The first U.S. outbreak of rabbit hemorrhagic disease (RHD), an acutely lethal, pandemic disease affecting adult rabbits, has been reported.

**RHD**

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**ABSTRACT:** Rabbit hemorrhagic disease (RHD) is responsible for the deaths of millions of pet, commercial, and free-ranging rabbits worldwide. This article reviews the clinical signs, history, etiology, pathology, diagnosis, and control methods of RHD. The details surrounding the first outbreak of RHD in the United States and its implications are also explored.

With mortality rates as high as 100% in affected rabbits, rabbit hemorrhagic disease (RHD), also known as rabbit calicivirus disease, has become—for many rabbit producers—the most dreaded viral disease since myxomatosis. Paradoxically, in countries such as New Zealand and Australia where free-ranging rabbits are considered major vertebrate pests, RHD has been used and in some circles heralded as an acceptable biologic control agent to drastically reduce free-ranging rabbit populations.\(^1,2\) RHD is primarily a peracute to acute, highly infectious, and lethal disease of domestic and free-ranging adult rabbits of the species *Oryctolagus cuniculus*. Rabbits become febrile (with temperatures up to 105°F) within 16 to 48 hours of infection and generally die within 6 to 24 hours after the onset of clinical signs.\(^3\) The most common sign in peracute cases is sudden death with little or no distinct clinical findings. In acute cases, clinical signs are generally nonspecific and may include anorexia, depression, dyspnea, diarrhea, constipation, and, in late stages, central nervous system problems (i.e., paddling, ataxia, convulsion, opisthotonos). A foamy, bloody discharge from the nostrils has been reported in approximately 20% of affected rabbits (Figure 1).\(^3,5\) Rabbits that survive the acute phase may be persistently infected and shed the virus for 4 weeks or more. Infection occurs in rabbits of all ages, but clinical disease is generally not observed in rabbits younger than 5 to 7 weeks of age. The mechanism of resistance in young rabbits has yet to be elucidated.\(^4,6\)

**HISTORY**

Rabbit hemorrhagic disease has been reported in numerous countries in which both domestic and free-ranging rabbits thrive, including most recently the United States.\(^7\) The first report of this disease occurred in 1984 in a group of rabbits that had been sent from Germany to China.\(^8\) In a relatively short time, RHD spread to other parts of Asia (Korea, Russia, India, Taiwan), Europe (Austria, Belgium, Czech Republic, France, Germany, Italy, Portugal, Spain, Slovakia, Sweden, Switzerland), the United Kingdom, and Ireland. This disease has affected rabbits in North Africa and the Mediterranean region, including Benin,
The lethality of the FECAL–ORAL CONTACT
However, the piglets did not species) as well as hares (Micro-
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species), the cotton-
Piglets inoculated subcu-
More commonly, the virus is inoculated
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5,15
These properties contribute to the highly con-
Figure 1—A rabbit exhibiting a foamy, bloody nasal discharge
as a result of rabbit hemorrhagic disease. (Courtesy of the
Egypt, Morocco, Saudi Arabia, and Tunisia. RHD has also been reported in Australia, New Zealand, Mexico,
Conversely, in Australia, free-ranging rabbits are estimated to cause more than $100 million/year in lost production to farm industries (e.g., sheep production). These rabbits significantly damage native flora, which in turn endangers the native wildlife species. In this sense, RHD has had a positive economic impact in Australia.1

ETIOLOGY
Based on capsid morphology, physical chemistry, protein composition, nucleic acid type, and replication strategy, several laboratories have contributed to the definitive identification and characterization of the causative agent of RHD as a calicivirus.4,5 The International Committee for Taxonomy of Viruses recently subdivided the Caliciviridae into four genera. The RHD virus and the European brown hare syndrome virus (EBHSV), which causes a disease similar to RHD but only affects hares, were placed in the genus Lagovirus.12 Caliciviruses cause a broad spectrum of diseases, including respiratory diseases in cats, vesicular lesions in swine and sea mammals, gastroenteritis in humans, and necro-
thizing hepatitis in rabbits and hares.13

