). All sera were screened using an IFAT, according to OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Differences in seroprevalence values among the 3 Municipalities of the area (Baone, Arquà Petrarca, Cinto Euganeo) were investigated considering all tested dog population (n=501; dog tested more times were considered positive also if only one sample resulted positive), in order to establish the geographical spread of the infection. In 2010, a new specific questionnaire was designed to verify if the preventive measures promoted in Calaone area have been used. Considering dogs living in Calaone, differences in seroprevalence values among years (2006, 2007, 2010) and, in the year 2010 (n=62), among age classes (0-4; 5-7; >7 years) were investigated using a Pearson chi-square test.

RESULTS: Overall, seroprevalence was 9.6% (48/501), with Baone Municipality (14.1%) significantly higher than others ($p<0,001$). Besides, nearly all positive animals of Baone were found in Calaone village. Dog owners of this village reported to have started using preventive measures (collars, spot-on or both) since the year 2006 (65.9%) and for all the subsequent years (90.0% in 2007, 91.1% in 2008 and 88.9% in 2009). The seroprevalence of Calaone dog population clearly shows a decreasing trend (Fig. 1). In 2010, the age class 0-4 presented a value (4.2%) significantly lower ($p<0.05$) than 5-7 age class (32.0%) and >7 age class (28.6%).

CONCLUSIONS: The monitoring activity conducted during the 2006-2010 period in the Colli Euganei area clearly demonstrated that the CanL infection is limited to Calaone village. The results of the epidemiological study performed in this village are still preliminary and strongly affected by the limited size of the dog population, but suggest that dog owners were highly sensitised on the disease and on the need to implement appropriate measures to control the infection. The massive use of collars and spot-on seems to act positively, considering the decreasing trend of the seroprevalence and the nearly absence of new cases among young animals. This trend has to be confirmed by further serological survey (one is planned for 2013) and the level of sensitization of dog owners has to be maintained high.

Updates on canine filariosis in Sardinia

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AIM: The aim of the present study was to update the prevalence of Dirofilariosis infection in dogs and to examine the relationship between host factors (gender, age and breed) in Sardinia.

MATERIALS AND METHODS: From January 2010 to April 2012, a total of 591 dogs of various breeds coming from all Sardinia provinces were examined with Knott's technique to determine prevalence of microfilaremia. Dogs were stratified per gender, age (<3 years, 3 - 6 years, > 6 years) and attitude/use (hunting, pet, watchdog). The identification of microfilariae was carried out according to morphometric keys of Euzeby J, 1981 (Diagnostic Experimental des Helminthoses animales. Livre1, Edition Information Tecniques des services Vétérinaires, Paris).
Pearson's chi-square (χ^2) test was performed to compare prevalence among sex, age, and attitude/use categories. Statistical comparisons were carried out using EpiInfo ver 6.4 statistical software.

RESULTS: The presence of microfilariae was found in 20.8% of examined dogs (n.123). These belonged to the species *Acanthocheilonema* (sin. *Dipetalonema*) *reconditum* (14%; n.83), *Dirofilaria immitis* (8.3%; n.49) and *D. repens* (8.3%; n.49). Plurispecific infections were observed in 35 animals (5.9%) (n.10 *D. immitis +D.repens*, 1.7%; n.12 *D. immitis + A. reconditum,* 2%; n.13 *D. immitis + D.repens + A. reconditum,* 2.2%). The microfilaremia was detected mainly in males (23% - 73/317) compared to females (18,2% - 50/274) however, this difference is not significant ($\chi^2 = 2.04$; P= 0.153); On the other hand it was significant the difference between between males and females for *A. reconditum* (17% *vs* 10.6% - $\chi^2 = 5.07$; P< 0,024). In dogs that live mostly in apartments or in the countryside microfilaremia was respectively 6.3% (4/64) and 23.8% (101/424) (Yates corrected $\chi^2 = 9.90$, P = 0.002). Five-hundred and seventy dogs of known age, were stratified into three age groups and prevalences for the different species of microfilariae calculated and reported in the following table:

Age groups	Examined dogs	Microfilarie n. (%)	D. immitis n. (%)	D. repens n. (%)	A. reconditum n. (%)
0.5-3 years	258	51 (19.8%)	19 (7.4%)	18 (7%)	39 (15.1%)
>3-6 years	163	39 (23.9%)	12 (7.4%)	17 (10.4%)	23 (14.1%)
>6 years	149	29 (19.5%)	14 (9.4%)	10 (6.7%)	20 (13.4%)
Significance P		>0.05	>0.05	>0.05	>0.05

Among 295 dogs with different aptitude we have calculated the prevalences for the different species of microfilariae and the associated values of odds ratio (OR), that were listed in the table below:

Category	Examined dogs	D. immitis n. (%)	D. repens n. (%)	A. reconditum n. (%)
Hunting	200	15 (7.5%) - 1.00	15 (7.5%) - 1.00	46 (23%) - 1.00
Pet	80	7 (8.8%) - 1.18	4 (5%) - 0.65	3 (3.8%) - 0.13
Watchdog	15	4 (26.7%) - 4.48	5 (33.3%) - 6.17	4 (26.7%) - 1.22
P value		>0.060	0>0.063	0>0.022

CONCLUSIONS: The survey confirms the presence in Sardinia of *A. reconditum, D. immitis* and *D. repens*, as in the period 1998-2003 (Scala et al, 2004, Atti SISVet, 58: 120-121), although the prevalence of microfilaremia has undergone a highly significant increase (20.8% *vs* 12.3% - $\chi^2 = 12.78$; P = 0.0003), as the species found have almost doubled their rates of prevalence (14% *vs A. reconditum* 7.4%; *D. immitis* 8.3% *vs* 3.8%; *D. repens* 8.3% *vs* 2.6%). This scenario requires greater attention for the implementation of pharmacological prophylaxis. No significant difference was highlighted in this survey instead of the dogs in the three age groups examined, while it is evident as the outdoor life of the dog is still a major risk factor for the disease.

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Nested-PCR conjunctival swab as a diagnostic tool for epidemiological surveys on feline leishmaniasis

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AIM: Dogs are the domestic reservoir of zoonotic visceral leishmaniasis (ZVL) caused by *Leishmania infantum*, however the role of other hosts in the parasite life cycle cannot be ruled out (Quinnell RJ and Courtenay O, 2009, Parasitology, 136: 1915-1934). Cats may suffer from feline leishmaniasis (FL), a syndrome which appears less frequent and severe than canine leishmaniasis (CanL). Several FL seroprevalence surveys performed in Mediterranean countries have lead to propose cats as secondary hosts for ZVL (for a review, see Gramiccia M, 2011, Vet Parasitol, 181: 23-30). In general, cats exhibit faint antibody responses consistently lower than in dogs affected by CanL, which does not help to elucidate the complexity of the disease. Therefore more accurate and reliable approaches are necessary to estimate FL infection prevalences. The present work aimed to investigate on the spreading of *L. infantum* infection in a feline population resident in a CanL endemic focus of Central Italy, by using a molecular approach applied to conjunctival swabs (CS), a low-invasive sample successfully proposed for CanL diagnosis (Di Muccio T et al., 2012. J Clin Microbiol, in press).

MATERIALS AND METHODS: From April through August 2010, 98 stray cats of both genders, aging 6 months-14 years, were randomly selected from an area of the Arezzo Province (Tuscany) recently reported at medium-high risk for CanL (10-20% seroprevalence) (Franco AO et al., 2011, Parasitology, 138: 1878-1891). Cats were examined for signs suggestive for FL, and peripheral blood and CS samples were obtained during the sterilization/castration sessions of the regional straying's program. Sera were analysed for anti-*Leishmania* IgG antibodies by IFAT at the cut-off dilution of 1/20 (Mancianti F, 2004, Parassitologia, 46: 203-206). Total genomic DNA extracted from buffy coat (BC) and CS eluted material was submitted to a nested (n)-PCR assay (Gramiccia M *et al*, 2010, Vet Parasitol, 181: 23-30). Cross-sectional prevalence of *Leishmania* infections was calculated as the rate of animals positive to each test; the non parametric McNemar test was used to evaluate differences between CS (n)-PCR and IFAT, and K statistic for their agreement.

RESULTS: No cats showed clinical signs of FL. IFAT revealed anti-*Leishmania* antibodies in 48 of them (49.0%) at titers ranging from 1/20 to 1/160. Thirty-eight cats were found positive to CS n-PCR (38.8%), whereas none of the animals was positive by BC n-PCR. Considering results obtained by IFAT and CS (n)-PCR, the rate of *Leishmania* positives to at least one test was 68.4%, and that of positives by both tests 19.4%. CS (n)-PCR positives only were 19.4%, and IFAT-positive only 29.6%, with slight agreement between the 2 tests (K=0.14).

CONCLUSIONS: Results of the serosurvey showed an elevated prevalence of *Leishmania*-specific antibodies in the investigated cats, with a rate (49.0%) much higher than the estimated CanL seroprevalence for the study area (Franco AO et al, 2011, ibid), and even higher than seroprevalence rates for CanL recorded in the most endemic areas of southern Italy (40.4%) (Maroli M et al, 2001, Med Vet Entomol, 15: 358-363). Our results also pointed out the limited diagnostic value of BC n-PCR, as observed previously (Fiorentino E et al., 2008, Parassitologia, 50 (Suppl. 1-2): 159). CS n-PCR findings also indicated an elevated level of exposure to *Leishmania,* although not concordant with serological findings. As to this last point, it should be noted that the survey period including the transmission season could reveal *Leishmania*/cat contacts that may not reflect a true prevalence of established infections. The absence of suggestive FL clinical signs confirms the low clinical susceptibility of cats to *Leishmania* infections.

