Network for wildlife health surveillance in Europe

Diagnosis Card

Lagovirus diseases: European Brown Hare Syndrome and Rabbit Haemorrhagic Disease

Authors (*corresponding author)
Antonio Lavazza*, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy; antonio.lavazza@izsler.it
Aleksija Neimanis, Department of Pathology and Wildlife Diseases, National Veterinary Institute (SVA), Uppsala; Sweden; aleksija.neimanis@sva.se

Reviewers

Dolores Gavier-Widén, National Veterinary Institute, Uppsala, Sweden
Gudrun Wibbelt, Leibniz Institute for Zoo and Wildlife Research (IZW), Berlin, Germany

Last update

11.01.2021

Etiology

Rabbit haemorrhagic disease (RHD) and European brown hare syndrome (EBHS) are similar diseases caused by two related but phylogenetically distinct non-enveloped positive-strand RNA viruses with a diameter of about 32–35 nm, belonging to the genus *Lagovirus* of the Caliciviridae family. They are highly robust viruses able to resist pH between 2 and 10 and remain infectious for at least 3–4 months in the environment.

Affected species (wildlife, domestic animals, humans)

RHDV was considered to have a restricted host specificity affecting only the European rabbit (*Oryctolagus cuniculus*) but a new serotype, RHDV2, emerged in 2010 with a broader host spectrum. RHDV2 can also cause disease in the Sardinian cape hare (*L. capensis* var *mediterraneus*), the Italian hare (*L. corsicanus*), the brown hare (L. europaeus) and the mountain hare (*L. timidus*), albeit with probable differences in degree of susceptibility. In the USA, RHDV2 infection has been recently confirmed in four wild lagomorph species: black-tailed jackrabbit (*Lepus californicus*), desert cottontail (*Sylvilagus audubonii*), antelope jackrabbit (*Lepus alleni*) and mountain cottontail (*Sylvilagus nuttallii*).

EBHS is a highly contagious disease of European brown hare (*Lepus europeaus*), mountain hare (*L. timidus*) and Italian hare (*L. corsicanus*). The disease was diagnosed also in the Eastern cottontail (*Sylvilagus floridanus*) in Italy both after natural and experimental infections. EBHS has not been commonly reported in other hare species like *L. granatensis*, and *L. capensis*, present in European countries. EBHS does not affect domestic and wild rabbits.

Epidemiological characteristics and disease course

RHDVs and EBHSV are transmitted directly or indirectly, mainly by oro-faecal route. Carnivores, insects, birds and humans can facilitate virus spread and insects can act as mechanical vectors, but no reservoir hosts other than lagomorphs have been definitively identified. Indirect transmission through contaminated fomites including equipment, cages, clothes, vehicles, utensils likely occurs, especially in outbreaks in farmed animals. Infection via contaminated green forage and vegetation is also possible and lagomorph carcasses are believed to contribute to environmental persistence of the virus.
RHD and EBHS are characterised by up to 100% morbidity in immunologically naïve populations but with a variable mortality rate depending on the type of virus and the age of the animals. For infection with RHDV/RHDVa, mortality approaches 80–90% and, although rabbits of all ages can be infected, the infection is subclinical in animals younger than 6–8 weeks of age. The disease caused by RHDV2 can have a slightly longer duration and the mortality rate is highly variable (50-80%) depending on the viral strain. Here, death may occur even in suckling rabbits from 7–15 days of age. EBHS mortality is close to 50% and is observed only in adult hares. Clinical disease is not observed in hares younger than 2-3 months of age. These young hares may contract the infection but do not usually develop clinical disease and show long-lasting protective immunity.
After the initial epidemic peak, characterised by widespread mortalities, the infection tends to become endemic in wildlife. Areas where EBHS and RHD are endemic appear to maintain stable hare and rabbit populations in which most animals are immune and mortality rates are low.

Clinical signs

The RHD and EBHS incubation period varies between 1 and 6 days. In acute cases, infected animals develop fever (>40°C) and die suddenly within 12–36 hours of the fever's onset. Death may be sudden

without clinical signs, but more often behavioural changes are observed, such as lack of fear, dullness, jumping into the air, circling, staggering, incoordination, and convulsion before death. In farmed rabbits and hares, anorexia, apathy alternated with excitement, cries, and respiratory distress during agony may be observed. During an outbreak, around 5-10% of rabbits and 30–50% of hares may show a chronic course of the disease, characterized by generalized jaundice clearly visible in the mucosae. Such chronically affected animals may die after several days or finally recover. A specific and relevant protective IgM response appears within 3 days, immediately followed by an IgA and IgG response 2–3 days later.

Gross lesions

At necropsy, the principal findings are an enlarged, friable and discoloured (tan to bronze) liver with an accentuated reticular pattern, and enlarged and often dark red spleen, oedema and congestion of the tracheal mucosa with foamy haemorrhagic contents in the airways, and variable areas of congestion and haemorrhage in tissues. Infrequently, generalized jaundice is seen.