Although the RHD virus has not been propagated in an established cell culture system, the virus was recently inoculated and recovered from isolated primary rabbit hepatocytes.14 More commonly, the virus is inoculated and grown in live rabbits. Only rabbits of the genus Oryctolagus are susceptible to the RHD virus. Rabbits species such as the volcano (Romeroiagus species), the black-tailed jackrabbit (Lepus species), and the cotton-

PATHOLOGY
Although not specific to RHD, the most severe gross pathologic lesions are observed in the liver, trachea, and lungs. The liver is generally pale to yellowish-brown, friable, and degenerated or congested with a marked lobular pattern (Figure 2). The tracheal mucosa is usually hyperemic and contains a frothy blood-tinged fluid. The lungs are often edematous and congested. The spleen is usually enlarged and congested.6 Disseminated intravascular coagulation is a characteristic feature of the pathogenesis of RHD.19 Multifocal petechial hemorrhages are evident in most organs, including the liver, lungs, kidney, heart, and visceral serosa (Figure 3). Hematologically, fibrin thrombi, lymphopenia, platelet reduction, and exhaustion of blood clotting factors are typically observed.19 Microscopic lesions of the liver are usually described as multifocal to coalescing areas of necrosis with or without intrasi-

Figure 1—A rabbit exhibiting a foamy, bloody nasal discharge as a result of rabbit hemorrhagic disease. (Courtesy of the Armed Forces Institute of Pathology, Washington DC)
nusoidal microthrombi. In a recent study, hepatic cell death induced by RHD viral infection was attributed to apoptosis (programmed cell death) as opposed to necrosis. Hepatocyte apoptosis produced extensive parenchymal destruction resulting in a lethal, acute hepatitis in experimentally inoculated rabbits. Apoptosis of intravascular monocytes and endothelial cells was also observed along with fibrin thrombi in blood vessels. Microscopic lesions in the trachea and lungs are characterized by edema and microthrombosis. The most consistent microscopic lesions observed are microthrombosis and karyorrhexis of the lymphoid tissue.

**DIAGNOSIS**

The liver, which contains the highest viral titer, is the tissue of choice for viral identification in suspected cases of RHD. The spleen and serum, which are also rich in virus, can serve as alternative diagnostic materials. The HA test, using either human type O or guinea pig erythrocytes, is used for routine laboratory diagnosis of RHD. In this test, agglutination at an end-point dilution of greater than 1/160 is considered positive. Positive results are confirmed by ELISA, electron microscopy (EM), or immunostaining. Because the virus cannot be grown in established cell lines, experimentally reproducing the disease remains a vital diagnostic tool, especially in difficult cases that may be both HA negative and ELISA positive. The method of choice for diagnostic purposes, especially when other methods give doubtful results, is immunoelectron microscopy technique. This method uses an immunologic reaction to induce clumping of viral particles into aggregates that can be quickly and easily identified by EM. It is recommended that unfixed liver, heparinized blood and serum, and fixed liver, spleen, kidney, lung, small intestine, and brain be sent to a state diagnostic laboratory to confirm all suspected cases. In the United States, diagnostic questions can also be directed to the National Veterinary Services Laboratories, Animal and Plant Health Inspection Service (APHIS), Foreign Animal Disease Diagnostic Laboratory (Plum Island), U.S. Department of Agriculture (USDA), P.O. Box 848, Greenport, NY, 11944; phone: 631-323-3017; fax: 631-323-3366; attn: Tom McKenna.