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SESSIONE 12

EPIDEMIOLOGIA DELLE MALATTIE PARASSITARIE DELL'UOMO

Intestinal parasites impair immigrant children's growth

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Parasitic infections in children are an important public health issue, particularly in developing countries where social and economic deprivation, poor hygienic conditions and warm climates favour the spread of intestinal parasites. Chronic malnutrition makes children more vulnerable to intestinal parasites, and this in turn leads to poor nutritional status, creating a synergistic relation which impairs growth (Quihui-Cota L et al, 2004, Trans Royal Trop Med Hyg, 98: 653-659; Yap P et al, 2012, Parasit Vectors, 5: 50). In industrialized countries, intestinal parasitosis combined with malnutrition is limited to those living in disadvantaged and marginal situations, i.e. immigrants or nomads in precarious sanitary conditions (Gutiérrez-Cisneros MJ et al, 2010, Ann. Trop. Med. Parasitol, 104: 145-149).

AIM: There is a lack of information about European countries, and so the aims of the present study were to evaluate the correlation between parasitic infections and nutritional status in immigrant children. We also investigated the risk factors linked to endoparasites.

MATERIALS AND METHODS: The study was carried out at the Poliambulatorio della Medicina Solidale e delle Migrazioni (PMSM) in Rome, which offers free health care to immigrants and/or deprived people. Between January 2008 and September 2010, we examined 247 children (aged 0 to 15) and collected their medical and personal histories. Anthropometric analysis and parasitological tests were perfomed on each child. We used the following nutritional indexes as standard reference values of growth: height-for-age (HA); weight-for-age (WA) expressed in terms of a "z score" for children up to 10 years of age (WHO 1986); only height-for-age (HA) for children aged 10 to 15 (WHO 1995). Nutritional indicators were calculated using "WHO Anthro" PC Software, version 2. Parents were given sterile containers to collect stool samples from the children on three consecutive days; these samples were then delivered to PMSM. Fecal samples were examined using standard copro-parasitological analysis methods, including wet mount Lugol iodine staining and formol ethyl-acetate concentration techniques. The samples were stained by the Ziehl-Neelsen modified technique for *Cryptosporidium* oocyst detection, and confirmation of *Cryptosporidium* and *Giardia* was obtained using an immunofluorescence test (Kit MERIFLUOR® *Cryptosporidium/Giardia*, Meridian Diagnostic, Cincinnati, OH, USA). A Scotch test for detection of *Enterobius vermicularis* was performed only when specific symptoms were present. The parasite prevalence differences in relation to HA and WA were tested by χ^2 or Fisher exact tests. The other epidemiological variables were offered to binary logistic regression models, in order to evaluate possible risk factors associated with positivity.

RESULTS: Thirty-seven children (15%) resulted positive for protozoan oocysts (i.e., *Blastocystis hominis, Entamoeba coli* and *Giardia duodenalis*) or helminths (i.e., *Enterobius vermicularis, Ascaris lumbricoides* and *Strongyloides stercoralis*) with a monospecific (81%) or multiple infection (19%).Nutritional status evaluation revealed that none of the parasitized children was suffering from acute malnutrition (W/A); 2 (5.4%) were overweight and 17 (46%) were significantly affected by chronic malnutrition (H/A) ($p<0.01$). Children classified as stunted were parasitized by one or more parasites, belonging to both nonpathogenetic (*Entamoeba coli*), and pathogenetic species (*B. hominis, G. duodenalis, S. stercoralis, E. vermicularis, A. lumbricoides*). Accordingly, children classified in the lower height z-scores presented a significantly higher prevalence of parasites (30.9%) than the others ($p<0.001$). The highest risk factors were living in shanties and cohabitation with other families ($p<0.01$), whereas less relevant risk factors were increasing age and cohabitation with other people.
The frequency and severity of parasitic diseases is a significant factor in limiting children's growth, mainly in developing countries (Casapia M et al, 2006, Int J Parasitol, 36: 741-747).

CONCLUSIONS: This study suggests that the association between parasite infection and stunted growth also occurs in developed countries, and in particular in immigrant communities in Italy. There is an urgent need for extensive improvements of the social and economic conditions of immigrants in order to overcome overt discrimination.

Factors associated with *Giardia duodenalis* prevalence in a rural area of Grand Bassam Department of Côte d'Ivoire

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Giardia duodenalis is a widespread parasite causing diarrhoeal diseases in developed and developing countries. Different researches from developing countries evidenced for an association between the occurrence of intestinal parasites and different socio–economic factors (Basualdo JA et al, 2007, Rev Inst Med Trop, 49 : 251-255; Nyarango RM et al, 2008, BMC Pub Health, 8: 237).

AIM: To contribute to the knowledge of the role of environment and social conditions on the occurrence of giardiasis, people living in an area of the South of Côte d'Ivoire, where giardiasis is highly endemic among children (Berrilli F et al., 2012, Trans R Soc Trop Med Hyg,106: 191-195), was analysed for the presence of *G.duodenalis*.

MATERIALS AND METHODS: During five-cluster samplings from August 2008 to May 2010, 343 stool samples were collected from symptomatic and asymptomatic persons aged <20 years from 6 rural villages (37 samples were from Assouindè, 81 from Imperiè, 31 from Kimoukro, 37 from Beniakrè, 14 from Tchenchebè, 11 from Wehou and 132 from Bonoua, a semi-urban village). Anamnestic data were acquired from all investigated people and informed verbal consent was obtained from children's parents or tutors and from adult patients. All investigations were performed in accordance with the principles of the Declaration of Helsinki.

The analysis for *G.duodenalis* presence was performed by Lugol's iodine staining method at the Don Orione Center Laboratory in Bonoua.

For the evaluation of risk factors the following environmental and social village's conditions were considered: housing settings (bricks, mud/bamboos houses), sanitation infrastructure (cohort masonry latrines, toilets, open yards), water supply sources (public water delivery system, wells, rivers/ponds), education (children attending or not primary school), distance from healthcare facility. Seasonality of infection was also investigated. For statistical analysis data were collected and analyzed using the SPSS statistical package (SPSS Inc., Chicago, IL, USA). Differences in proportions were determined using the Pearson Chi square test or Fisher's exact test (if cells<5).

RESULTS: Out of 343 investigated people 21.6% were infected by *G. duodenalis* with differences among the villages (Assouindè 16.2%, Bonoua 11.4%, Imperiè 23.5%, Kimoukro 41.9%, Tchenchebè 42.9%, Beniakrè 32.4%, Wehou 27.3%). People living in Bonoua village had the lowest probability to acquire giardiasis. The prevalence of *G. duodenalis* was statistically higher among people living in Kimokro, Tchentchebe, Beniakrè e Weho villages, with a relative risk (vs Bonoua) of 2.4-3.8 while people living in Assouindè and Imperiè have a relative risk (vs Bonoua) of 1.4-2.1. Thus, the prevalence of giardiasis decreased in villages with better water delivery system and sanitation ($p<0.001$), higher percentage of primary schools ($p<0.001$) and lower distance of households to the road and dispensaries ($p<0.05$). Then, a lower prevalence was observed in the small rainy season compared to the heavy rainy season ($p<0.05$).

CONCLUSIONS: This study provides useful information on the factors favouring the persistence and spreading of giardiasis in South of Côte d'Ivoire. Statistical analysis confirms that improving infrastructures and education as well as increasing healthcare facilities reduce the risk of giardiasis. In this view, due to its basic low-cost diagnosis, *G.duodenalis* can be considered a good indicator for measuring the likelihood of transmission of intestinal parasitic infection in developing countries. Interdisciplinary research is needed in order to provide integrated and appropriate solutions to prevent exposure to parasites and vulnerability to neglected diseases, among people living in disadvantaged countries.

Association of Matrix Metalloproteinase (MMP)-2 and MMP-9 activity in neurocysticercosis

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AIM: Matrix metalloproteinase (MMP)-2 (MMP-2, gelatinase A) and 9 (MMP-9, gelatinase B) are endopeptidases of the MMP family produced by a variety of cells including macrophages and monocytes, that take part in the degradation of extracellular matrix (ECM) and basement membranes. These enzymes are involved in a wide spectrum of both physiological and pathological processes as well as in disruption/proteolysis of blood brain barrier during central nervous system (CNS) infections. Neurocysticercosis (NCC) is a parasitic disease of the CNS caused by the larval stage of *Taenia solium* tapeworm, (identified as the most frequent cause of acquired epilepsy, at global level) whose severity of the symptoms depends on several factors including the degree of inflammatory reaction in the host brain. This study was aimed to detect the levels of MMP-2 and MMP-9 in the serum of patients with NCC (and to investigate the relationship between the levels of MMPs and clinical out come of the disease, i.e. in symptomatic and asymptomatic individuals).

MATERIALS AND METHODS: Sera from individuals from three South American countries, India and Italy, aged 4-78 years, with diagnosed NCC (n=17) and healthy controls (n= 10) were collected. All sera were tested for antibodies specific for *T. solium* with a commercially available western blot kit. For the detection of MMP-2 and MMP-9 activities, gelatin zymography was performed. Serum samples, diluted in SDS buffer (2x) were separated under non-reducing conditions in 10% SDS-PAGE gels polymerized with 1mg/ml porcine gelatin (Sigma, Milan, Italy) using the Mini-PROTEAN™ III system (Bio-Rad Las, CA, USA). Gels were washed twice for 30 min in 2.5% Triton X-100, incubated at 37°C overnight in activation buffer and stained with 0.2% Coomassie brilliant blue. Gelatinolytic activity was quantified by measuring band intensities with Image J-Fiji software.

