



European Brown Hare Syndrome

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Etiology

European Brown Hare Syndrome (EBHS) is a viral disease caused by a non-enveloped positive-strand RNA virus with a diameter of about 32–35 nm, belonging to the genus lagovirus of the Caliciviridae family. EBHS virus (EBHSV) is antigenically and genetically related to Rabbit Hemorrhagic Disease virus (RHDV) of rabbits (*Oryctolagus cuniculus*). It is a highly robust virus that is resistant to pH, and remains infectious for 3–4 months in the environment.

Affected species (wildlife, domestic animals, humans)

EBHS is a highly contagious disease of European brown hare (*Lepus europeus*), mountain hare (*Lepus timidus*) and Italian hare (*L. corsicanus*). The disease was diagnosed by specific EBHS methods in Eastern cottontail (*Sylvilagus floridanus*) in Italy both after natural and experimental infections, and seropositive reactors were observed in free-ranging populations. 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

EBHSV is transmitted directly or indirectly, mainly by oro-faecal and respiratory routes. Carnivores, insects, birds and humans can act as animate vectors, but no reservoir hosts other than hares have been yet identified. Indirect ways of transmission, including equipment, cages, clothes, vehicles and utensils likely occur, especially in outbreaks in farmed hares. Infection via consumed vegetation is also likely, with the virus being excreted and spread in the faeces of predators that have consumed infected hares.

EBHS is characterized by 100% morbidity in immunologically naïve susceptible populations, but mortality (varying rate, but often close to 50%) is observed only in adult hares. The clinical disease is not observed in hares younger than 2-3 months of age. These may contract the infection but do not usually develop clinical disease and show long-lasting protective immunity.

Mortality is highest in autumn when the population is most dense and the young of the year become susceptible. After the initial epidemic peak, the infection tends to become endemic. Areas where EBHS is endemic appear to maintain a stable hare population in which most hares are immune and mortality rates are low.

An epidemiological model that considers the peculiar characteristics of EBHS (juvenile resistance to the disease till 60-90 days of age, high environmental resistance of the virus and quick diffusion of infection) has shown that the impact of the infection on the population dynamic can be dramatic when the hare density is low (<8 hares/km²). In fact, in such case, there are lower possibilities that young hares that are naturally resistant to the disease become infected and develop long-lasting immunity. The mortality can therefore be reduced and attain endemic stability by increasing the host density (over 15 adults/km²). This situation in high density areas supports the infection of young animals when resistant to the disease and the development of immunity and thus the high (60-90%) seroprevalences of populations.

Clinical signs

The peak of mortality in experimentally infected hares is commonly observed between 72 and 90h after infection. Death may be sudden, lacking clinical signs but more often behaviour changes are observed, such as lack of fear, dullness, jumping into the air, circling, staggering, incoordination, and convulsion before death. In farmed hares, it is also possible to observe anorexia, apathy alternated with excitement, cries, and respiratory distress during agony. During an outbreak, around 30–50% of hares may show a chronic course of the disease, characterized by generalized jaundice clearly visible in the mucosae. Such chronically affected hares may die after several days or finally recover.

Gross lesions

At necropsy, the principal findings are oedema and congestion of tracheal mucosa with foamy haemorrhagic contents, liver enlargement, degeneration and discoloration with sharply demarcated and friable lobes, enlargement of the spleen, and generalized jaundice.

Histological lesions

Liver: Necrotic hepatitis. Diffuse acute coagulative hepato-cellular necrosis of hepatic periportal and midzonal areas (sometimes the whole lobule may be affected) along with formation of acidophilic bodies. In the sub-acute or chronic clinical course, lesions in the liver include: less extensive necrosis, more pronounced fatty change, more inflammatory changes, and proliferation of bile ducts.

Spleen: Congestion and oedema of red pulp. Often follicular depletion, but sometimes follicular hyperplasia. 25% of affected animals have hyaline-like changes in the cords and sinuses and splenic cellular depletion.

Respiratory: Tracheal hyperaemia and sometimes submucosal haemorrhages. Congestion, oedema and haemorrhages in the lungs.

Kidney: nephrosis with tubular degeneration and necrosis in about one third of the cases. Proteinaceous casts in the tubules.

Brain: cerebellar Purkinje cells and cerebral neurons exhibit granular and vacuolar degeneration.

Differential diagnosis

Pasteurellosis: especially in the septicaemic form characterized by diffuse haemorrhages.

Toxoplasmosis and 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. The confirmative diagnosis is based on recognition of typical microscopic liver lesions together with demonstration of the presence of the EBHSV by virological methods.

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 anti-EBHSV serum or specific anti-EBHS MAbs, histopathology and immunohistochemistry (with hyperimmune serum or specific anti-EBHS MAbs, ELISA and PCR).

The adoption of an EBHSV-specific sandwich ELISA technique using a high-titre positive anti-EBHSV hare serum as catcher and cross-reacting RHDV MAbs or specific EBHSV MAbs as tracer, is highly recommended, notably for the simplicity of implementation and the low cost. Such sandwich ELISA in association with a panel of MAbs is also useful for antigenic characterisation of different viral strains.

RT-PCR and real-time RT-PCR appear to be very sensitive methods for the detection of EBHSV and are at least 104-fold more sensitive than ELISA. They are also more convenient and rapid than other tests. Even if they are now often used, RT-PCR methods are not strictly necessary for routine diagnosis.

EBHSV can also be identified in diagnostic samples by EM examination of liver homogenate in veterinary laboratories equipped with this device. In particular, to increase the sensibility, the immune-EM method using convalescent anti-EBHSV serum or specific anti-EBHS MAbs can be used to identify EBHSV.

Due to the significant antigenic differences existing between RHDV and EBHSV, the serological techniques, which use RHDV as antigen, are not recommended for the serological diagnosis of EBHSV. However, a direct ELISA method could be employed for the detection of positive and negative EBHSV hare sera; in fact, the adsorption of RHDV on to the solid phase of an ELISA microplate exposes cross-reactive internal antigenic determinants. This method is sensitive but has a very low specificity. Thus, to detect specific anti-EBHSV antibodies, a specific competition-ELISA for EBHSV

using specific antigen and antisera or MAbs should be used.

APHAEA protocol (for harmonization at large scale)

Direct diagnosis: PCR is the universal method able to ascertain viral positivity in short time with high sensitivity.

Indirect diagnosis: the detection of specific antibodies against 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: Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia Romagna Via Bianchi 9, 25124 Brescia – Italy

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Recommended literature

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