**RABBIT HEMORRHAGIC DISEASE IN THE UNITED STATES**

The first suspected outbreak of RHD in the United States was confirmed on April 7, 2000, by the National Institute for Agrarian Research, Madrid, Spain. This case typifies the sudden onset and devastating nature of the RHD virus. The outbreak occurred in Dennison, Iowa, on a small, rural farm where rabbits were raised for exhibition purposes. In all, 25 of 27 susceptible rabbits died of RHD; the two remaining rabbits were euthanized and incinerated by federal officials. The first death was a companion rabbit that was allowed to roam outside the family home. On March 9, the rabbit died without exhibiting recognized clinical signs. One week later, rabbits maintained in outdoor hutches also began dying abruptly without showing clinical signs. On March 22, a veterinarian forwarded tissue samples to the Iowa State University Veterinary Diagnostic Laboratory. Based on clinical history and microscopic lesions of the liver, RHD or toxic hepatopathy was suspected. On March 24, a second rabbit was submitted with similar lesions. State and federal offices were notified on March 27 and a foreign animal disease investigation was initiated. On March 31, the USDA’s Foreign Animal Disease Diagnostic Laboratory (FADDL) tentative-
ly made a diagnosis of RHD based on HA analysis and EM of liver homogenate from inoculated rabbits.

Using polymerase chain reaction analysis on rabbit liver samples received from FADDL, the National Institute for Agrarian Research found that the viral (Iowa) isolate was indistinguishable from the viral subtype (RHDVa) that was previously identified in Italy and Germany. This finding was important in that it eliminated New Zealand and Australia as sources of the virus found in the United States and pointed to Europe, where RHDVa originated. However, the source and pathway of the virus into the United States, particularly to the remote Iowa location, has yet to be determined. Rabbits are not considered livestock in the United States; therefore, importation is not regulated. The importation of live rabbits or rabbit products from RHD endemic areas remains a potential source for introduction of the virus into the United States. To complicate matters, the Australia RHD isolate can currently be mail-ordered from a company in Dunedin, New Zealand.

The investigation of RHD in Iowa is considered closed by both state and federal officials. In order to contain the outbreak, the remaining rabbits were destroyed, the site was quarantined, the hutches and equipment were burned, and the area was decontaminated. Epidemiologic inquiries at surrounding farms and businesses in the county appear to support the USDA-APHIS position that the disease no longer exists in the United States. Technically, however, according to Article 2.8.3.2. in the International Standards published by the Office International des Epizooties regarding RHD, a country may be considered free from RHD when the disease has not been present for at least a year. It also requires that no vaccinations have been carried out in the previous 12 months, and that virologic or serologic surveys in both domestic and free-ranging rabbit populations confirm the absence of the disease. The 1-year period can be reduced to 6 months if the country adopts a stamping-out policy. Although it is difficult, regaining disease-free status is not unprecedented. A large-scale rigorous slaughter and disinfection regimen, in conjunction with movement restrictions, sentinel rabbits, and subsequent surveillance, was successfully employed in the eradication of RHD from Mexico.

CONTROL AND PREVENTION

Because of the sudden onset and rapid progression of RHD, no treatment exists. Consequently, three options—prevention, eradication, and vaccination (or a combination)—are available for managing this disease. Preventing the entrance of virus into a country or rabbitry remains the first line of defense against RHD. As implied earlier, however, this can be problematic because clinically normal animals (e.g., recovered from disease but still shedding virus) can be legally imported into the United States without restriction, testing, or quarantine. Private owners and commercial vendors of rabbits should establish strict quarantine procedures (e.g., isolation of incoming animals, disinfection of objects that rabbits come into contact with, erection of fencing around outside hutches). A minimum of 1 week of isolation should be instituted for new additions or for rabbits returning (e.g., shows, escapes) to a facility.

Eradication is most feasible in countries in which free-ranging rabbit populations are not susceptible or the disease has not been established. In epizootic areas, as was the case in Mexico, RHD can be eradicated by depopulating susceptible rabbits from the affected areas, disinfecting facilities and equipment with appropriate agents (e.g., 10% household bleach), use of sentinel rabbits after 30 days, and repopulation with RHD virus-free rabbits. Vaccines have not been employed in these areas because an antibody response to the natural disease cannot be distinguished from a vaccine response. Moreover, if not fully protected by the vaccine, rabbits could present as clinically normal yet may be persistently infected. In enzootic areas such as Europe, RHD is controlled by maintaining closed colonies, adhering to strict sanitation, and by vaccination of breeding stock.