RESULTS: No significant difference was observed in the serum activity of MMP-9 between patients (both symptomatic and asymptomatic) and healthy controls (Fig. 1 right). In three NCC patients it was also observed the presence of the active form of the enzyme. Also serum MMP-2 activity resulted not significantly different compared to healthy controls, however some patients showed values which were never recorded in healthy controls (Fig. 1 left).

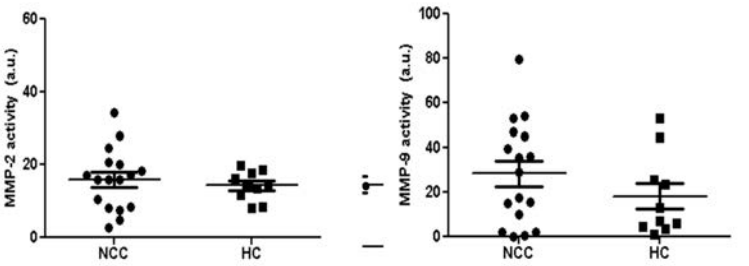

Fig. 1. Data derived from zymographic analysis of metalloproteinase-2 (MMP-2) and metalloproteinase-9 (MMP-9) activities in sera of neurocysticercosis (NCC) and healthy controls (HC). a.u.= arbitrary units

CONCLUSIONS: A previous study, carried out in India, had shown that serum MMP-2 activities were significantly higher in symptomatic and asymptomatic NCC compared to healthy controls whereas serum MMP-9 activity was significantly associated with symptomatic NCC compared to healthy controls and asymptomatic NCC (Verma A et al, 2011, Parasitology, 138: 1423-1428).
Our preliminary results show that in NCC patients MMP-9 and MMP-2 serum levels are not modified compared to healthy controls, however especially as regards MMP-2 the distribution of values is much higher than in controls and it is likely that by increasing the number of patients evaluated, significant differences might be found.

Pattern of imported malaria in Italy: 2000-2010 analysis

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Although the total number of imported malaria cases has been decreasing in Italy since 2000, malaria still represents the main health threat for people visiting tropical countries. The Ministry of Health and the Istituto Superiore di Sanità are in charge of the malaria surveillance system monitoring the epidemiological situation. This information is essential to orientate appropriate malaria prevention measures and recommendations for international travelers (Romi R et al, 2009, Travel Med Infect Dis, 8: 144-154; Romi et al, 2010, G It Med Trop, 15: 35-38).

AIM: The purpose of this study is to up-to-date the main epidemiological features of the imported malaria cases in Italy in the 2000-2010 period and to assess the trends over time.

MATERIALS AND METHODS: Using a dedicated database, all imported malaria cases notified by Local Health Authorities to the Ministry of Health and microscopically confirmed by the Istituto Superiore di Sanità, were analyzed.

RESULTS: In the 2000-2010 decade, Lombardy, Veneto, Emilia Romagna and Piedmont were confirmed to be the Regions with the highest number of malaria notifications. In Central Italy the highest number of cases was notified in Latium (7.1%). No significant increase in the number of notifications was observed in the Southern peninsular and island Regions. In the 2000-2010 period a total of 7,695 malaria cases were recorded; 13 cases were autochthonous, 9 arising from accidental events (transfusion, transplantation, nosocomial infections, "malaria" luggage) and 4 classified as cryptic cases, two of which suspected to be transmitted by indigenous vectors (Romi et al, 2012, this volume); 7,682 cases were imported, 2,019 (26.3%) involving Italian citizens and 5,663 (73.7%) foreigners, most of them (80%) were settled immigrants visiting relatives and friends (VRFs). From 2000 to 2010, cases of imported malaria have steadily decreased from 977 to 677, respectively, corresponding to an overall 31% reduction. In particular, a reduction in cases was reported both among Italian travelers (-42%) and foreigners (-27%). The majority of malaria cases were contracted in Africa (93%), most originated from West Africa, resulting Nigeria, Ghana, Senegal, Ivory Coast, Burkina Faso, and Cameroun the countries mainly involved. *Plasmodium falciparum* was the most frequently parasite found (83.6% of total cases), with 82% of infections acquired in Africa. *P. vivax* was responsible for 8.5% of the reported cases, predominant in Asia (80%), Latin America (85%) and Papua New Guinea (85%). *P. ovale* was responsible for 6.0% of total infections, 99% of them acquired in West Africa. *P. malariae* accounted for 1.6% of total cases, 95% of them contracted in Africa. A few numbers of mixed infections was observed (0.3%). Thirty fatal cases were reported in the study period, all being *P. falciparum* infections acquired in Africa. Cumulative fatality rate was 0.5%, with a highly significant difference in fatality rate between Italians (1.5%) and foreigners (0.2%) ($p<0.001$).

CONCLUSIONS: The number of malaria imported cases in Italy is known to be underestimated because of considerable underreporting that we estimated to be around 20% in whole country with a dramatic disproportion in the Southern Italy, in spite of a massive immigration from malaria endemic countries in that area. Efforts are needed by the Local Health Services to increase reporting of malaria cases; improving the accuracy of the annual data improves the surveillance of malaria in Italy. Anyway, considering the results reported above, despite the apparent decrease in number of imported malaria cases in 2000-2010 both among Italians and settled-immigrant groups, malaria remains the tropical disease most frequently imported in Italy, with a small but constant contribution of deaths due to *P. falciparum*. The level of alert for travelers visiting countries with endemic malaria must remain high in particular for VFRs that continue to be a population difficult to reach by effective malaria recommendations. Most of the VFRs still tend to ignore or underestimate the risk of contracting malaria by returning to their home countries. Therefore, as the lack of prophylaxis compliance is, in general, the major source of concern, new prevention strategies, in particular targeted at VFRs, should be implemented in order to have a substantial impact on the magnitude of imported malaria in Italy.

Seroprevalence of some parasitic zoonoses in the Saharawi refugee camps (Algeria)

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AIM: Parasitic zoonoses such as cystic echinococcosis (CE) and toxoplasmosis represent relevant public health problems in several geographical areas North Africa but no information is available about the Saharawi population which lives in the refugee camps in Algeria. This ethnic group occupies a restricted area of the desert in cohabitation with several domestic animals (dogs, sheep, goats, camels, etc.). For these reasons, we aimed to study the prevalence of human CE as well as that of toxoplasmosis, in this population. Previous studies in this population has shown high prevalence of both intestinal protozoa (*Giardia duodenalis*) and helminthes (*Hymenolepis nana*) in paediatric population (Gatti S et al, 2007, Ital J Trop Med, 12: 21-26).

MATERIALS AND METHODS: 397 sera from individuals of both sexes (246 female and 151 male) with an age ranging from 1 to 72 years were collected in the refugee camps of Tindouf region (West Algeria), in the period 2009-2010, in collaboration with the NGO Africa 70. Subjects were enrolled in the study when they were admitted to the hospitals of the refugee camps, after giving their permission.

A questionnaire was used to have personal data, to investigate the presence of a specific symptomatology, as well as possible risk factors. Blood samples were either collected on filter papers and, after drying, stored at room temperature in plastic bags with hermetic seal, until use or centrifuged at 500 x g to collect the serum, then stored at -20°C, until use. Samples collected on filter paper were reconstituted with the appropriate buffer before their use in serological tests. Antibodies specific for *Echinococcus granulosus* were evaluated in 397 individuals by means of an immunoenzymatic assay, commercially available, as a screening test and sera with positive results were then evaluated by immunoblot (WB), using a commercial and *in house* (c/o Laboratory of Dr. A. Siracusano, ISS, Rome) kit.

Toxoplasma gondii specific IgG were assayed by use of an ELFA system in 198 individuals of the above cited group. In sera resulted positive for IgG (63 individuals) specific IgM were also tested.

RESULTS: On 397 sera evaluated, a positivity rate of 1%.was found by means of ELISA, however the positivity was confirmed by WB in only 0,46%. Positive sera recognized only antigens of 7 and 28 kDa, which do not make possible to identify the *Echinococcus* species. To exclude a low sensitivity of the commercial WB, sera resulted negative with this kit, but with an O.D. in ELISA near the cut-off, were also tested by an *in house* WB which confirmed the negativity.

Toxoplasma-specific IgG were evaluated in 198 individuals and a positivity rate of 32% was recorded. The highest positivity for IgG was found in females in the age class 5-10 years. The positive sera were also tested for specific IgM which were found in only 12.5% of cases.

CONCLUSIONS: Our study, therefore, reveal a scarse circulation of the parasite in the human adult population, however it will be necessary to evaluate serologically a higher number of subjects to draw more accurate conclusions, since, previous data from the pediatric population (Giorgetti P. et al, 2010, Parassitologia, 52: 320) as well as from animal surveys (data not shown) show unequivocally the circulation of the parasite in the refugee camps.

A different situation was found as regards toxoplasmosis, in fact a significant part of the population is exposed to the parasite, particularly in younger ages, differently from what happens in industrialized countries such as Italy (Pinto B et al, 2011, Eur J Clin Microbiol Infect Dis, 31: 1151-1156).

The difference between female and male in the age class 5-10 years deserves further analyses.

Cystic echinococcosis: a neglected zoonosis in the Mediterranean

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AIM: The underestimated or misunderstood disease Cystic Echinococcosis (CE) continues to be a serious public health problem, especially in the Mediterranean. WHO have recently included echinococcosis and cysticercosis in the Neglected Zoonosis subgroup (http://www.who.int/neglected_diseases/NTD20Global20plan_20January202007.pdf) for its 2008–2015 strategic plan for the control of NTDs.
Apart from the parasite- (species, strain, biotic potential) and host-related (susceptibility/resistance) factors, environmental (temperature, humidity, zoonotic agents), social-economic and cultural (structural organisation, animal raising and butchering practices, disposal of butcher waste, strays, man-dog interrelation, population awareness) factors also govern the spread of this zoonosis. An accurate picture of the spread and impact of CE in the Mediterranean, as well as a knowledge of the spreading scenarios, would be very useful for evaluating the benefits of control measures in a region whose countries have profoundly different demographic, social and economic dynamics.

