Health monitoring program for the control of laboratory animal diseases

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Abstract
Pathogens present in the environment are the biggest source of diseases and epidemics in the breeding of laboratory animals. In fact, the presence of microorganisms can critically influence the animal health status and, consequently, the validity and reproducibility of experimental data. In accordance with the 3Rs principle (Refinement, Reduction, Replacement), this study is part of the Refinement concept. The FELASA guidelines, formulated with the aim of guaranteeing the best animal health state, are a valid support for researchers. In this preliminary study, health-monitoring program was carried out within the breeding of laboratory animals in IZSLER facility. The main murine viruses were analyzed through molecular biology techniques (PCR, RT-PCR) and enzyme immunoassays (indirect ELISA). The established surveillance program steadily guarantees animal health and ensures the most controlled environmental and sanitary conditions. Further investigations will be needed to develop virus control strategies.

Materials and Methods
In this preliminary study, a virus surveillance program was carried out within the breeding of laboratory animals in IZSLER facility. The main murine viruses analyzed were:
- Polymyx virus of mice (POLY)
- Adenovirus type 1 (ADENO)
- Murine Hepatitis Virus (MHV)
- Murine Teiovirus (TMEV)
- Parvoviruses (Minute Virus of Mice, MVM and Mouse Parvovirus MPV)
- Pneumonia Virus of Mice (PVM)
- Ectromelia virus (ECTY)
- Polymyx virus of mice (PVM)
- Sendai virus (SENDAI)
- Reovirus type 3 (REO-3)

Viruses were analyzed through molecular biology techniques and enzyme immunoassays (indirect ELISA). In particular, for molecular biology analysis, organs and faeces were used. Spleens, hearts, lungs, livers and kidneys were disrupted using a lysis buffer and an automatic homogenizer, while the faeces were processed with PBS and mixed with a stirrer. RNA and DNA were extracted by an automatic spin column system and PCR internal methods were performed with specific primer pair and probes for each virus. The experimental conditions for RNA virus amplifications were as following: 1 cycle of reverse transcription at 50°C for 20 min, 1 cycle of initial denaturation at 95°C for 5 min, 40 cycles of denaturation (95°C for 15 sec) and annealing/extension (60°C for 45 sec). The same protocol, except for the reverse transcription, was used for the DNA viruses. The quantifications of the target sequences were analyzed through a Real Time thermal cycler.

Commercial kits were purchased in order to carry out the ELISA assay for the detection of Immunoglobulins G (IgG). The serum was incubated in an adsorbed plate with each viral antigen at 37°C for 45 min. The conjugate was added to the reaction plate and incubated at 37°C for 45 min. The substrate was distributed in the wells and placed in the dark for 30 min at room temperature. Using a spectrophotometer an absorbance (OD) at 405 nm was read and the data was processed in order to calculate the absorbance differential (ΔOD).

Results
A total of 42 samples were analyzed. In Figure 1 the main results are shown. From serological analysis it was observed that the mice have been in contact with MHV, highlighting the presence of the specific MHV IgG in 42% of samples, followed by ADENO (16%). The two Paroviruses analyzed, MVM and MPV show 12% and 10% of prevalence, respectively, while REO-3, SENDAI, PVM and TMEV antibodies are present in less than 10% of the analyzed samples. Overlapping data were obtained from molecular biology tests, where in fact a
prevalence of MHV antigens is observed (38%). PVM is the second predominant virus in RT-PCR (18%), whereas the molecular detection of MVM and REO-3 replicates the ELISA assay test, showing 15% and 7% of predominance, respectively. The molecular analysis of ADENO and TMEV are in contrast with serological data (ADENO: 4% vs 16% and TMEV: 7% vs 3%). Finally, ECTV and POLY, whose antibodies were absent in the ELISA assay, show a prevalence of 4% and 7% of respective antigens in RT-PCR.

**Conclusions**

This preliminary work emphasizes the importance of welfare and care of laboratory animals in experimental research. The established surveillance program steadily guarantees animal health for the entire duration of the housing and ensures the most controlled environmental and sanitary conditions. More samples will be processed in order to perform a statistical analysis of the data obtained. However, further investigations will be needed for animal welfare monitoring and virus control strategies developing (e.g. quarantine).

**References**