Epidemiology and diagnosis of the European brown hare syndrome in Scandinavian countries: a review

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Summary: Outbreaks among European brown hares (Lepus europaeus, Pallas) of a fatal disease associated with severe liver damage have occurred in Sweden since the beginning of the 1980s. The disease, called the European brown hare syndrome (EBHS), was recognised in Denmark in 1982 and is today widespread in Denmark and southern Sweden. It has not been reported in Norway or Finland. Two species of hares are affected in Sweden, the European brown hare and the varying hare (Lepus timidus, Linnaeus). EBHS occurs both in free living and farmed hares. The disease is clearly seasonal, occurring most frequently in October, November and December.

A virus related to the viral haemorrhagic disease of rabbits virus has been shown to cause EBHS. All attempts to isolate the virus in cell culture have been unsuccessful. Diagnosis can be made by histopathology and detection of the virus in organ homogenates by haemagglutination, negative staining electron microscopy and enzyme-linked immunosorbent assay (ELISA). Antibodies can be detected early by haemagglutination inhibition and ELISA.

KEYWORDS: Diagnosis - Epidemiology - European brown hare syndrome - Hares - Lepus - Liver disease - Scandinavia - Viral haemorrhagic disease of rabbits.

INTRODUCTION

Outbreaks of a disease of unusually high mortality occurred among European brown hares (Lepus europaeus, Pallas) in Sweden at the beginning of the 1980s. The disease was called Fältharesjuka, or the European brown hare syndrome (EBHS). Although the nutritional condition of most of the affected hares was good, death apparently occurred in the acute form; the most consistent finding was severe liver damage. In Denmark, a similar condition among free living European brown hares was observed for the first time in 1982. Mortality continued to be reported in Sweden and Denmark in the following years, and EBHS was subsequently detected in other European countries. Numerous studies were conducted to determine the cause of the disease. Mycotoxins, agricultural pesticides, selenium deficiency and new varieties of O.O-oilseed rape (Brassica napus) with low glucosinolate content were among the possible causes investigated; however, no definitive association could be found linking any of these suspected factors to EBHS. A possible viral cause was also considered.

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The liver lesions of EBHS are similar to those which characterise certain types of viral hepatitis, such as yellow fever and human haemorrhagic fevers (3). Although viral isolation was attempted, no positive results were obtained; serological studies to detect antibodies to hepadnavirus (human hepatitis B), picornavirus (human hepatitis A), yellow fever and a number of haemorrhagic fever viruses were also negative.

In October 1988, an inter-European seminar on the pathology of the European brown hare syndrome was held in Uppsala, Sweden. The participants in Uppsala concluded that the disease was, in fact, a new pathological entity and that it had been recognised in other European countries, such as West Germany and Italy. A variety of possible aetiologies was discussed. Dr A. Lavazza from Italy was the first to suggest that the aetiology of EBHS was related to the virus causing viral haemorrhagic disease (VHD) of rabbits, a then newly emerging disease in Europe. Lavazza had found viral particles, morphologically similar to those of rabbits with VHD, in the liver of hares which had died of EBHS. The observations by Lavazza are now recognised as accurate, and the diseases of hares and rabbits are known to be closely related.

The EBHS virus (EBHSV) has not yet been classified and the VHD virus (VHDV) is currently under study. The classification of this virus has been controversial. Most European researchers have concluded that VHDV is a calicivirus (1, 7), while American research workers believe it to be a parvovirus (4). In any case, VHDV shares some antigenic determinants with EBHSV. In addition, the liver lesions in the two diseases are remarkably similar.

**EPIDEMIOLOGY**

A descriptive epidemiological study was conducted to determine the geographical and time distribution of EBHS in Sweden. EBHS was diagnosed in 275 of the 2,818 hares received for post-mortem examination at the Department of Wildlife Diseases of The National Veterinary Institute in Uppsala, between January 1980 and December 1989. Diagnosis was based on typical histological changes observed in the liver. (The results of this study are currently being prepared.) The first cases of EBHS were identified in 1980 on the island of Gotland, off the southeast coast. EBHS appeared in southern Sweden in 1981 and spread northwards in the following years. It is not known if EBHS occurred earlier than 1980. Today, EBHS is widespread in most parts of Denmark (2). EBHS has not been reported in Norway or Finland.