MATERIALS AND METHODS: Data on socio-economic structure, the anthropological and cultural factors affecting the spread of this disease, the most significant transformations that have occurred over time and any control measures put in place, have been collected for each country. The data on spread of CE have been gleaned from the national and international literature as well as from national databases (including regional and provincial) and databases of international organisations. The data were analysed in terms of reliability and epidemiological significance and of the trend of disease spread, also in relation to any prevention measures implemented.

RESULTS: The data gathered confirm the widespread presence of CE throughout the Mediterranean and the fact that its impact is grossly underestimated. Though in some countries epidemiological studies have improved in recent years, generally the literature data, and likewise the official statistics based on mandatory notification, do not provide a reliable measure of the spread of CE. Often the sources consist of occasional, partial surveys, personal case studies, surveys of rare cyst locations or particular clinical cases or specific branches of surgery, suggesting that the disease is far more widespread than believed. As far as animals are concerned, the surveys often concern single abattoirs, often with no indications as to animal provenance, etc. The amount of information recorded in an area is not necessarily proportional to the significance of the disease: better organised health and animal husbandry services can provide more accurate information, while where these are lacking, data may elude observation. The scant official notifications compared to actual cases, means that statistics fluctuate and vary randomly over time depending on the degree of success of disease awareness raising. Notwithstanding these limitations, the results suggest a very high parasite burden both in humans and animals.

CONCLUSIONS: The paucity of detailed epidemiological data together with the long latency of the disease contribute to underestimating CE which is widespread in all Mediterranean countries. Clearly there is a pressing need for an epidemiological network in the region. Indeed cystic echinococcosis should be considered as a case for mandatory notification when the parasite is detected in animal intermediate hosts as well as in humans. Particular attention should be given to enhancing the efficiency of data collection and flows. This would make it possible to increase awareness not only of the general public but also of health authorities, to estimate the real costs and to consider the benefits of control also at a supranational level.

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Overview of the epidemiological status of cystic echinococcosis (CE) in Sardinia

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Cystic echinococcosis (CE) is one of the most important and underestimated worldwide-distributed diseases. This zoonosis is endemic in areas where the long lasting "livestock–dog–human" interaction is more likely to exist, as in the Mediterranean, and is emerging or remerging in vast areas. In Sardinia sheep farming is an 8000 year-old tradition. Today around 3 million dairy sheep, ~40% of the national total, are reared and pastoralism continues to play a pivotal role in the social organization and culture specific to the island. Notwithstanding the intermittent efforts and investments spent in the past in an attempt to control this zoonosis, Sardinia has one of the highest CE rates both in livestock and in humans. Extensive or semi-extensive grazing, the large number of "stray-", "community-" and "sheep-dogs", the persistence of illegal home slaughtering, along with improper disposal of dead animals and poor disease awareness, combined with climate and ecological conditions have contributed to favouring CE transmission on the island. Major demographic, socio-economic and structural changes have actually modified the CE scenario in the last decades, though not in a uniform manner.

AIM: The purpose of this study is to assess the current situation of CE in humans and in the major intermediate host (sheep) in Sardinia, also through a retrospective analysis, to gain a better understanding of the current epidemiological factors associated therewith.

MATERIALS AND METHODS: Data on human CE incidence obtained in different surveys carried out by our research group, collecting data from all public and private hospitals up to 1995, and examining hospital inpatient discharge records over the period 2001-2005 have been compared. Data on prevalence in sheep have been gleaned from epidemiological surveys conducted in different Sardinian abattoirs over the years.

RESULTS: Human incidence rates in Sardinia, as high as 14.3per 100,000 inhabitants in the 1940-50s (Giromini & Granati, 1954, Folia Med, 37:746–70) varied, according to the different surveys conducted over thirty years or so, from 14.6 in 1969-73 (Ferretti et al, 1977, Epidemiol Prev, 1: 33-45) to 9.8 in 1990-95 (Ecca et al, 1998, Parassitologia , 40:49), to 6.62 in the most recent 2001-05 survey (Conchedda et al, 2010, Parasitol Int, 59: 454–459). Despite the discontinuous efforts and financial resources invested over the years, CE still poses a serious health risk in Sardinia. Between 2001-2005 mean annual hospital admission rate was 8.9 per 100,000 inhab., amounting to an estimated cost of hospitalization alone of roughly € M. Prevalence in sheep on the other hand varied from ~80% or more in the 1950-60s (Tanda , 1960, Vet It, 11:3-14; Serra, 1963,Vet It, 14:521-31) to 83-88% in the period 1979-89 immediately preceding the last control attempt (Arru et al, 1980, Atti Tav Rot "Echinococcosi / Idatidosi" Alghero, 29–31; Mura et al, 1981 N Ann Ig Microbiol , 22:159–76; Gabriele et al, 1998, Ig Mod, 110:13–22; Conchedda et al, 1997, Parassitol, 39:359–66). More recent studies, carried out during 1998-2010, some 10 years after the last campaign, indicate a reduction in prevalence to about 75% in northern Sardinia (Scala et al, 2006, Vet. Parasitol,15: 33–38), the highest prevalence in the most pastoral province of Nuoro (83%), diminishing to about 65% in southern Sardinia (Conchedda et al, 2012, Acta Trop, 122: 52– 58), with a CE rate of 78% in the former Oristano province and 58% in the Cagliari province where the greatest reduction was observed.

CONCLUSIONS: The persistence of CE in livestock and humans in Sardinia, especially in less favoured areas, is substantiated by epidemiological data. Though substantial, but not permanently balanced, improvements have been achieved, a major effort is needed to identify positive direct and indirect actions that are able to change the situation permanently if effective prevention is to be achieved. Failing this, the problems in Sardinia will remain unsolved, potentially resulting in a reversal of the decreasing trend in parasite pressure, as has happened in other parts of the world where CE has re-emerged even in areas where it was once thought to be under control.

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Cystic echinococcosis in humans: degree of calcification and cyst activity

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AIM: As studies on the natural history of CE in humans have demonstrated (Bortoletti et al., 2002, Parassitologia, 44, 159,171; 2004, Parassitologia, 46, 363,366; Conchedda et al, in press.), because of its long latency particularly in the liver, the CE metacestode in humans goes through a series of complex transformation processes, to become severely degenerated and ultimately resulting in the death of the parasite, presenting a number of morpho-functional types of cysts (unilocular, multivesicular, transitional, hyperlaminated, hyperlaminated- degenerated) . On the other hand, on the basis of ultrasonographic imaging, CE lesions are classified into six types: CL, CE1, CE2, CE3, CE4, and CE5 (WHO, 2003, Acta Tropica, 85, 253-261) from a very early stage of development to involute, necrotic inactive parasites. During the transformation processes that CE cysts undergo over time, calcification has been generally regarded as the terminal stage of parasite degeneration during its natural evolution, and believed to be an index of cyst inactivity.

As therapeutic management may vary depending on cyst activity, the aim of the present study was to determine the proportion of cysts with calcification of the cyst wall and cyst content in a sample of surgically removed human CE specimens at successive levels of involution, to clarify to what extent calcification contributes to the assessment of cyst activity and to investigate the correlation with specific antibody responses.

MATERIALS AND METHODS: In this study, 96 surgical specimens from 69 CE patients diagnosed by ultrasonography and treated for surgical excision of one or multiple cysts removed "en bloc" by means of total closed pericystectomy, have been studied. Attention was focused on morphostructural and functional state, with particular regard to extent of calcification of cyst wall and/or cyst content.

Correlation of the specific antibody response and the disease stage has been evaluated by determining serum levels of specific IgG, IgG1, IgG4, IgE antibodies by means of enzyme-linked immunosorbent assay (ELISA) using a Protoscolex somatic antigen.

RESULTS: Calcification of the cyst wall and/or cyst content was detected in 69/96 cysts (71% of all cysts) in 51/69 patients. Scattered and coarse calcification and more or less calcified walls were present in various cyst types, from "classic" and "multivesicular" to the more complicated stages and obviously in advanced and totally degenerated forms where the parasite wall may appear totally and massively calcified. The extent of calcification may be such that in some cases the entire wall actually has a bony consistency. Out of the 69 cysts with calcification detected by morphological examination, only 12 were indicated by US classification as WHO type CE5, 31 as type CE4, 14 as type CE3, 9 as CE2 and 3 as CE1 respectively. Note that morpho-structural comparison of parasitological examination with US classification showed a mis-classification in a 3 out of 14 CE1, in 1 out of 12 CE2, in 1 out of 19 CE3 and in 4 out of 39 CE4 cysts. Ab response in patients divided into 3 groups according to extent of calcification (1° group: patients with no-calcified cysts, 2° with scattered calcification and 3° with coarse calcification showed that IgG were present in 92%, 100% and 71% of cases, IgG1 in 77, 72 and only 14%; IgG4 in 85, 91 and 43%, IgE in 85, 82, 43% of cases respectively, with O.D. for all isotypes invariably decreasing in advanced calcification.

CONCLUSIONS: Calcification of the cyst is not restricted to the inactive WHO cyst types CE4 and CE5, but occurs in all cyst stages. Thus, the detection of calcification is not in itself sufficient to evaluate parasite activity and can be misleading, since this process may coexist with metabolically active parasites. Immunological tests, particularly for IgG1 IgG4 and IgE may help in evaluating cyst activity, as they are detected in a greater proportion and with higher O.D. in active than in inactive stages.

