Hepatitis of viral origin in Leporidae: introduction and aetiological hypotheses

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Summary: In less than ten years, two very serious viral hepatic diseases have spread through Leporidae populations (rabbits and hares) in numerous countries. In May 1989, the Office International des Epizooties designated this new disease of rabbits “viral haemorrhagic disease” and entered it as a List B disease in the International Animal Health Code.

Clinically, the disease is very similar to the European brown hare syndrome. However, numerous uncertainties prevail today on the true nature of the viruses of the two species. Although they are related, the viruses appear to be different and cross infection between species has given contradictory results.

Hepatitis of Leporidae have probably existed in Europe for several years, although their viral aetiology has been demonstrated only recently. The acute form has occurred in hares in Northern Europe since approximately 1980, while the inapparent (or ignored) form has been present in rabbits in Czechoslovakia since 1975. These diseases of Leporidae are true viral hepatitis which, in their fulminating forms, bear a remarkable resemblance to human viral hepatitis (B and non-A non-B) with regard to clinical symptoms, pathological lesions and mode of transmission.

The dominant faecal-oral transmission observed for types A and E hepatitis would explain the particular susceptibility of family-kept rabbits, as they are fed potentially contaminated fodder.

As the clinically similar fulminating hepatitis in human beings is caused by a diversity of viruses (both RNA and DNA), the disease in Leporidae might also be caused by different viruses.

Although there has been no report of human infection from an animal source, sufficient similarities exist between Leporidae and human viral hepatitis to encourage joint research in the specialised medical fields.


INTRODUCTION

The development of rabbit farming over the past twenty years has been accompanied by an increase in the occurrence and severity of respiratory and digestive diseases resulting from intensive husbandry. There have been bacteriological
investigations of these two groups of diseases, and the role of many bacteria (*Pasteurella, E. coli, Clostridia*) is often associated with environmental factors (close confinement, feeding, increased productivity).

Although a number of viruses have been recovered from rabbits, particularly through laboratory tests, virologists have tended to focus on the only disease of economic importance known to be caused by a virus, myxomatosis.

In 1984, reports from the People’s Republic of China (16, 27, 34) of the rampant spread of a viral haemorrhagic disease (or viral haemorrhagic pneumonia) marked a turning point in the study of the diseases of rabbits (*Oryctolagus cuniculus*).

Not until 1988 was a link established between this disease and a clinically identical disease which had broken out in various European countries, beginning in Southern Italy in 1986, and which also affected Africa (Egypt and Tunisia) and even the Americas (Mexico).

The haemorrhagic nature of the lesions of apparently nutritional origin, and with the liver being prominently affected, led to interpretations of a toxic aetiology. This hypothesis, espoused by the media, was presented as a result of environmental pollution (a notion which defied logic, as only rabbits were susceptible to the toxin or toxins) (3, 5, 21).

The press in Italy blamed the trouble on radioactive fallout from the Chernobyl accident, thus bringing an economic disaster upon rabbit keepers.

Epidemiological and virological observations, as well as pathological studies, eventually demonstrated that the origin of the disease was clearly infectious and, in particular, that this primary hepatic necrosis, which brought about coagulation disorders (14, 15, 19, 21, 23, 33), had a viral origin.

In May 1989, the OIE named this disease *viral haemorrhagic disease (VHD) of rabbits*, and added it to List B of the *International Animal Health Code*.

Although viral particles are easily seen in hepatocytes, attempts to cultivate them have long failed. Chinese research workers (notably C.-Y. Ji) were the first to do so successfully, as reported by W.-Y. Xu and H.-B. Huang in this issue of the *Review*. Previously, the inability to obtain the virus by culture meant that the characteristics of this virus were studied in homogenates of liver. Certain conflicting results were reported; for some authors, it was a DNA virus of the genus *parovirus* (6, 10, 34), while for others it was an RNA virus of the genus *calicivirus* (13, 23, 24).

This ambiguity persists unless two different viruses, which induce a very similar disease, exist.

Since 1985 (and probably earlier), other species of the order Leporidae — the brown hare (*Lepus europaeus*) and the mountain hare (*Lepus timidus*) — have also suffered heavy losses in many countries of Northern and Western Europe (8, 11, 17, 18).