Histological lesions

Liver: Necrotic hepatitis. Periportal to massive, acute hepatocellular necrosis with variable apoptotic bodies and inflammatory cell infiltrate, activation of Kupffer cells and occasional areas of lytic necrosis. Hepatocellular mineralization can be seen in hares. In the sub-acute or chronic clinical course, lesions in the liver include less extensive necrosis, more pronounced fatty change in hepatocytes, more inflammatory infiltrates, and proliferation of bile ducts.
Spleen: Congestion, oedema and necrosis of the red pulp. Often lymphocytolysis and follicular depletion.
Respiratory system: Tracheal hyperaemia and sometimes submucosal haemorrhages. Congestion, oedema and variable haemorrhages in the lungs.
Kidney: Tubular nephrosis with degeneration and necrosis and proteinaceous casts in the tubules are variably seen.
Multisystemic fibrin thrombi, particularly in the renal glomeruli and pulmonary capillaries, are often seen in rabbits.

Differential diagnosis

Pasteurellosis: especially the septicaemic form characterized by generalized haemorrhages.
Toxoplasmosis, Yersiniosis, Tularaemia: enlargement of the spleen is a common finding.

Criteria for diagnosis

Presumptive diagnosis is based on post-mortem examination, but gross pathological changes are not specific for EBHS and/or RHD. Although lagoviral disease can be diagnosed if characteristic microscopic liver lesions are observed, confirmative diagnosis requires demonstration of the presence of a lagovirus. Further typing of the virus is required to identify the lagovirus responsible, particularly for hares that can be infected by both viruses.

Recommended diagnostic method(s) and preferred samples (incl. recommended amount and appropriate storage)

Identification of the virus in liver homogenate or other tissue can be done using haemagglutination test (with human O red cells), electron microscopy (EM), immune EM using convalescent antisera or specific MAbs, ELISA and PCR. Histopathology and immunohistochemistry (with hyperimmune serum or specific MAbs) can be conducted in formalin fixed liver,
The adoption of specific sandwich ELISA techniques using a high-titre positive homologous serum as catcher and cross-reacting RHDV/RHDV2 MAbs or specific EBHSV MAbs as tracer, is highly recommended, notably for the simplicity of implementation and the low cost. Such sandwich ELISAs in association with a panel of MAbs are also useful for antigenic characterization of different viral strains.
RT-PCR and real-time RT-PCR are very sensitive methods for the detection of lagovirus (RHDV/RHDV2, EBHSV or even other non-pathogenic lagoviruses) and are at least 10^4-fold more sensitive than ELISA. They are also more convenient and rapid than other tests. Although they are now often used, RT-PCR methods are not strictly necessary for routine diagnosis.
Due to the significant antigenic differences between RHDV, RHDV2 and EBHSV, regarding serological techniques for detecting and titrating antibodies, different competition-ELISAs for respectively RHDV, RHDV2 and EBHSV using specific antigen and antisera or MAbs should be used.

EWDA proposed protocol (for harmonization at large scale)

Direct diagnosis: PCRs are the universal method able to ascertain viral positivity in a short time with high sensitivity and can characterise the lagovirus responsible.
Indirect diagnosis: the detection of specific antibodies against RHDV/RHDVa and EBHSV is achieved by cELISA that should be considered the elective method at population level.

Laboratories that can be contacted for diagnostic support

O.I.E. Laboratory reference for Rabbit haemorrhagic disease: IZSLER Via Bianchi 9, 25124 Brescia – Italy antonio.lavazza@izsler.it; lorenzo.capucci@izsler.it;
ANSES Ploufragan-Plouzane Laboratory. 41, rue de Beaucemaine - BP 53 - 22440 PLOUFRAGAN – France. Ghislaine.LEGALL-RECULE@anses.fr

Recommended literature

1. OIE, Rabbit Haemorrhagic Disease Chapter 2.6.2 (version adopted in May 2016) (2018). OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. 8th ed. OIE., Paris, pp.1389-1406 https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.06.02_RHD.pdf
2. Frölich K, Lavazza A, 2007. European Brown Hare Syndrome. In: Lagomorph Biology, Alves PC, Ferrand N, Hacklaender K, Springer Berlin, pp. 253-262.
3. Lavazza A., Cooke B.D. (2018) "Chapter 5: Diseases of Lagomorphs" in: Smith A.T, Johnstione C.H. Alves P., Hackländer K. “Lagomorphs: Pikas, Rabbits and Hares of the world” Johns Hopkins University Press, Baltimore, Maryland (USA), pp. 18-21.
4. Le Gall-Reculé G., Lavazza A., Marchandeau S., Bertagnoli S., Zwingelstein F., Cavadini P., Martinelli N., Lombardi G., Guérin J.L., Lemaitre E., Decors A., Boucher S., Le Normand B. & Capucci L. (2013). Emergence of a new lagovirus related to Rabbit Haemorrhagic Disease Virus. Vet. Res., 44, 81. doi: 10.1186/1297-9716-44-81.
5. Yon L., Duff P., Ãgren E., Erdelyi K., Ferroglio E., Godfroid J., Hars J., Hestvik G., Horton D., Kuiken T., Lavazza A., Markowska-Daniel I., Martel A., Neimanis A. S., Pasmans F., Price S., Ruiz-Fons F., Ryser-Degiorgis M-P-, Widen F., Gavier-Widen D. Recent changes in infectious diseases in European wildlife. Journal of Wildlife Diseases, 55(1), 2019, pp. 000–000 DOI: 10.7589/2017-07-172