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Intestinal parasites isolated at INMI L. Spallanzani, Rome, during the years 2009-2011

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AIM: This study is concerned with the distribution of intestinal parasites detected in patients who presented at the Parasitology Laboratory of the INMI L. Spallanzani from January 2009 to December 2011, and with the investigation of the prevalence of intestinal parasites among immigrates and Italian patients with and without HIV infection.

MATERIALS AND METHODS: All commercial products and devices were used in accordance with manufacturers' instructions. DNA-based methods were used to differentiate *E. histolytica* from *E. dispar*.

RESULTS: 5743 faecal samples, 163 urine and 118 scotch tests from 6024 patients were examined. 166/5743 faecal samples resulted positive (2.9%); 3/163 urine resulted positive (1.8%); 8/118 scotch tests resulted positive (6.8%). Helminths detected were: *Enterobius vermicularis* (9), *Taenia spp*. (6), *Schistosoma haemotobium* (3), *Strongiloides stercoralis* (2), *Ascaris lumbricoides* (1), while Protozoa detected were: *Giardia duodenalis* (53), *Entamoeba dispar* (17) and *Isospora belli* (3). 8/21 patients infected with Helminths were immigrants (38%), and 34/73 patients with enteric protozoan infections were also immigrants (46.6%). Overall, co-infection of HIV and intestinal parasites was 4/21 (19%) for Helminths, and 43/73 (59%) for Protozoa. Non pathogenic *Entamoeba coli* was found in 31 cases, 19 were Italians and 12 immigrants. HIV prevalence was 10.5% among Italians and 16.7% among immigrants.

CONCLUSIONS: Intestinal parasites are a serious problem in developing countries, but should not be underestimated in industrialised countries. Moreover, this study showed that parasitic infections are common among HIV positive patients.

Detection and genotyping of *Giardia duodenalis* in Pemba Island, Tanzania

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Giardia duodenalis represents one of the most widespread human enteric parasite. Human infection exerts a deep impact on public health because of high prevalence and its possible effects on growth and cognitive status in infected children. This is particularly relevant in endemic area where access to safe water is precarious and the lack of effective sanitation, along with improper hygiene conditions, improves the risk of infection. In view of the lack of data about *Giardia* assemblages in Tanzania, that may reflect different biological and epidemiological aspects

AIM: To analyze the prevalence and genotypes of *G. duodenalis* in both human and animal samples, collected in the Archipelago of Zanzibar, in order to deepen the knowledge on transmission patterns and zoonotic potential.

MATERIALS AND METHODS: The study area incuded the district of Chake in Pemba Island and the smallest Island of Kojani. Between October 2009 and October 2010, 67 human faecal samples were collected: 45 from paediatric patients from two primary schools in the district of Chake Chake; the remaining 22 samples (picked from the ground) in Kojani Island. Moreover, in Kojani Island 62 stool samples from freely roaming animals were also collected directly from the ground: 19 from zebus (*Bos taurus indicus*), 41 from goats (*Capra hircus*) and 2 from chickens (*Gallus gallus domesticus*). Detection and molecular identification of *G. duodenalis* was performed by multilocus analysis, amplifying fragments of the *18S*, *gdh*, and *tpi* genes. DNA was purified and sequenced by Biofab Research (Italy) and *consensus* sequences were determined by reverse and forward sequence alignment using the software ClustalW2. Genotype assessment was based on both sequence comparison and phenetic analysis conducted with the MEGA (version 5.0) software.

RESULTS: Based on the molecular analysis, 37 out of the 67 fecal human samples were positive to *Giardia* DNA (55.2%), 16.2% of isolates have been identified as Assemblage A, 83.8% as Assemblage B. Specifically, 28/45 positive samples were from Pemba (62.2%) and 9/22 from Kojani (40.9%). 21.4% of the isolates from Pemba was assigned to Assemblage A-AII, 78.6% to Assemblage B (both BIII and BIV). All isolates from Kojani were identified as Assemblage B (both BIII and BIV). As to animals, 10 out of the 62 fecal animal samples were positive, 8 from goats (21.9%), and 2 from zebus (21.0%). Samples from chickens were all negative. Genotype frequencies observed in goats were: 55.6% Assemblage E, 11.1% Assemblage A, 22.2% Assemblage B-BIV and 11.1% of mixed Assemblage B+E; in zebus were 75% Assemblage A and 25% Assemblage B-BIV

CONCLUSIONS: Despite the close contact between humans and animals, the high rate of human infection with Assemblage B-BIV, the high proportion of Assemblage A in zebus and the presence of host-specific Assemblage E in goats indicate that the risk to public health from animals infected by *G. duodenalis* may be minimal and the zoonotic transmission could have a limited epidemiological role in the transmission of giardiasis in the area. Further studies based on multilocus genotyping are required to confirm these results.

A family outbreak of fasciolosis evidenced in Italy

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Fasciolosis is a major veterinary health problem of herbivorous such as cattle, sheep, and goats, whereas human infections were considered, up to 1990, a disease of secondary relevance (Chen and Mott, 1990, Trop Dis Bull, 87: R1-R38). In the last decade, however, the number of cases reported has increased, with up to 17 million people infected (Mas-Coma et al, 1999, *Fasciolosis*, Dalton JP Ed, CABI Publishing, New York: 411-34), living in 51 countries (Esteban et al, 1998, Res Rev Parasitol, 58:13-42), Italy included (Caprino et al, 2007, Chir Ital, 59: 891-4).

AIM: To report two cases occurred in Italy, to present clinical and diagnostic features of the infections, and to investigate on the genetic relationships between the flatworm specimen recovered and that isolated in Europe.

MATERIALS AND METHODS: A nine–year-old Caucasian child (female), coming from Romania and living in Perugia for 2 years, was admitted to the Bambino Gesù Children Hospital in Rome for severe microcytic hypochromic anaemia. The medical history included an unspecified anaemia occurred when she was living in her Country, which was treated with blood transfusion. The blood tests performed at the admission in the Bambino Gesù Children Hospital showed slightly elevated liver and pancreatic enzymes with normal bilirubin and elevated peripheral eosinophils. An abdominal ultrasound study triggered the suspicion of biliary parasitosis: dilatation of the biliary tract with multiple hyperechoic intraluminal filaments aggregating to form a single conglobates in choledocus, minimal swelling of the head of the pancreas with ectasia of the Wirsung and Santorini ducts, presence of ribbon-shaped hyperechoic striations moving within the lumen of the small intestine. The patient underwent an endoscopic retrograde cholangiopancreatography and a pre-cut sphincterotomy that caused the release of abundant grey gelatinous material mixed with bile and an active parasite that was gathered. Stool samples were also collected and submitted, together with the parasite, to the D.O.U. "Diagnostic Parasitology"of the Umberto 1° Teaching Policlinic in Rome. Stool analyses were performed by means of direct and after Ridley concentration microscopic examination. Further data about the family lifestyle were collected, and parasitological analyses were extended to other family members complaining same sanitary problems.
The parasite gathered was measured, examined by microscopy and then submitted to molecular diagnostics (PCR using primers DSJ3 and DSJf and sequencing) (Lai et al, 2010, Ann Trop Med Parasitol, 104: 65-72).

RESULTS: Macroscopic and microscopic features of the removed parasite (a flat, brownish leaf-shaped organism measuring 2.5x1cm) confirmed the endoscopic suspicion of *F. hepatica*, whose eggs were also identified in stool samples. One of the child sisters too proved positive to parasitological and radiological analyses, and both children started a pharmacological treatment. By a genetic point of view, the flatworm isolated was a bit more closely related to *F. hepatica* specimens collected from Italian (Lazio region) than European cattle present in GenBank. Unfortunately, no data are available for Romania.

CONCLUSIONS: Notwithstanding preliminary phylogenetic results indicating a close relation with Italian strains of *F. hepatica*, the anamnesis suggests this little family outbreak is not autochthonous. In fact, the two sisters spent in Romania most their life, in a breeder family and in precarious hygienic conditions; moreover, time necessary to develop such severe clinical manifestations is long and, overall, both children yet suffered of anaemia that required even a blood transfusion, a sign strongly indicative of fasciolosis. Further genetic analyses are needed to better investigate on the origin of these human infections evidenced in Italy.

Cyst stage and dimension influence serological response in hepatic *E. granulosus* infection

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AIM: To assess the correlation, if any, between cystic stage, as seen at US, and serological test (IHA and ELISA).

MATERIALS AND METHODS: This was a retrospective investigation of a cohort of patients diagnosed with CE and followed-up in our clinic from May 2000-June 2012. Cysts were sorted by their WHO classification stage (active, transitional and inactive cysts) and by their dimension (S≤ 5 cm, M 5-10 cm and L≥ 10 cm).

RESULTS: Of 339 patients evaluated in 812 visits, 249 had 1 parasitic cyst and 90 a non parasitic cyst. IHA and ELISA were positive in 87 and 83% of active cysts, 90 and 93% of transitional and 60 and 50% of inactive cysts and post-surgical residual cavities respectively. Specificity was 99%. A statistically significant difference (p< 0,0001) for median values of IHA and ELISA was found between active and inactive, active and residual cavities, transitional and inactive cysts. Both test were positive in 58% of examinations, specifically in 81% of active, 90% of transitional and 51% of inactive cysts. Both tests were negative in 99% of non parasitic cysts. Serological response turned out to be influenced by cyst dimensions. Both test were positive in 76% of L, 62 and 48% of M and S cysts respectively, with statistically significant differences between serological response to L and S cysts and M and S cyst.

CONCLUSIONS: Serological response to hepatic CE with routine test used in our Laboratory is influenced by dimension and biological activity of the cyst. Future clinical studies on the use of serology in CE should take this into account.