This disease, known as the *European brown hare syndrome (EBHS)*, is accompanied by the same lesions as VHD. As with the disease in rabbits, various
toxic causes were at first postulated, including the ingestion of mercaptodimethur (methiocarb), a molluscicide applied to beet crops, and overeating the “O.O.” variety of oilseed rape.

A bacterial aetiology was also suspected because of the fairly frequent isolation of *Clostridium sordellii* from carcasses (17).

Since 1988, the relatively easy demonstration of viral particles morphologically identical to those of rabbit VHD virus (VHDV) in the liver, together with the similarity of the pathological lesions, led certain research workers to propose a single name for both syndromes: “infectious necrotic hepatitis of Leporidae” (19).

Taking current research into account, the acronyms VHD and EBHS are used in this paper to refer to the disease in rabbits and hares, respectively.

**GEOGRAPHICAL DISTRIBUTION**

Reported for the first time in the People’s Republic of China and in the Republic of Korea in 1984, VHD has occurred in numerous countries on four continents (Table I).

The first cases of EBHS in France were observed in 1985 (17, 18) and the first cases of VHD in July 1988 (21). Since then, the spread of both diseases has been monitored by a network of epidemiological surveillance, based on information received by Departmental diagnostic laboratories throughout France. Diagnosis is mainly post mortem, in some cases supported by virological tests (haemagglutination, immuno-electron microscopy, immunofluorescence) performed at the National Centre for Veterinary and Food Research (CNEVA) in Ploufragan (15).

This provides information on the distribution of the conditions, but prevalence is unknown due to the low percentage of cases submitted for diagnosis. Figure 1 shows how VHD in domestic and wild rabbits overlaps in the same geographical areas with EBHS in hares.

**ORIGIN AND MODE OF TRANSMISSION OF VHD AND EBHS**

**Origin**

Although the clinical form of VHD in rabbits was first observed in the People’s Republic of China and the Republic of Korea in 1984, it is uncertain that the virus originated in Asia.

Tests performed in Czechoslovakia on serum samples kept since 1975 have revealed the presence of antibodies to VHDV in a high percentage of animals (Rodák, article in this issue of the *Review*). This proves that the infection must have existed at that time, either in an inapparent form or in a subacute form which passed unobserved. The very early presence of the virus in Europe is linked to the observation of certain
TABLE I

List of countries reporting the presence of hepatitis of viral origin in Leporidae

<table>
<thead>
<tr>
<th>Country</th>
<th>VHD</th>
<th>EBHS</th>
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<tr>
<td><strong>Asia</strong></td>
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<tr>
<td>People's Republic of China</td>
<td>1984</td>
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<tr>
<td>Republic of Korea</td>
<td>1984</td>
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<td><strong>Europe</strong></td>
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<tr>
<td>Austria</td>
<td>1989</td>
<td>1986</td>
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<tr>
<td>Belgium</td>
<td>1989</td>
<td>1985?</td>
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<tr>
<td>Czechoslovakia</td>
<td>1987</td>
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<td>Denmark</td>
<td>1990</td>
<td>1982/1983</td>
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<td>France</td>
<td>1988</td>
<td>1985/1986</td>
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<td>Germany</td>
<td>1989</td>
<td>1987</td>
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<td>Great Britain</td>
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<td>Greece</td>
<td>1990</td>
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<td>Hungary</td>
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<td>Italy</td>
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<td>Malta</td>
<td>1990</td>
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<td>Poland</td>
<td>1989</td>
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<td>Portugal</td>
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<td>Romania</td>
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<td>Spain</td>
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<td>Sweden</td>
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<td>Switzerland</td>
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<td>USSR</td>
<td>1986/1987?</td>
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<td>Yugoslavia</td>
<td>1989</td>
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<tr>
<td><strong>Africa and Indian Ocean</strong></td>
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<td>Egypt</td>
<td>1988</td>
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<td>Lebanon</td>
<td>1989</td>
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<tr>
<td>Reunion Island</td>
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<td>Tunisia</td>
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<td><strong>Americas</strong></td>
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<tr>
<td>Mexico</td>
<td>1988</td>
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Chinese authors that the disease occurred in their country following the introduction of Angora breeding stock from Germany (34).

