

# RABBIT HAEMORRHAGIC DISEASE

Aetiology Epidemiology Diagnosis Prevention and Control References

## AETIOLOGY

### **Classification of the causative agent**

Family Caliciviridae, Genus *Lagovirus*, Rabbit haemorrhagic disease virus (RHDV)

RHDV was identified in 1984 as agent of a highly contagious, acute and fatal disease of rabbits (RHD). As consequence of its worldwide spread, RHDV differentiated into many strains, all highly virulent and with a close genetic relationship. There is a single serotype with two subtypes: RHDV and the antigenic variant RHDVa (identified in Europe in 1996 but probably already present in China since 1985). In addition to RHDV, other non-virulent caliciviruses have been identified, both in commercial and wild rabbits, that are genetically related to RHDV in varying degrees. Among these viruses, those most closely genetically related to RHDV (identified in several European rabbit farms) induce a high level of cross-protection towards RHD. Recently a new non-pathogenic calicivirus genetically related to RHDV has been identified in Australian wild rabbit populations but infected rabbits show a very limited degree of protection, if any, when challenged with RHDV.

A closely related calicivirus that emerged in the 1980s causes a similar disease in the hare (*Lepus europaeus*) termed European brown hare syndrome (EBHS). In spite of the overt genetic relationship between the two viruses and their almost contemporaneous identification, RHDV and EBHSV represent two viral species, antigenically distinct and with non-overlapping host ranges.

### **Resistance to physical and chemical action**

Temperature: Survives heat of 50°C for an hour, and freeze–thaw cycles.

pH: Stable at pH 4.5–10.5. Survives pH 3.0 but inactivated at pH >12.

Chemicals: Inactivated with sodium hydroxide (1%) or formalin (1-2%). OIE Code recommends formalin (3%) for disinfecting pelts. Treatment with 1.0–1.4% formaldehyde or 0.2–0.5% beta-propiolactone at 4°C inactivates the virus but does not reduce its immunogenicity and is therefore indicated for the production of vaccines.

Disinfectants: Other suggested disinfectants include substituted phenolics (e.g. 2% One-stroke Environ®) and 0.5% sodium hypochlorite. Viral infectivity is not reduced by ether or chloroform and trypsin.

Survival: RHDV and EBHSV are very resistant to inactivation, particularly when protected by organic material. Virus may persist in chilled or frozen rabbit meat, as well as in decomposing carcasses in the environment, for months. It is protected within tissues, and can survive >7 months in organ suspensions stored at 4°C, at least 3 months in the dried state on cloth at room temperature, for up to 20 days at 22°C in decomposing rabbit carcasses and at least 2 days at 60°C in an organ suspension and the dried state.

## EPIDEMIOLOGY

Rabbit haemorrhagic disease (RHD) is an extremely contagious and fatal viral disease of domesticated and wild rabbits belonging to the *Oryctolagus cuniculus* species. Severe losses are common in unvaccinated animals; on some farms, most or all of the rabbits may die (80–90% lethality). This disease has also caused dramatic declines in some wild rabbit populations, particularly when it is first introduced. RHD spreads very readily.

### **Hosts**

- Rabbit haemorrhagic disease affects wild and domesticated members of the species *Oryctolagus cuniculus*, the European rabbit.
- Other rabbit species including cottontails (*Sylvilagus floridanus*), black-tailed jackrabbits (*Lepus californicus*) and volcano rabbits (*Romerolagus diazzi*) are not susceptible.
- Similarly, European brown hares (*Lepus europaeus*) and other hare species (*Lepus timidus*, *Lepus corsicanus*, *Lepus granatensis*, *Lepus capensis*) are not affected by RHDV, although

they are affected by a disease caused by a different calicivirus (European brown hare syndrome – EBHS).

- Although rabbits of all ages can be infected, in animals younger than 40–50 days of age the infection is subclinical
- Virus replication has not been reported in other mammals, including rabbit predators, although seroconversion can occur. Inoculation of tissue suspensions from infected rabbits into 28 different vertebrate species other than rabbits failed to produce disease and no replication of the virus was detected by reverse-transcription polymerase chain reaction.