Intestinal parasites in children of urban areas of the Cordillera province (Bolivia)

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The World Health Organization estimates that over 3.5 billion people worldwide are infected with intestinal parasites responsible for malnutrition and anaemia, particularly in poor communities of developing countries, where children are the most vulnerable subjects.

AIM: In this study we report the results of a survey (MAE project ''Fortalecimiento de la red de salud del Chaco Boliviano: una perspectiva comunitaria'') carried out to detect intestinal parasites in children living in some municipalities of the Cordillera province (Bolivia), where control programs started about 25 years ago.

MATERIALS AND METHODS: During 2011 a coproparasitological survey was carried out on 267 apparently healthy children 3-12-year-old, living in the municipalities of Cabezas, Lagunillas, Charagua, Cuevo, Camiri, Boyuibe and Gutierrez. Fecal samples were submitted to microscopic examination directly and after Ridley concentration. Parasites were identified on the basis of their morphological features, and samples positive to *Blastocystis* and *Entamoeba histolytica/dispar* complex were further analysed to identify the subtype and species, respectively, involved. Molecular diagnostics (PCR followed by sequencing, and nested PCR, respectively) were performed following protocols described by Bohm-Gloning et al (1997, Trop Med Int Health, 2: 771-778) and by Solaymani et al (2006, J Clin Microbiol, 44: 2258-2261).

RESULTS: Intestinal protozoa and/or helminths were recovered in 69.7% of the samples, and multiple infections were observed in 39.7% of the subjects. No significant differences were observed between males and females, whereas a significant higher infection rate was recorded in 4-8-year-old children. The protozoon most commonly found was *Giardia intestinalis* (38.5%), followed by *Entamoeba coli* (37.8%) and *Blastocystis* spp. (15.7%). Other protozoa as *Endolimax nana, Entamoeba hartmanni, E. histolytica/dispar/moskowskii*, *Iodamoeba butschlii* and *Chilomastix mesnili* were also identified in 3.7%, 2.6%, 2.6%, 1.1% and 0.7% of the subjects, respectively. Coinfections *E.coli-Giardia, E.coli-Blastocystis, E.coli-Blastocystis-Giardia* were detected in 15.3%, 4.8%, and 2.9% of the children, respectively. Helminth eggs more often identified were those of *Hymenolepis nana* (5.6%), followed by those of *Taenia* spp (1.1%), *Ascaris lumbricoides* (0.7%), and hookworms (0.3%). A total of 15/42 (35.7%%) *Blastocystis*-microscopically positive samples yielded the expected amplicon of 1,100bp. Preliminary sequences obtained from 9 purified amplicons showed high identity (98%-100%) to homologous sequences of *Blastocystis* sp. subtypes 4 (n=3) and 5 (n=6) deposited in GenBank. Nested PCR identified 2 infections due to *E. histolytica* and 2 to *E. dispar.*

CONCLUSIONS: Our findings, even if only based on children, indicate that implemented control programs (sanitary education included) resulted successful, as evidenced by the infection rate significantly lower than that observed in the same area in 1988 by Cancrini et al (Parassitologia, 30: 263-269). Geohelminths are almost absent, zoonotic opportunistic species like *Balantidium coli* and *Cryptosporidium* disappeared, and parasites like *H. nana* are strongly reduced, indicating a lower exposure to contamination with human faeces. The high prevalence of water/vegetable-transmitted protozoa suggests that hygienic conditions, mainly water supply, should be further improved.

As for *Blastocystis*, subtype 4 was found usually in humans, whereas subtype 5, found also in Italian population (Mattiucci S et al, 2010, Parassitologia 52: 207), is thought to be specific to pig and cattle (Noël C et al., 2005, J Clin Microbiol, 43: 348-355). Unfortunately, *E. histolytica* is present and may represent a serious sanitary problem.

An urban outbreak of severe dermatitis by the red mite, *Dermanyssus gallinae* De Geer, 1778 (Mesostigmata: Dermanyssidae) in Southern Italy, Apulia region

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AIM: The poultry red mite, *Dermanyssus gallinae* is a temporary blood-sucking ectoparasite of several birds worldwide, including feral pigeons. It can also bite mammals, including humans and causes nonspecific pruritic skin disorders. We report a case of urban red mite dermatitis (RMD).

MATERIALS AND METHODS: In October 2011, the Istituto Zooprofilattico di Puglia e Basilicata (IZSPB) was contacted to identify arthropods suspected to be the origin of pruritus and bites in three persons (55 and 51 years old, males, lawyers and their 95-year-old mother) living in the same old building, in a city in Northern Apulia. For about 3 weeks, all of them had been complaining of annoying pruritus and diffuse skin lesions with biting and stinging sensations. Symptoms were more severe on the two brothers, particularly on the trunk, abdomen and arms. Initially, the patients ascribed the skin lesions to mosquito bites. In order to relieve the symptoms, they used to take a shower, after which they would cover their bodies with talcum powder but the treatment proved to be ineffective. Later on, the patients consulted two general practitioners who attributed the dermatitis to *Sarcoptes scabiei* and to wood mites, respectively. Cortisone cream and antiparasitic shampoo were prescribed but even this treatment was ineffective. Following another episode of intense pruritus, the elder brother collected a reddish parasite from his underwear. Alarmed by this finding, he carefully inspected the house collecting several dust-like arthropods from various pieces of furniture.

RESULTS: All the parasites were identified as *D. gallinae* according to Baker's (Baker AS, 1999, Mites and ticks of domestic animals, The Natural and History Museum, London, The Stationery Office) morphological keys. Interviewed by IZSPB team, the patients reported that an active pigeon nest had been removed two months earlier, close to the infested room. The use of pyrethroids in two cycles of fumigation, preceded by thorough vacuuming, led to complete regression of the symptoms.

CONCLUSIONS: Physicians and dermatologists are often unaware of the role of red mite in urban areas. In fact, case reports of RMD are rarely recorded in the human medical literature due to the difficulty of detecting and accurately identifying the red mite, and of matching the symptoms with the parasite. However, feral pigeons are among the most successful avian settlers in urban environments due to the abundance of food and the absence of predators. They are distributed worldwide and live close to human populations. Their breeding and roosting sites harbour zoonotic ectoparasites, including *D. gallinae* (Haag-Wackernagel D, Bircher AJ, 2010, Dermatology, 220: 82-92), the main ectoparasite acquired by humans from feral pigeons, together with the *Argas reflexus* tick (Haag-Wackernagel D, 2005, Ann of Appl Biol, 147: 203-210). Red mites are well adapted to host absence and can survive without food for up to nine months (Kirkwood AC, 1963, Exp Parasitol, 14: 358-366), so they can emigrate from pigeons' nests into the nearest houses long after the natural avian hosts have disappeared. This infestation may occur in private habitations but also in hospitals, schools and other public edifices leading to a mass outbreak such as those recorded in scattered reports from a few parts of Europe (Bellanger AP et al, 2008, Infect Control Hosp Epidemiol, 29: 282-283) as well as from Italy (Gelati A et al, 2007, Atti XLV Convegno SIPA; Cafiero MA et al, 2008, J Eur Acad Dermatol Venereol 22: 1382-1383). Failure or delay in diagnosis can also result in economic loss due to the different and numerous sanitation measures that must be taken. As recently registered in recurrent episodes of red mite infestation in the delivery room at a maternity unit in Southern Italy, the measures included relocating hospital equipment and sealing off infested rooms for disinfestations, leading to temporary work stoppages and loss of income. The loss of working days due to discomfort and alarm in health-care workers verified, too (Galante D et al, 2011, Atti 21stECCMID/27thICC, 17: 286-287).The possibility that red mites may also be vectors/reservoirs of zoonotic pathogens is an additional concern associated with the parasite (Chirico J et al, 2003, Med Vet Entomol, 17: 232-234; Valiente-Moro C et al, 2007, Vet Paras, 146: 329-336). We therefore consider that urban RMD is far more common than the few reports in the literature would suggest, and that it may in fact be an emerging public health problem requiring a multidisciplinary approach.

A retrospective study on burden of human echinococcosis based on hospital discharge records from 2001 to 2009 in Sardinia, Italy

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AIM: The purpose of this study was to estimate the burden of human CE in Sardinia. Such an estimate is imperative since it can be used as a tool to prioritize control measures for this essentially preventable neglected disease. Recent studies suggest that this disease has a large social impact on endemic areas, and estimates of burden in terms of monetary and no-monetary impact on human health are essential to allocate financial and technical resources.

MATERIALS AND METHODS: In this work, a retrospective study was carried out using public Hospital Discharge Records drawn from the regional database between 2001 and 2009. During these years, a total of 1,409 discharges were recorded, 1,196 (84.88%) records corresponding to primary diagnosis, that is, patients hospitalized for symptoms directly correlated to CE, and 213 (15.11%) records corresponding to secondary diagnosis, that is, patients hospitalized for symptoms not directly correlated to CE and with an afterwards or concurrent diagnosis of echinococcosis made during the hospitalization, with an annual regional average record of 9.5 per 100,000 inhabitants. Direct cost associated with diagnosis, surgery or chemotherapy, medical care, and hospitalization in humans were evaluated in this work. Furthermore, indirect costs were also evaluated by using the disability-adjusted life years (DALYs), the preferred disease-burden measure of the World Health Organization.