Moreover, the presence in Northern Europe of a hepatopathogenic virus with high pathogenicity for populations of hares (L. europaeus and L. timidus) probably dates from 1980 (8, 11).

Hypothesis of transmission between species

The similarity of shared macro- and microscopic lesions, in addition to numerous characteristics held in common (e.g., viral morphology, resistance of young animals, oral transmission), supports the hypothesis of transmission from one species to another.

Various authors in the People’s Republic of China (16), France (22) and Italy (5) have reported successful transmission experiments from hares to rabbits, and also from rabbits to hares.
FIG. 1

Distribution of VHD and EBHS in France in 1990

Number of outbreaks reported during 1990:

- ▲ domestic rabbits  570  VHD
- △ wild rabbits     216  VHD
- ○ hares           406  EBHS

Source: VHD-EBHS Surveillance Network, CNEVA, Ploufragan, France.
However, many more authors insist that cross transmission attempts have completely and consistently failed and that, despite a shared origin, the two calicivirus are different (13).

Knowledge of the characteristics of different strains of virus is still too fragmentary to explain this paradox. Although some epidemiologists affirm that, despite the coexistence of the two species in certain countries, only one of the species is affected, although this is far from being the general rule. Heavy losses from infection of both species simultaneously have been recorded in many countries, including Italy, France and Germany. Finally, ELISA has detected antibodies to VHDV in clinically healthy hares in Czechoslovakia (L. Valíček, personal communication).

Direct transmission within a species

There have been few studies of wild animals, but the pattern of virus circulation among such populations would seem to be identical to that occurring among hares and domestic and wild rabbits. In all cases, development of the virus in hepatocytes and its transport in the bile to the intestine explain why the preferential mode of transmission is the faecal-oral route.

Young animals are resistant to the virus in each of these cases. Experiments recently conducted in France on groups of ten young rabbits aged 4-10 weeks and born of antibody-free dams have shown that their susceptibility, zero at four weeks, increases rapidly thereafter (unpublished personal results) (Fig. 2).

Plasma assays conducted on young control rabbits have revealed a considerable increase in hepatic transaminases after weaning (at five weeks). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities are three times greater at six weeks, demonstrating a considerable change in liver function. This might indicate hypersensitivity of hepatocytes at this age, or the acquisition of a hepatic function capable of eliciting the pathogenic potential of the virus.

Both VHD and EBHS seem to evolve in the same way among wild populations. Following a clinical episode shown by the occurrence of numerous outbreaks in a given area, the population is restored by surviving adults and, in particular, by the naturally resistant young animals. This immunised population multiplies rapidly. In 15 to 18 months, a new wave of mortality, due to weakened immunity aggravated by an expanding population, has often been observed.

There seems to be a juxtaposition of populations of differing immune status among wild rabbits and hares. Fluctuations in immune status may explain the cyclic appearance of EBHS among hares and the persistence of infection in populations, as has been witnessed for some time in certain countries of Northern Europe (8, 11).

Indirect transmission and the possible role of frozen carcasses

The great ease with which the disease is transmitted by all routes employed experimentally explains the rapidity and frequency of natural infection to which domestic and wild Leporidae are exposed. Any equipment or feed contaminated by secretions or excreta of a sick animal, or by simple contact with a carcass, is potentially infective. This explains the heavy losses caused by VHD among rabbits kept on small farms when their diet is composed of green feed and forage.
Fig. 2

Mortality following experimental infection with VHD virus
7 groups of 10 animals aged 4-10 weeks *

Physiological evolution of hepatic transaminases
6 groups of 10 animals aged 3-10 weeks **
Although there is no specific information on this subject, the possibility of insects playing an active or passive role in the transmission of these diseases cannot be dismissed.

With regard to the possible role of frozen carcasses of rabbits, it should be recalled that the virus is extremely resistant in the environment. The virus maintains its infectivity within carcasses in more or less advanced putrefaction and remains remarkably stable during freezing and thawing. Although it appears that the virus has never been detected in frozen carcasses, this mode of infection cannot be excluded, particularly in the light of the following two incidents:

In Mexico, the occurrence of VHD in December 1988 was associated chronologically and topographically with the storage of frozen carcasses imported from the People's Republic of China (9, 33). However, epidemiologists of the Mexican Directorate General of Animal Health obtained no evidence of contact (such as by waste water or rodents) between the carcasses, which were stored properly, and the first farms to be affected, which were at least 200 m from the storage place (9).