Commercially available virus antigen harvested from experimentally infected rabbits, inactivated (e.g., formalin), and given parenterally with or without adjuvants (e.g., oil, aluminum hydroxide), provides effective protection of adult rabbits from RHD. In most cases, immunity develops a few days postinoculation and requires revaccination every 6 months. Currently, there are three manufacturers licensed to sell killed (inactivated) RHD vaccine in Europe. The available products are Cylap® (Fort Dodge Veterinaria, SA; Spain), Cinical® (Merial, France), and Lapinjet® (Sanofi-Synthelabo, France). The source of the vaccine antigens for these products is liver tissue from infected animals because the virus has not been grown in established cell lines. A number of recombinant virus vaccines using VP60 (the major capsid protein of the RHD virus) have been reported to be protective against lethal challenge. Expression systems have included viruses (e.g., canary pox, vaccina, myxoma), yeast (i.e., Saccharomyces), transgenic potato plants, and bacteria (i.e., Escherichia coli). Recombinant vaccines could certainly reduce the risk of disease arising from the use of improperly prepared killed vaccines; however, no commercially recombinant vaccines are currently available. In addition, no vaccine is legally available for use in the United States.

The USDA is currently soliciting input from rabbit
interest groups in the hopes of building a consensus in regard to the use of vaccines. The information gathered will be forwarded to the Secretary of Agriculture’s Advisory Committee on Livestock and Poultry, which will make recommendations for developing a U.S. policy on RHD. Given the nature of the virus and the susceptibility of the U.S. rabbit population, a comprehensive RHD policy, including import restrictions, surveillance, and vaccination procedures, should be explored.

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REFERENCES


### ARTICLE #3 CE TEST

The article you have read qualifies for 1.5 contact hours of Continuing Education Credit from the Auburn University College of Veterinary Medicine. Choose the one best answer to each of the following questions; then mark your answers on the test form inserted in *Compendium*.

1. RHD is caused by a
   a. rotavirus.   c. calicivirus.
   b. picornavirus. d. parvovirus.

2. Which of the following rabbit genera are susceptible to the RHD virus?
   a. *Lepus*  
   b. *Oryctolagus*  
   c. *Sylvilagus*  
   d. a and b

3. Which of the following animals are not resistant to RHD infection?
   a. humans  
   b. dogs and cats  
   c. mice and hamsters  
   d. none of the above

4. ______________ is not a characteristic feature of RHD.
   a. Sudden death  
   b. Hepatic necrosis  
   c. Disseminated intravascular coagulation  
   d. Bloody nasal discharge in 60% of affected animals

5. Which tissue is commonly used for viral identification of RHD?
   a. liver  
   b. brain  
   c. kidney  
   d. whole blood

6. __________ is the method of choice used in diagnosing RHD.
   a. The HA test  
   b. ELISA  
   c. The immunoelectron microscopy technique  
   d. The injection of suspect tissue into live rabbits.

7. An outbreak of RHD has been confirmed in
   a. China.  
   b. Australia.  
   c. the United States.  
   d. all of the above

8. Which of the following statements regarding the RHD virus is false?
   a. The virus can readily be propagated in established cell culture lines.
   b. The virus remains viable in adverse environmental conditions.
c. The virus is spread by fecal–oral contact.
d. The virus is extremely contagious.

9. Of the following methods, ____________ was not used to manage the outbreak of RHD that occurred in the United States.
   a. quarantine
   b. vaccination
   c. depopulation
   d. decontamination

10. Why are vaccines against the RHD virus not recommended for use in epizootic areas?
    a. No vaccines are currently available.
    b. Vaccines provide only partial protection.
    c. Vaccine antibody response cannot be distinguished from the natural disease response.
    d. Vaccines are too costly to adequately cover the entire area.