RESULTS: During the reporting period, the direct cost for 1,266 OH was € 6,625,453.40 distributed as € 4,561,244.00 (range € 381,555.77 in 2005 and € 783,628.43 in 2001) for 515 OH with surgical procedures and € 2,064,209.40 (range € 87,660.83 in 2001 and € 320,444.86 in 2009) for 751 OH with medical care. The mean cost of a single OH was € 5,316.85 (range € 4,171,43 in 2006 and € 7,161.37 in 2002) considering an average length of hospital stay of 15.1 days. The direct cost for 143 DH was € 91,396.53 (range € 5,175.35 in 2003 and € 15,996.88 in 2006) with a mean cost for each DH of € 653.77 (range € 345.02 in 2003 and € 1,143.00 in 2009). From 2001 to 2009 the total direct cost (OH plus DH) for echinococcosis in Sardinia was € 6,716,849.93 corresponding to a mean cost of € 746,316.65 per year. Mean direct costs of treating a case of human echinococcosis in Sardinia have been calculated to be € 5,970.92, corresponding to US$ 8,434.69.We calculated the number of patients hospitalized at least once in the whole period: the total of 1,409 records corresponded to 938 single patients, of which 667 (71%) patients hospitalized once and 271 (29%) patients with multiple hospitalizations. Patients hospitalized once were included into 71.10% of patients improving after surgery or medical care, respectively 313 and 354; 271 patients with multiple stays were grouped in 225 (23.98%) patients with less than 3 and more than 1 stay and 46 (4.9%) patients with more than 3 stays, of which 22/46 (2.34%) patients with medical treatment and 24/46 (2.55%) for surgical procedure (Murray CJL, 1994, Bull World Health Organ,72: 429-445).Total DALYs were 505.40 with a range of 169.32 in the age cluster 41-60 and 20.76 in the age cluster 81 >90. For each age cluster and patients group, the DALY value was calculated separately. The group of 225 patients developing morbidity and with less than 3 and more than 1 hospitalization, showed the higher DALY value. Age cluster with the higher DALY value was 41-60.

CONCLUSIONS: In Sardinia CE persists as a public health problem with a cost that is 746,316.65 euro/year. These data confirm the high prevalence of human echinococcosis in Sardinia and highlight the importance of implementing a continuous and more effective control programme. More accurate data on CE prevalence in humans (particularly undiagnosed or asymptomatic cases) are needed, and the activation of correct reporting measures for this infectious disease, together with the implementation of the Community Network under Decision n° 1219/98/EC of the European Parliament and Council, is of considerable importance. We stress that CE is a neglected and preventable zoonosis that remains a human health concern; additional funding is needed to reduce human and animal infection rates through improved disease surveillance, regular treatment of dogs and greater cooperation among agencies.

Molecular diagnosis of eight cases of Gastric Anisakiasis in Italy, with the first evidence of Gastro-Allergic-Anisakiasis (GAA) associated to *Anisakis pegreffii* (Nematoda: Anisakidae)

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AIM: Human Anisakiasis is a fish-borne zoonosis caused by larval stage of *Anisakis*. Humans are accidental hosts in the life-cycle of *Anisakis*; they become infected by consuming fish and squids harboring larval stages in their muscle. The larvae in humans do not develop to the adult stage; they may penetrate the gastro-intestinal tract, often with severe pathological consequence, as the formation of eosinophilic granulomas. There is a growing report that these parasites are able to produce a strong allergic reaction: this aspect seems to be more evident when an alive larva reaches the gastric submucosa (Gastro-Allergic Anisakiasis). Several new cases of gastric human anisakiasis occurred in recent years in Italy are here reported.

MATERIALS AND METHODS: The patients originated from the following regions: Abruzzo (4), Latium (1), Campania (2), Tuscany (1). All the patients suffered of acute gastric pain and nausea after some hours/one day they ate raw fresh marine fish; two of them had an allergic reaction. In all patients larvae of nematodes were collected during esophagoduodenoscopy. Their identification was performed by means of DNA sequencing of mitochondrial (mtDNA *cox2* and *rrnS*) and nuclear (ITS1-ITS2 of the rDNA) genes. In addition, sera of patients were tested for IgE-*Anisakis* by ImmunoCAP (Phadia, Sweden) and by WB to detect specific antigens (allergens) of *A. pegreffii*.

RESULTS: Sequences analysis at the three genes showed overall highest nucleotide homology with those of *A. pegreffii* available in GenBank. The sera available showed a value at the ImmunoCAP of >100 IgE-As. At the Immunoblotting (WB); only two showed IgE antibody reaction to the larval antigen ANI s1.

CONCLUSIONS: The first case of gastric Anisakiasis in Italy was described by Stallone et al, 1996 (MedJSurg 4:13-16). Development of molecular has resulted with an increase in the frequency of the reports of this zoonosis in many parts of the world. To date, two species of the genus *Anisakis* are found to be causative agents of human infections; they are *A. simplex s.s.* and *A. pegreffii* (Umehara et al, 2007, Parasit Int,56:211-215; D'Amelio et al, 1999, Parassitologia, 4:591-593; Fumarola et al, 2009, Foodborne Pathog Dis 6:1157-1159; Mattiucci et al, 2011 BMC Infect Dis 11:82). This represents the first report of (GAA) due to *Anisakis pegreffii* in Italy.

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Malaria: data collected in Naples from 2000 to 2011

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AIM: Malaria is still common imported tropical disease in Italy. The present paper, focus on its main epidemiological aspects, making an overview of the data collected from 2000 to 2011, by "D. Cotugno ", hospital of Naples, the Regional Reference Center of Infectious Diseases. In these years, we have examined 94 cases of malaria, 43(46%) related to Italian patients and 51(54%) to migrants. Furthermore, we had, in November 2010, a case of transfusion-induced malaria in an Italian patient affected by Lepore's anaemia. Malaria, in 92% of our cases, was acquired in Africa and, *Plasmodium falciparum* was responsible of approximately 84% of global infections. Our data, combined with the growing phenomenon of immigration and globalization, forced the Italian health authorities to maintain a high level of attention on this disease.

MATERIALS AND METHODS: Patients suspected of having contracted malaria, from 2000 to 2011, were admitted to "Cotugno" hospital. Diagnoses were made by the Parasitology Laboratory of Cotugno, through research of the parasite in samples of peripheral blood smear and thick films, after disinfection and puncture of patient's fingertip. The smears were allowed to dry at room air for about 1 hour, and stained with May-grumwald-Giemsa. The thick films have been made to dry for about 12 hours and stained with Giemsa diluted 1/20.

RESULTS: From 2000 to 2011 we had 377 requests of exams for *Plasmodium spp.* We found it in 94(25%) samples, 43(46%) coming from Italian patients and 51(54%) from migrants, 65(69%) males and 29(31%) females, age range from 1 to 66 years of life. A total of 42(45.7%) cases were Italian: tourists (12;12%), workers (23;24%) and missionaries (7;7%), 51(54.2%) foreign migrants (visit friends and relatives) and 1(1%) cases of malaria induced by blood transfusion in an Italian patient. The number of the cases of malaria has been constant on year. 10(10%) patients take pharmacoprophylaxis, 4(4%) of theme with correct drug. The data show that in 86(92.6%) cases, Malaria was contracted in Africa, in 7(7%) cases in Asia (India, Pakistan, Indian ocean, 2 Indonesia and 2 China) and we had just 1 case of introduced malaria in Italy (1%). *P. falciparum* was isolated in 79(84%) cases of malaria; 76 of these cases was contracted in African countries; *P. vivax* was responsible of 3(3.3%) cases of malaria contracted in India, Indonesia and Indian ocean; *P. ovale* was responsible of 8(8.5%) cases of malaria contracted in several African countries; *P. malariae* was responsible of 1(1%) case of malaria contracted in Guinea; we have found 3(3%) mixed infections, 2 from *P. falciparum* + *P. vivax* contracted in Pakistan and Indonesia and 1 from *P. falciparum* + *P. Ovale* contracted in Nigeria. During the period of study we had 2(2%) death (1 Nigerian female patient with double infection: *P. falciparum* + HIV and 1Italian patient with severe Malaria). Although the majority of cases had favorable outcome, we report 8 cases of severe Malaria (*P. falciparum*).

CONCLUSIONS: Imported malaria remains a major health problem in Naples as well as in Italy. Mainly, the disease was acquired in several African countries. *P. falciparum* was responsible of 82(87%) cases of malaria. In the south of Italy, Campania region has the greatest numbers of migrants (147.057 about 150 differently nationality), 45% live in Naples province and 25% in Naples city.

The case of malaria caused by *P. falciparum* following a transfusion, imposes a large and constant attention to these pathogens not routinely present in our Country. Their presence could increase as a result of large and continuous migration flow. Thanks to the rapid and correct diagnosis, to the skill and expertise of our clinicians, malaria cases had all a successful outcome, with 2 exception. In conclusion, Malaria is an Italian health authorities concern. We must pay attention on autochthonous Malaria cases registered in Spain and Greece; being some malaria's vector present in some areas of Italy, we might expect autochthonous case in our country too. Considering the above reasons, the steady increase in average temperature and environmental changes caused by human work, we must remain vigilant in monitoring the epidemiological picture of our country.

Echinococcus granulosus: evidence of G1-G3 and G6-G10 complexes in humans in Southeastern Romania

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AIM: The cestode *Echinococcus granulosus*, (family Taenidae), is the etiological agent of cystic echinococcosis (CE), a neglected chronic infection with a worldwide distribution (Brunetti E et al, 2011, PLoS Negl Trop Dis, 5: e1146). CE is an extremely important public health problem in Eastern Europe, in particular in Romania, where is considered the most important zoonotic disease (Neghina R et al, 2010, Foodborne Pathog Dis, 7: 613-618). Genotyping of human CE is useful both to confirm diagnosis and to collect data on parasite transmission patterns and susceptibility of humans to a particular genotype of *E. granulosus*. In this work, we aimed: i) to identify the *E. granulosus* strains that cause CE in 60 patients from Romania; ii) to determine the possible correlations between the patients' epidemiological and clinical data and the genotype of their parasite.