Reunion Island imports 380-400 tonnes of frozen meat of Chinese origin every year. In 1989, the Rabbit Producers Cooperative reported outbreaks of VHD under very unusual circumstances (V. Demergers, personal communication). The outbreaks apparently occurred after a stock of frozen meat had been disposed of in an open field. The meat might have been spoilt by a breakdown in refrigeration caused by the passage of a cyclone in January 1989 and there was no rendering plant on the island. The large number of dogs on the island may have provided the contact between the rejected carcasses and the many farmed colonies.

Although the occurrence of VHD in these two cases is indisputable, the origin of the disease, although probable, cannot be confirmed. It seems that in neither Mexico nor Reunion Island were the carcasses of Chinese origin directly or indirectly tested for virus.

COMPARISON BETWEEN HUMAN VIRAL HEPATITIS AND VIRAL HAEMORRHAGIC DISEASE OF LEPORIDAE

It is important to note that no cases of transmission of VHD or EBHS to human beings have been reported, even among such persons regularly exposed to animals as farmers, hunters, abattoir workers and research workers.

Regardless of their viral aetiology, however, infectious necrotic hepatitis of Leporidae have a certain number of similarities with their human counterparts.

Classification of human viral hepatitis

In this rapidly developing field, the present classification of viral hepatitis and their causal agents are as follows (7, 12, 25):

- **hepatitis A**: RNA virus of the **picornavirus** type;
- **hepatitis B**: DNA virus of the **hepadnavirus** type;
- **hepatitis Delta**: the delta particle is a defective RNA virus requiring the surface antigen of hepatitis B virus (HBsAg) for replication (30).
Other hepatitis which are neither A nor B are grouped as non-A non-B, in particular (26, 28, 29):

- hepatitis C: attributed to a flavivirus (RNA virus), and
- hepatitis E: attributed to a calicivirus (RNA virus).

Neither of the latter two viruses has yet been isolated in culture, and vaccinal protection is available only against hepatitis B (although a vaccine against hepatitis A is being developed).

**Virological aspects**

When considering the diversity of both the DNA and the RNA viruses implicated in human hepatitis, and the fact that some of them produce an almost identical clinical picture, it should be noted that these observations could clarify the ambiguity surrounding the identity of the virus (or viruses) of hepatitis of Leporidae, namely: an RNA virus of the calicivirus type or a DNA virus of the parovirus type.

As research of equal value has led to diametrically opposed conclusions, the existence of two or several viruses expressed clinically as very similar forms of necrotic hepatitis could be conceivable.

**DNA virus**

Although there is no risk of confusing the viral particles of VHD or EBHS with those of hepatitis B (since hepadnavirus has characteristic polymorphism, with small particles measuring 22 nm, large Dane’s particles of 42 nm and filaments of 100 nm), it is interesting to recall that the hepadnavirus group includes viruses capable of inducing hepatitis in ducks (duck hepatitis virus) and in species phylogenetically close to Leporidae: the American marmot (woodchuck hepadnavirus) and squirrel (ground squirrel and tree squirrel hepadnaviruses) (1, 4, 20).

Many young people acquire inapparent infection with hepatitis B virus and, independently of an enhanced risk of primary hepatic cancer, subsequent activation of the B virus by a delta particle (defective RNA virus) may result in clinical acute or hyperacute hepatitis. Initial tests conducted in France for anti-B and anti-Delta antibodies in serum from convalescent rabbits have given negative results (M. Menault and J. Pillot, personal communications).

**RNA virus**

Among the RNA viruses responsible for human hepatitis, hepatitis A virus shares the following characteristics with the agent of hepatitis of Leporidae:

- both belong to the same, or a similar, virus family, certain Chinese workers having identified VHD virus as a picornavirus (16);
- both have exceptional resistance in the environment and can withstand heat (30 min at 60°C and 10 min at 80°). These characteristics make hepatitis A virus one of the most resistant viruses (31).