### **Transmission**

- Direct contact with infected animals through the oral, nasal or conjunctival routes.
- Exposure to an infected carcass or hair from an infected animal.
- By means of fomites, including contaminated food, bedding and water.
- Experimental transmission by oral, nasal, subcutaneous, intramuscular, or intravenous routes.
- Importation of infected rabbit meat. This could be one of the main means of transmission of RHD to a new area. Meat contains high levels of virus infected blood with which survives freezing well.
- Mechanical transmission. Flies and other insects are very efficient mechanical vectors; only a few virions are needed to infect a rabbit by the conjunctival route. Wild animals can transmit the virus mechanically. Although virus replication does not seem to occur in predators or scavengers, these animals (dogs, foxes, etc.) can excrete RHDV in faeces after eating infected rabbits.
- How long RHD recovered rabbits may remain infectious is still uncertain. Indeed, low level of serum antibodies is sufficient to protect rabbits from the disease but infection at intestinal level could occur with the shedding of the virus in the faeces. High sensitivity PCR demonstrated a long-term persistence (up two months) of the viral RNA in recovered or in vaccinated and the infected rabbits. Whether this is due to real and active persistent or latent RHDV infections is still to be demonstrated.

### **Sources of virus**

- Liver has the highest titre of virus, followed by the spleen and serum.
- Most or all excretions including urine, faeces and respiratory secretions are thought to contain virus.
- Rabbit meat contains virus by virtue of its high blood supply

### **Occurrence**

- RHD was first reported in 1984 in the People's Republic of China and in 1986–88 in Europe.
- RHD has been reported in over 40 countries in all the Continents. It is still endemic in most parts of the world, certainly in commercial and wild rabbits in Europe and in wild rabbit populations of Australia and New Zealand.
- Outbreaks have also been recorded in Saudi Arabia and West and North Africa.
- In 2000 and 2001, three independent outbreaks were recorded in the United States of America (USA).
- At the end of 2004 it was reported in Uruguay and in 2005 and 2008 again in the USA.

**For more recent, detailed information on the occurrence of this disease worldwide, see the OIE World Animal Health Information Database (WAHID) Interface** [<http://www.oie.int/wahis/public.php?page=home>] or refer to the latest issues of the World Animal Health and the OIE *Bulletin*.

### **DIAGNOSIS**

The incubation period is one to three days, and death usually occurs 12–36 hours after the onset of fever.

- A presumptive diagnosis can be made in an unvaccinated rabbitry when there are multiple cases of sudden death following a short period of lethargy and fever, and characteristic hepatic necrosis and haemorrhages are visible at necropsy
- A field diagnosis is more difficult when there are few rabbits on the premises, rabbits are relatively isolated, as in research colonies, or the rabbitries are partially vaccinated.

- The clinical manifestations have been described mainly in the acute infection (nervous and respiratory signs, apathy and anorexia). Clear and specific lesions, both gross and microscopic, are present. There is primary liver necrosis and a massive disseminated intravascular coagulopathy in all organs and tissues. The most severe lesions are in the liver, trachea and lungs. Petechiae are evident in almost all organs and are accompanied by poor blood coagulation.
- The morbidity rate varies from 30% to 100%, and the mortality rate is 40–100%. The highest morbidity and mortality rates are seen in adult rabbits from naïve populations. Young rabbits less than eight weeks old are less likely to become ill or die. Rabbits four weeks of age and younger are unaffected. The age-related resistance in very young rabbits is still poorly understood. Surviving rabbits develop immunity and become resistant to related strains of RHDV.
- In wild rabbits, outbreaks can be seasonal. In some populations, they have been associated with the breeding season.
- The morbidity and mortality rates vary between populations. In Europe, rabbit haemorrhagic disease has caused dramatic declines in wild rabbit populations in Spain, Portugal and France, but wild rabbits in the U.K. and some other Northern European countries have been less severely affected. Such different evolution is most probably related to the circulation and presence in wild rabbits of non-virulent RHDV-like strains, which induce cross protection.