There are two species of hares in Sweden, the European brown hare and the varying hare (*Lepus timidus*, Linnaeus). Both species are affected by the disease. Currently, EBHS is diagnosed in approximately 10% of the varying hares and 30% of the European brown hares received for post-mortem examination at the National Veterinary Institute. The distribution of EBHS in Sweden corresponds to that of the population of European brown hares; the disease does not occur in areas where only varying hares are present. Wild rabbits in areas where EBHS is endemic do not appear to contract the disease. So far, EBHS has not been recognised in Sweden in any species but hares (genus *Lepus*). No serological studies to detect antibodies to EBHS have been conducted in other species.
The mortality of EBHS is clearly seasonal. Most deaths occur in October, November and December, with somewhat fewer cases in January, February and September; death is relatively rare during the rest of the year. A similar temporal distribution is observed in Denmark (Dietz, personal communication).

EBHS also affects farmed hares in Sweden and Denmark. The mortality rate in the initial outbreak is quite variable and can reach 100% on small farms (5). Mortality is concentrated into a period of approximately two weeks. Most of the hares which survive develop antibodies. In subsequent years, mortality due to EBHS becomes sporadic on farms which have had outbreaks of the disease. In Denmark, EBHS has been diagnosed as the cause of death of 25.6% of the farmed hares subjected to post-mortem examination at The National Veterinary Laboratory (2).

Little is known about the routes of infection of EBHS. The virus is shed in faeces, and contamination of food and water with excreta from infected animals is probably the most common means of transmission.

Affected hares may die from the peracute form of disease without clinical signs. In the acute form, the hares show signs of nervous disorders: they run in circles, fall on one side and suffer blindness, paralysis of the hind legs and terminal convulsions. Abnormal behaviour, such as the loss of fear of dogs and people, is often observed. Death occurs within hours after the onset of such signs.

In some cases the course of the disease is longer, and the affected hares die after a few days of depression and anorexia.

**Histopathological diagnosis**

Histological examination of the liver is a reliable method of diagnosing EBHS. Typical histological changes include periportal to massive coagulation necrosis of the hepatocytes, the presence of acidophilic bodies formed by cells undergoing acidophilic degeneration and necrosis, and a scant portal infiltrate of pseudo-eosinophils and lymphocytes. In many cases, there is basophilic stippling in the cytoplasm of the hepatocytes of periportal areas, represented by a granular accumulation of calcium. Recognition of excessive calcium by special stainings, such as Van Kossa, is useful for diagnosing autolytic material. Immunostainings, such as immunofluorescence and peroxidase-antiperoxidase, have been used to detect virus in tissues. The histological liver lesions caused by other common pathogens of hares can be readily differentiated from EBHS. Bacterial infection of the liver associated with systemic infections, such as tularemia, pseudotuberculosis, listeriosis and pasteurellosis, produces randomly distributed foci of hepatocellular necrosis. Toxoplasmosis produces a marked reticuloendothelial hyperplasia and the toxoplasmal organisms can be demonstrated in the lesions. Liver necrosis produced by most toxins is centrolobular rather than periportal. Hepatic biotransformation of most toxins involves mixed-function oxygenases, enzymes which are abundant in centrolobular hepatocytes.

**Virological diagnosis**

Isolation of EBHS virus is very difficult. Repeated attempts by the authors to isolate the virus in rabbit and hare primary cell cultures and in certain cell lines (RK-13, PK-15, FL) have not given consistent results. Detection of EBHS virus therefore remains limited to other methods. The virus can be demonstrated in organ homogenates; because the liver has high viral content, homogenates are usually made.
from this organ. The homogenate is prepared by triturating 2 g liver with 4 ml saline solution, 1 ml chloroform and 0.5 ml of a solution of penicillin (100 IU/ml) and streptomycin (100 µg/ml). The triturate is centrifuged at 5,000 rpm for 15 minutes; the supernatant is frozen, then thawed and centrifuged. The supernatant is used for the detection of virus by the following methods:

- negative staining electron microscopy using the standard procedures which enable detection of viral particles of 25 to 40 nm, some of them having a central core;
- haemagglutination of human type "A" or "O" red blood cells at pH 6.4 and at 4°C;
- enzyme-linked immunosorbent assay (ELISA) with a cross-reactive rabbit hyperimmune anti-VHD serum or a mixture of monoclonal antibodies (6).