Hepatitis A virus differs from VHD virus in that it produces relatively benign hepatitis.

Among the RNA viruses responsible for severe hepatitis in man, hepatitis E virus (recently identified as a calicivirus, as were VHDV and EBHSV) is capable of producing hyperacute hepatitis, which is often fatal in certain populations at risk.
Great care has to be taken when storing hepatitis E virus (−80°C), which is not the case for VHDV. The fragility of the virus in the laboratory poses a particular problem for epidemiologists as, logically, this hinders, or prohibits, the spread of the virus among human populations.

Clinical and histological aspects

The haemorrhagic syndrome which occurs in animals due to a failure to synthesise certain coagulation factors of hepatic origin is also characteristic of some forms of human hepatitis, called "fulminating hepatitis" (types B and E). When hyperacute, these forms of hepatitis can be fatal; this is particularly true of hepatitis E in pregnant women in certain countries of South-East Asia (12, 25, 26, 28, 29).

As with hepatitis E in human beings, animals are also affected by disseminated intravascular coagulation, attributable to secondary involvement of bacterial endotoxins following viral infection of hepatocytes (19, 28).

There are various reports on the similarity of lesions observed in hepatocytes in Leporidae and non-A non-B hepatitis in man (19, 28). Necrosis, with accumulation of bile pigments and haemosiderin in hepatocytes and in Kupffer's cells, occurs in both cases. The hepatocytes are hypertrophied ("ballooned") and the nuclei contain fine granulations, with movement of chromatin to the margins.

Epidemiological aspects

The parenteral route, mainly through transfusion, is the principal route of transmission of viral hepatitis B, Delta and C, while hepatitis A and E are transmitted mainly by the digestive route (7, 12).

In both the latter forms, and in VHD and EBHS as well, infection occurs after ingestion of contaminated water or food. The virus develops in hepatocytes, is carried in the bile to the intestine and is excreted in large quantities in faeces.

In the case of human viral hepatitis transmitted by the faecal-oral route, the frequency of infection is governed by the standard of living and particularly by the level of hygiene. In most developing countries, cases of hepatitis A are generally recorded during the first ten years of life. In many European countries, 80-90% of persons aged between 40 and 50 are immune (32).

It should be noted that, when hepatitis of viral origin in Leporidae occur, at least among domestic rabbits, the method of feeding has a decisive influence on the frequency of the disease. The forage and green feed used exclusively in small family rabbitries are the main sources of infection (21; J.P. Filleul, personal communication).

Although current knowledge of non-A non-B human hepatitis is still incomplete, prolonged excretion of virus and asymptomatic carriers of hepatitis B are known to occur. The persistence of infection among rabbits in certain Central European countries since 1975, and among hares in Sweden since 1980, would be explained if such an asymptomatic state occurred in animals.

To date, it has not been possible to cultivate the agent of hepatitis E; the virus is propagated by inoculating infective material into various primate species (Rhesus or Cynomolgus). Very recently, attempts to infect piglets have succeeded, resulting in clinical hepatitis, increased ALT activity and viral particles found in the faeces.
This raises the important question of the potential role of animals, particularly of pigs, in spreading the virus (2).

CONCLUSIONS

There are certain pathological, clinical and epidemiological similarities between hepatitis of viral origin in Leporidae and certain viral hepatitis of human beings.

These similarities lead to certain hypotheses concerning hepatitis of Leporidae, particularly the possibility that:

- there is more than one virus responsible
- there are chronic and/or asymptomatic carriers
- the asymptomatic carrier state could hypothetically be activated by a defective virus
- endotoxins have a role in pathogenesis.

Further research is required to confirm or reject these possibilities.

Although the problem of the survival of rabbit farming has already been resolved by a first generation of inactivated vaccines, the problem of control of the disease among wild populations remains unanswered. To the ecological and economic significance of the disease may be added the interest aroused by new diseases of animals which closely resemble certain diseases of man. Leporidae may not serve as a perfect experimental model, but they are susceptible to hepatitis sufficiently similar to certain viral hepatitis of man to encourage joint research in specialised medical circles.

**

REFERENCES