### ***Clinical diagnosis***

While the clinical evolution of the disease can be peracute, acute, subacute or chronic, clinical manifestations have been described mainly in the acute infection, as there are usually no clinical signs of disease in the peracute form, and the subacute form is characterised by similar but milder signs. The incubation period varies between 1 and 3 days; death may occur 12–36 hours after the onset of fever (>40°C). During this phase, various signs could be observed such as anorexia, apathy, dullness, prostration, nervous signs (convulsion, ataxia, paralysis, opisthotonos, paddling), groans and cries, respiratory signs (dyspnoea, frothy and bloody nasal discharge), cyanosis of mucous membranes. During an outbreak, a limited number of rabbits (5–10%) may show a chronic or subclinical evolution of the disease, which is characterised by severe and generalised jaundice, loss of weight and lethargy. These animals often die 1 or 2 weeks later, probably due to liver dysfunction.

### ***Lesions***

Due to the rapid course of this disease, the animals are usually found in good condition after death. Gross pathological lesions are variable and may be subtle and include circulatory and degenerative disorders. Liver necrosis and splenomegaly are the primary lesions. The liver appears yellowish-brown in colour, brittle and degenerated, with a marked lobular pattern. The tracheal mucosa is hyperaemic, containing abundant frothy fluid, and the lungs are oedematous and congested. The spleen is engorged, with rounded edges and enlarged (splenomegaly). The presence of clotted blood in blood vessels is due to disseminated intravascular coagulation (DIC). Such massive coagulopathy is usually the cause of haemorrhages in a variety of organs and sudden death. In subacute and chronic disease, an icteric discoloration of the ears, conjunctiva and subcutis is clearly evident.

### ***Differential diagnosis***

- septicaemic pasteurellosis
- poisoning
- heat exhaustion
- other causes of severe septicaemia with secondary DIC

### ***Laboratory diagnosis***

#### **Samples**

- Fresh liver, spleen, and blood
- Formalin-fixed samples of liver, spleen, lung, kidney and other organs

The liver contains the highest viral titre (from  $10_3$  LD<sub>50</sub> [50% lethal dose] to  $10_{6.5}$  LD<sub>50</sub>) in acute or peracute disease, and is the organ of choice for viral identification of both RHDV and EBHSV. Serum and spleen may also contain high levels of virus. In rabbits with chronic or subacute disease, RHDV may be easier to

find in the spleen than the liver. RT-PCR can detect viral RNA in a many organs, urine, faeces or serum. Serum should be collected for serology.

## Procedures

### *Identification of the agent*

- Haemagglutination (HA) test. First test used for routine laboratory diagnosis of RHD, but it is less sensitive and specific than other assays, and requires human type O red blood cells – now replaced by virus detection ELISA. HA test is performed on 10% tissue homogenate of liver or spleen. HA may give false negative results in the chronic form of RHD.
- Electron microscopy: Negative-staining EM, immuno-EM, and immunogold EM. For diagnostic purposes and when other methods give doubtful results, the best EM method is an immuno-EM technique (IEM). This induces clumping of viral particles into aggregates that are quickly and easily identified by EM.
- Virus detection enzyme-linked immunosorbent assay (ELISA). Performed on 10% liver homogenate. A monoclonal antibody-based ELISA developed at the OIE reference laboratory for RHD enables the subtyping of RHDV isolates
- Immunostaining. Tissue fixed in 10% buffered formalin and embedded in paraffin can be immunostained using an avidin–biotin complex (ABC) peroxidase method with intense staining mainly in periportal areas of the liver, macrophages of the lungs, spleen and lymph nodes, and mesangial cells of the kidney. Tissue cryosections of liver, spleen and kidney fixed in methanol can also be directly immunostained for specific fluorescence.
- Western blotting. Used when HA or ELISA are inconclusive.
- Reverse transcription polymerase chain reaction (RT-PCR) tests. Ideal rapid diagnostic test for RHD because of high sensitivity and low level of sequence variation among RHDV isolates. This method is performed on organ specimens (optimally liver), urine, faeces and sera. A similar RT-PCR method has been used to identify the nonpathogenic RCV and EBHSV. Not strictly necessary for routine diagnosis, but it is more sensitive (10<sup>4</sup>-fold more sensitive than ELISA), convenient and rapid than other tests. Considering its high sensitivity and the complicated epidemiological picture of rabbits caliciviruses, PCR results require a careful interpretation.
- *In-situ* hybridisation. Highly sensitive and can detect RHDV as early as 6–8 hours after infection, but this technique is mainly used in research.
- Never grown in cell cultures. Rabbit inoculation remains the only way of isolating, propagating and titrating the infectivity of RHDV. Not a practical method for the routine diagnosis of RHD, but could be useful for the testing of samples that give equivocal test results (e.g. HA negative/ELISA positive) or for the initial diagnosis of the disease in countries where RHD is not known to exist.