Serological diagnosis

Antibodies to EBHS can be detected as early as four to five days after infection. Some of the methods used are haemagglutination inhibition and ELISA (6).

DISCUSSION

EBHS is endemic among free living hares in Denmark and southern Sweden, although little is known about prevalence rates. Morbidity and mortality rates in free living hares are unknown, and the effect of EBHS on the size and dynamics of the population of hares has not been quantified. European brown hares in endemic areas are developing active resistance: antibodies are found both in apparently healthy adult and newborn hares. Little is known about reservoirs of EBHS. Probably, European brown hares which survive infection continue to shed virus for long periods and the disease is thus maintained in particular areas. This could explain why EBHS does not occur in northern Sweden, Finland or Norway, where only varying hares are found. In the epidemiological study conducted by the authors, the 275 diagnosed cases actually underestimate the number of hares which die of EBHS in the wild. Each case represents a number of other cases of hares found dead in the wild; such cases should therefore be considered as a series of outbreaks. The reason for the higher frequency of cases during the autumn and winter is unclear.

It is important to note that EBHS cannot be diagnosed only by clinical signs or gross pathological findings. The presence of changes which indicate shock, such as lung oedema and hyperaemia in various organs, is not specific to EBHS. Liver lesions are often very subtle macroscopically and diagnosis should be confirmed by histological or virological methods. Histological diagnosis is reliable because the characteristic lesions in the liver differ from those produced by other known hare pathogens and by most toxins. During routine pathological examinations of hares which die of trauma or other causes, liver changes with variable degrees of chronicity (fibrosis and bile duct proliferation) are found. These lesions may represent a chronic form of EBHS. Similar changes have also been found in farmed hares which survived an outbreak of EBHS in Denmark (Dietz and Henriksen, personal communication), and in free living hares in other European countries where EBHS is endemic.
The relationship between EBHS and VHD of rabbits in Scandinavia has not been completely clarified. There is no epidemiological evidence, however, that cross infection between hares and rabbits occurs. Moreover, on the Swedish island of Gotland, where there is a dense population of wild rabbits, European brown hares and varying hares, the authors have observed the occurrence of EBHS in hares since 1980, while VHD did not appear in wild rabbits until 1990.

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Le virus responsable de l'EBHS a été identifié comme étant apparenté au virus de la maladie hémorragique virale du lapin. Toutes les tentatives d'isolement du virus sur culture cellulaire se sont soldées par un échec. Le diagnostic peut être réalisé par histopathologie ; la détection du virus dans des broyats d'organes s'effectue au moyen du test d'hémagglutination, par observation en microscopie électronique en coloration négative, et par la technique ELISA. On peut détecter précocement les anticorps au moyen de l'épreuve d'inhibition de l'hémagglutination et de la technique ELISA.


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Resumen: Al principio de los años 1980, aparecieron en Suecia los primeros focos de una enfermedad mortal que ocasionaba graves lesiones hepáticas en las liebres pardas europeas (Lepus timidus, Pallas). Esta enfermedad, llamada síndrome de la liebre parda europea (EBHS), fue también reconocida en Dinamarca en 1982; actualmente se ha propagado en toda Dinamarca y en el sur de Suecia. Sin embargo, no se ha señalado ningún caso en Noruega ni en Finlandia. Dos especies de liebres son afectadas en Suecia: la liebre parda europea y la liebre variable (Lepus timidus, Linnaeus). El EBHS afecta indistintamente las liebres en libertad y las liebres en cautiverio; reaparece de manera estacional, durante los meses de octubre, noviembre y diciembre.
El virus responsable del EBHS fue identificado como aparentado al virus de la enfermedad hemorrágica viral del conejo. Todas las tentativas que se realizaron para aislar el virus en cultivo celular han fracasado. El diagnóstico puede realizarse por histopatología. La detección del virus en trituraciones de órganos se efectúa mediante la prueba de hemaglutinación, la observación en microscopía electrónica por coloración negativa, y la técnica ELISA. Se puede detectar precozmente la presencia de los anticuerpos mediante la inhibición de la hemaglutinación, y la técnica ELISA.


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REFERENCES