MATERIALS AND METHODS: Cyst samples (endocyst or cyst fluid) were collected from 60 Rumanian patients suffering from CE of the liver or the lung from 2008 to 2011. Epidemiological and clinical data were collected for all patients. Liver cysts were classified as active (CE1-2), transitional (CE3a-3b), or inactive (CE4-5) cyst. All patients were tested for anti-*Echinococcus* antibodies by ELISA commercial kits. Every sample was examined to assess the presence of viable protoscoleces in the cyst fluid. Endocyst samples were fixed in 95% ethanol, whereas cyst fluids were centrifuged and pellets collected. Each sample was stored at -20°C until used for the molecular analysis. Genomic DNA was extracted using a commercial kit (Qiagen). A fragment of cytochrome c oxidase subunit 1 (cox1) and NADH dehydrogenase subunit 1 (nd1) mitochondrial genes were amplified by PCR using specific primers (Bowles J et al, 1992, Mol Biochem Parasitol, 54: 165-173; Bowles J, McManus DP, 1993, Int J Parasitol, 23: 969-972). Analysis of nucleotide sequence data was performed by BLAST.

RESULTS: The obtained coxI and ndI nucleotide sequences from 59/60 patients, showed maximum homology (>99%) with the G1- and G3-genotype sequences registered in GenBank and were classified as belonging to the G1-G3 cluster (*E. granulosus* sensu strictu). Only one sequence showed maximum homology (>99%) with the *E. canadensis* complex (G6-G10). The cyst with this G6-G10 genotype belonged to a 52-year-old woman who underwent radical surgery to remove a CE1 medium-sized cyst located in the liver; she lives in a urban area and reported frequent contact with stray dogs; data regarding travels abroad were not available. The striking prevalence (98%) of G1-G3 complex among the samples analyzed prevented us from evaluating any correlations between the epidemiological and clinical data of the patients and the genotype of their cysts.

CONCLUSIONS: Our findings show that human CE in Romania is mainly restricted to *E. granulosus* G1-G3 genotype complex. This study also presents the first human case in Romania infected by a G6-G10 genotype complex. The real extent of *E. canadensis* among animals and humans should be further investigated to understand its real role in human CE and, finally, to adopt proper preventive measures against the infection.

Epidemiology of human opisthorchiasis in Italy

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AIM: *Opisthorchis felineus* was described in cats and dogs for the first time in the XIX century, then the presence of this zoonotic trematode in Italy remained in oblivion for over 100 years, with few sporadic reports in cats and dogs and in a rabbit in central and northern Italy. In the human beings, opisthorchiasis has been never documented in Italy up to 2003 when the first cases occurred in the Umbria region. The aim of the present work was to describe the outbreaks and single cases of human opisthorchiasis in Italy from 2003 up to 2011.

MATERIALS AND METHODS: Epidemiological investigations based on index cases were carried out by parasitological detection of *O. felineus* eggs in stools, serology by ELISA using excretory/secretory antigens of *O. felineus* adult worms, and by PCR to amplify the ITS2 region of the rDNA in fecal samples.

RESULTS: From 2003 to 2011, four single cases and nine outbreaks of opisthorchiasis were documented with a total of 207 infections in humans. Most of the infected people claimed to have consumed raw marinated fillets of tench (*Tinca tinca*) at restaurant (no. 148), at a private dinner (no. 20), served by catering (no. 31), at home (no. 5), or unknown place of infection (no. 3). Epidemiological investigation allowed to trace back the fish origin to the Trasimeno lake (no. 10 persons), the Bolsena lake (no. 134 persons), and the Bracciano lake (no. 60 persons). The three infected persons for which the origin of infection was unknown, lived in villages close to the Bolsena lake. In some restaurants, raw tench fillets were served instead of raw fillets of the common whitefish (*Coregonus lavaretus*) due to the reduced availability on the market of the whitefish caused by the increased request in the summertime. All confirmed infected persons showed a positive serology and most of them had *O. felineus* eggs in their stool. About 2/3 of infected persons developed signs and symptoms of opisthorchiasis after 25-30 days p.i., but the symptomatology disappeared within 3-4 months even if they did not received any treatment. About 1/3 of infected persons did not develop any sign or symptom of the disease. All infected persons (both symptomatic and asymptomatic) developed eosinophilia and increased liver enzymes which slowly decreased only after the treatment. Infected persons were treated with albendazole (most of them) or praziquantel with a good compliance. After the first treatment, only some highly infected persons still shed eggs and they were successfully treated again using praziquantel.

DISCUSSION: The main problem of human opisthorchiasis is the lack of pathognomonic signs and symptoms, the high number of asymptomatic people and last but not least the very low number of persons who are skill in the microscopical diagnosis of gastroenteric parasitic infections. In fact in many cases, fecal samples positive for *O. felineus* eggs were considered as negative in private laboratories and the diagnosis of choice was reached only some months after the infection. The emergence of autochthonous opisthorchiasis infections in humans in Italy since 2003 is probably due to the changed food behavior characterized by the increased consumption of raw fish. At the same time, the increased consumption of tenches at restaurant along the shores of lakes, resulted in the availability of tenches and their remnants to stray cats which represent the main final hosts. After the occurrence of the 2007 outbreak of opisthorchiasis, the Italian Ministry of Health requested that all stocks of tenches should be marked with a warning label 'to be eaten after cooking or to be frozen at -20°C for 7 days', but this information was ignored or the warning label had been lost, resulting in outbreaks which, in some cases, occurred far from the lakes (e.g. Aosta in 2010).

CONCLUSIONS: *O. felineus* is present in a silent form in the Central regions of Italy; however, changes in the human behavior, as the increasing consumption of raw fish, can result in the emergence of opisthorchiasis. Tools aimed to control the liver fluke circulation and to avoid the transmission to humans should be urgently implemented.

Anopheles gambiae salivary proteins as epidemiological markers of human exposure to malaria vectors: humoral response to the gSG6 and cE5 proteins in a malaria hyperendemic area of Burkina Faso

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Hematophagous arthropods saliva injected into hosts during blood feeding evokes a humoral response which may be used as marker of human exposure to disease vectors and, therefore, may be a useful tool to evaluate disease risk and to monitor efficacy of vector control programs. We have previously analyzed the IgG response to the anopheline-specific salivary protein gSG6 in a population from a malaria hyperhendemic area of Burkina Faso and reported that the *Anopheles gambiae* gSG6 may be exploited as marker of human exposure to the three main Afrotropical malaria vectors: *An. gambiae*, *An. arabiensis* and *An. funestus* (Rizzo C et al, 2011, *PLoS ONE*, 6: e17980; Rizzo C et al, 2011, Malaria J, 10: 206). More recently we characterized the *An. gambiae* salivary protein cE5, a highly specific thrombin inhibitor of the anophelin family (Ronca R et al, 2012, Insect Biochem Mol Biol - in press).

AIM: (i) evaluate immunogenicity to humans of the cE5 protein; (ii) compare IgG, IgG1 and IgG4 response to the *An. gambiae* salivary proteins gSG6 and cE5; (iii) measure the seasonal variation of the anti-cE5 IgG response.

MATERIALS AND METHODS: Surveys were carried out in the village of Barkoumbilen, a malaria hyperendemic area of Burkina Faso ~35 km NE of Ouagadougou where the main malaria vectors are *An. gambiae* and *An. funestus*. Antibody levels (IgG, IgG1 and IgG4) were measured by ELISA in the sera of exposed individuals (Rimaibé and Mossi ethnic groups, n=208 or n=121) and of European unexposed controls (n=59 or n=68) at the beginning (Aug'94) and at the end (Oct'94) of the high transmission/rainy season, as well as during the following low transmission/dry season (Mar'95).

RESULTS: Despite the individual heterogeneity cE5 was more immunogenic than gSG6. Consistently with a previous report (Rizzo C et al, 2011, PLoS ONE, 6: e17980) the anti-gSG6 IgG response was high in children and progressively decreased with age, most likely because of tolerance due to prolonged exposure. On the contrary the anti-cE5 humoral response showed a trend to increase during adulthood. Intriguingly, the humoral response to these two proteins also differed for the dominant IgG subclass: the response to gSG6 was characterized by high IgG4 levels (IgG4>IgG1), whereas in the case of cE5 IgG1 was largely the prevalent antibody (IgG1>>IgG4). Finally, there was no significant variation of the anti-cE5 IgG response between the rainy (high transmission) and the dry (low transmission) seasons.

CONCLUSIONS: The relatively long-lasting nature of the anti-cE5 humoral response points out that cE5 is not a good candidate as marker of human exposure to malaria vectors. However, taking into account that IgG1 and IgG4 antibodies are often considered as markers of Th1- and Th2-type responses, respectively, we conclude that our study allowed to identify two salivary antigens with strikingly different properties. On one side gSG6 seems to triggers a short-lived response of the Th2-type, with high IgG4 levels and induction of tolerance. On the other side cE5 apparently elicits a Th1-type response which lasts longer and is characterized by high IgG1 levels and no development of tolerance. Previous reports in murine malaria models indicated that pre-immunization with *Anopheles* saliva may provide protection from *Plasmodium* infection by up-regulation of Th1-type response (Donovan MJ et al, 2007, Infect Immun, 75: 2523-2530; Fonseca L et al, 2007, Malaria J, 6: 77). In this context the *An. gambiae* gSG6 and cE5 salivary proteins may represent useful tools to study both the effects of mosquito saliva on early host response to *Plasmodium* infection and the mechanisms underlying the development of tolerance to insect saliva.

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