### *Serological tests*

Characterisation and titration of specific antibodies arising from natural infection or from immunisation are performed using the haemagglutination inhibition test or an indirect or competitive ELISA. Antibodies may be detected experimentally 4–6 days post-inoculation. Humoral response has great importance in protecting animals from RHD.

At least three basic techniques are applied for the serological diagnosis of RHDV:

- Haemagglutination inhibition (HI).
- Indirect ELISA (I-ELISA)
- Competitive ELISA (C-ELISA)

Each of these methods has advantages and disadvantages. With respect to the availability of reagents and technical complexity, HI is the most convenient method, followed by the I-ELISA and C-ELISA, respectively. However, both ELISAs are quicker and easier than HI, particularly when a large number of samples are tested. The specificity of the C-ELISA is markedly higher than the other two methods. Indirect ELISA (RHDV directly adsorbed onto the solid phase of the plate) is the test of choice to detect cross-reactive antibodies induced in rabbit also by non-pathogenic calicivirus and EBHSV.

An isotype specific ELISA (detecting IgM, IgA and IgG) has been very useful for epidemiological studies both in commercial rabbits and wild population.

For more detailed information regarding laboratory diagnostic methodologies please refer to Chapter 2.6.2 Rabbit haemorrhagic disease in the latest edition of the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* under the heading “Diagnostic Techniques”.

## **PREVENTION AND CONTROL**

### **Sanitary prophylaxis**

- In uninfected countries prevention of introduction is the optimal control method. Restrictions are placed on the importation of rabbits, meat and angora wool from endemic areas.
- In an outbreak, strict quarantine is necessary.
- If wild rabbits are not susceptible (i.e. *Sylvilagus* spp. and *Romerolagus* spp.), control through stamping out is possible.
- RHDV is extremely contagious; it can be transmitted on fomites and by insects, birds and scavenging mammals. Therefore eradication can be accomplished by depopulation, disinfection, surveillance and quarantines.
- Sentinel rabbits can be used on treated premises to monitor for persistent viruses.
- In regions where RHDV circulates in wild rabbits, eradication is not feasible. Instead, this disease is controlled in domesticated rabbits with biosecurity measures including sanitation and disinfection, the maintenance of closed colonies, and vaccination.
- Vaccination may be limited to breeding animals if RHD has not been reported on a farm, but all animals should be vaccinated if an outbreak has occurred. Even with strict sanitation and other control measures, the likelihood of reinfection is high after an outbreak, due to the possible persistence of the virus in the environment.

### **Medical prophylaxis**

- Immunity is solid following natural infection. However, because the virus is hardy in the environment and the disease becomes endemic in populations, it is probable that recovered animals are repeatedly exposed to the virus to re-boost immunity.
- In endemic areas where control is desirable, a vaccine consisting of clarified liver suspension that has been inactivated and adjuvanted is used. This inactivated vaccine is administered initially twice at a 2-week interval, and then annually.
- Vaccinated animals quickly produce strong systemic immunity but a low, if any, mucosal immunity (no IgA production). As consequence, animal are fully protected from the disease but not from infection that primary occurs at intestinal level.
- It is advisable to vaccinate only breeding stock; vaccination of meat animals is not necessary if disease has not occurred on the farm. In some countries, vaccine is commercially available.
- Recombinant vaccines administered by the parenteral and oral routes are being developed but are not yet registered and commercially available.

For more detailed information regarding vaccines, please refer to Chapter 2.6.2 Rabbit haemorrhagic disease in the latest edition of the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* under the heading “Requirements for Vaccines”.

For more detailed information regarding safe international trade in terrestrial animals and their products, please refer to the latest edition of the OIE *Terrestrial Animal Health Code*.

## **REFERENCES AND OTHER INFORMATION**

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The OIE will periodically update the OIE Technical Disease Cards. Please send relevant new references and proposed modifications to the OIE Scientific and Technical Department ([scientific.dept@oie.int](mailto:scientific.dept@oie.int)). Last updated October 2009.