Spillover event of recombinant *Lagovirus europaeus*/GI.2 into the Iberian hare *(Lepus granatensis)* in Spain

Roser Velarde [a], Joana Abrantes [b,c], Ana M. Lopes [b,d], Josep Estruch [a], João V. Côrte-Real [b,c], Pedro J. Esteves [b,c], Ignacio García-Bocanegra [e], Jordi Ruiz [f], Carlos Rouco [g,h]

[a] Wildlife Ecology & Health group (WE&H) and Servei d'Ecopatologia de Fauna Salvatge (SEFaS), Departament de Medicina i Cirurgia Animals, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain.
[b] Research Network in Biodiversity and Evolutionary Biology (CIBIO-InBIO), University of Porto, Vairão, Portugal.
[c] Department of Biology, Faculty of Sciences, University of Porto, Porto, Portugal.
[d] Instituto de Ciências Biomédicas Abel Salazar (ICBAS) / Unidade Multidisciplinar de Investigação Biomédica (UMIB), Universidade do Porto, Porto, Portugal.
[e] Grupo de Investigación en Sanidad Animal y Zoonosis (GISAZ), Departamento de Sanidad Animal, Universidad de Córdoba, Cordoba, Spain.
[f] Departament d'Agricultura, Ramaderia, Pesca i Alimentació de la Generalitat de Catalunya, Barcelona, Spain.
[g] Departamento de Botánica, Ecología y Fisiología Vegetal, Universidad de Córdoba, Cordoba, Spain.
[h] Sociedad, Ecología y Gestión del Medio Ambiente, UCO-IESA, Unidad Asociada al CSIC, Cordoba, Spain.

Background

RHD was caused by Lagovirus europaeus/GI.1 until the emergence some years ago of L. europaeus/GI.2, a virus with a great capacity to recombine with existing lagoviruses, replacing the former strains in the wild and contributing to increase spillover events detected among different lagomorph species, including hares[1]. Surprisingly, the endemic Iberian hare (Fig. 1), a key prey for many endangered predators in the Iberian Peninsula and focus of a severe population decline during the last few years, partly, due to the incidence of infectious diseases, seemed unaffected by GI.2.

Figure 1. An Iberian hare (*Lepus granatensis)* adult individual. Image: Juan Lacruz

Materials and methods

An adult pregnant female Iberian hare was found dead on November 2020th in north-eastern Spain (Fig. 2). Mortality in rabbits was also detected, including a female rabbit in the study.

No other Iberian hare carcasses were found in the study area

Necropsy: histopathology and Immunohistochemistry samples

Molecular analysis: RT-PCR for GI.2 and PCR to rule out EBHSV/GII.1 (duodenum, faeces, spleen and liver) at CIBIO (Portugal). Additional phylogenetic studies performed.

Figure 2. Map of the study area (Catalonia, North-East of Spain), in which some rabbits and one Iberian hare were found dead. The region is characterized by the sympatric presence of Iberian hares, European brown hares (*Lepus europaeus)* and rabbits.

Results and Discussion

Pathology alterations were consistent with *Lagovirus* disease in both rabbit and hare (Fig.3).

Gross findings	Histologic changes
Pale enlarged liver with reticular pattern and multifocal hemorrhages	Periportal to midzonal coagulative necrosis pattern, hemorrhages and a mild portal lymphocytic inflammatory reaction (Fig. 4a)
Mild splenomegaly	Moderate numbers of active macrophages within the red pulp
Congestion/hemorrhages in cervical lymph nodes	Mild lymphocytolisis with tingible body macrophages in several follicles
Congestion of the respiratory tract and petechial hemorrhages in the thymus	Acute focal hemorrhages in the lungs and thymus and tracheal mucosa congestion
Foetuses: no evident lesions	Focal subepicardial and myocardial hemorrhages and liver congestion

Figure 3. Gross and microscopic lesions observed in the Iberian hare and its foetuses.

Immunohistochemical analysis revealed distribution of the antigen similar to RHD natural and experimental infections[2], being detected mainly in the spleen and liver (Fig. 4b). Neither of the three fetuses nor placenta showed antigen positivity.

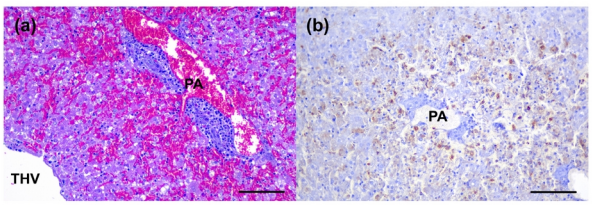

Figure 4. Microscopic lesions and viral antigen detection in the liver of the GI.2 infected Iberian hare. (a) Acute to subacute haemorrhagic periportal to midzonal hepatocellular necrosis and apoptosis (PA, portal area; THV, terminal hepatic venule) and mild inflammatory portal infiltrates (H&E stain, bar=200 µm). (b) Immunohistochemical visualization of viral capsid antigen (brown) (bar=200µm).

Genome characterization confirmed GI.2 in both specimens (except for the foetuses) and discarded GII.1 in the Iberian hare. Virus shedding was confirmed by faeces molecular analysis.

Phylogenetic studies demonstrated a close relatedness between hare and rabbit coding sequences, although they were not 100% identical, discarding contamination and suggesting a spillover, most likely from rabbit to the Iberian hare.

Both strains were GI.4P-GI.2 recombinant strains

Conclusions

- Iberian hares may be infected by GI.2 and show clinical disease.
- Vertical transmission has not been observed, but more research should be developed on this matter.
- This could be considered an isolated spillover event, suggesting the virus is not still well adapted to the new host.
- It is worth to study the role that species-specific factors may play in the epidemiology of GI.2 in the Iberian hare.

References

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