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# DEVELOPMENT OF A VACCINE PROTECTIVE AGAINST VIRAL HEMORRHAGIC DISEASE

Wang Yongkun, Zhu Guoqiang, Duan Yueyou, Ji Chuany, Yu Yonglin, Qin Aijian, Zhou Yangsheng, Sun Huaichang, Tian Huifang, Xu Yiming, Wang Baoan, Huo Jinfu, Li Jinbao, Gao Jinliang, and Shao Tianhuo

Jiangsu Agricultural College, Yangzhou, Jiangau, China

## Abstract

Rabbit viral hemorrhagic disease (VHD) is a new rabbit disease (3) characterized by high body temperature (18) and sudden death. Mortality is as high as 90-95% or even 100% of infected animals. Major symptoms are systematic congestion and hemorrhage and multiple vascular thrombi (14,15,16,17). Since the disease was first found in the southern areas of Jiangsu province in China in 1984, heavy losses have been reported in commercial and family rabbit farms. Since 1987, the disease has been reported, with other names, in other countries in Asia and Europe.

The VHD virus has been isolated successfully from liver extracts (6,7) of diseased rabbits. The virions are icosahedral, non-enveloped, and 32-34 nm in diameter. The virion has a core, 15-17 nm in diameter, and its capsid consists of 32 tube-shaped capsomeres which are 6.5 nm long and 4.5 nm in diameter, with a central hole about 2 nm in diameter. Among 32 capsomeres, 12 are pentamers and 20 are hexamers (a total of 180 subunits). The capsids are probably made up of two layers. VHD is more like calciviruses in morphology than other viruses (9).

The virus is acid-fast, and stable in 1 M $MgCl_2$ solution. It is resistant to treatment of ether and chloroform (4,7) and has the ability to agglutinate human red blood cells. The hemagglutination activity can be inhibited by a specific antibody against the virus (11). Therefore, the hemagglutination inhibition test (HIT) can be used to diagnose the disease (12,13). The LD50 of diseased rabbit liver tissues to susceptible rabbits is $10^7$-$10^{7.5}$ when 1 ml suspension is used. Three hybridoma clones secreting monoclonal antibodies to VHDV have been developed.

Two virus peaks are observed after cesium chloride equilibrium ultracentrifugation (8). Two peaks contain the empty particles and the full virion, respectively. The virions of the first peak have a buoyant density of 1.28-1.34 g/ml and the second peak has a buoyant density of 1.36-1.44 g/ml.

The structural polypeptides of purified virions were investigated by sodium dodecyl sulfate polyacrylamide gel electrophoresis. A major band (VP2) with a molecular size of 63-64 Kd and a minor band (VP1, 35-38 Kd) were visualized. The two polypeptides were

further electrotransferred onto a nitrocellulose membrane and probed with convalescent or normal rabbit serum. The VP2 was identified as a major polypeptide.

Digestion of viral genome extracted from purified VHDV with S1 nuclease indicated that VHDV was single-stranded. The effects of formaldehyde on the absorption spectrum of VHDV particles and the staining of VHDV nucleic acid with acridine orange also indicated that the viral genome was a single strand.

Under EM, the viral genome derived from purified viruses was ss-linear with a full length of 2.0-2.1 $\mu$m. The molecular mass of the viral genome was estimated to be 2.4-2.5 x $10^8$ dal.

Using cross-protection in rabbits, immunoeleotroscopical technique (7) and cross hemagglutination-inhibition tests, no antigenic differences have yet been found among 21 strains collected from 13 different areas in China. The virus preserved in -20° C for 18 months did not lose its infectivity. We failed to pass the virus in all tissue cultures (primary cells and established cell lines), embryonated eggs and laboratory animals.

It is inferred from the above the VHDV is a number of calciviridae, but classification of the virus remains to be made.

A formalin-inactivated tissue suspension vaccine developed in our laboratory has been proved to be very effective. Almost 100% of vaccinated rabbits were protected from challenge or infection. A significant level of protection can be developed within 3-5 days of vaccination and the protection lasts for eight months. Therefore, the vaccine has been used nation-wide in China and has very successfully controlled the disease.

## Introduction

In February, 1984, an acute fatal viral rabbit disease emerged in Jiangsu province of China. The disease spread quickly, emptying a rabbit farm within two or three days. Within six months the disease spread nearly throughout the country. It caused significant economic losses in commercial rabbit farms and attracted many scientists to investigate its transmissive sources, the properties of its causative agent, diagnostic methods and prophylaxes.

No similar reports or descriptions of such a disease dated before 1984 were found in China or other countries. At first, veterinarians tried all kinds of antibiotics but failed to save any diseased rabbits. Finally, the disease was demonstrated to be caused by a new virus through experimental transmission with the filtrate of infected rabbit liver tissue. Since 1984, we have been involved in research on the disease and have made fruitful achievements in epidemiology, symptoms, postmortem changes, etiological features, diagnostic methods and prophylactic measures.

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## Epidemiology

### Susceptible Animals

The rabbit is the only animal known to be susceptible to the VHD virus, and rabbits imported from Germany have been most susceptible (5,10). Other livestock and laboratory animals including chickens, ducks, geese, pigs, sheep, goats, cattle, guinea pigs, rats and mice are not susceptible. Rabbits aged over three months are more susceptible than those less than two months. In experimental infections, the incubation period is very short; most rabbits die within 24-72 hours. During the early epidemic, morbidity in animals aged 3 months, 2-3 months and 1 month was 90-100%, 80% and 50%, respectively. At present, morbidity and mortality have decreased. Hares can also be infected.

### Infectious Sources

Tissues of diseased rabbits including blood, muscles, and internal organs, wool, fur, excrement, contaminated food, and even wool scissors are possible transmissive sources or vectors.

### Portals of Entry

The gastrointestinal and respiratory tracts are the main entry for natural infection. Susceptible animals can also be infected subcutaneously, intramuscularly or intravenously. So far, no vertical transmission has been found.

### Symptoms

The disease can be divided into three types according to clinical symptoms:

Supper Acute Type. Rabbits infected naturally and experimentally show high body temperature (41° C) at first, then die suddenly without obvious clinical symptoms 18-24 hr after infection. Duration between onset and death is 6-8 hrs.

Acute Type. Sixteen to twenty-four hours after being infected, animals show some nonspecific symptoms such as poor appetite and high rectal temperature (41° C). A few hours later, the rabbits show obvious nervous symptoms such as sudden excitation, running and jumping in the cages, and biting the feeding tools or cages. Finally, they become exhausted and prostrate with paddling action before death. Sometimes the rabbit carcass is in opisthotonos position. In some cases noses of the dead animals are full of bloody foam and a yellowish secretion can be seen around the anus.

Chronic type. Infected animals show a rise in body temperature, poor appetite and depression in one or two days. About 50% die. Others recover gradually in one week.

A hemagglutination (HA) test can be done with conventional procedures using both glass slides (read results within 5 min) and 96-well HA plates or large-well (1 cm in diameter) HA

plates (at 37° C or room temperature, 0.9 % saline or pH 7.2, 0.01 M PBS as dilution; read results after 15-30 min.).

A total of 18 strains of the virus were tested and all agglutinated only human red blood cells (HRBC), especially type O HRBC, but HA titers were significantly different among different strains and passages of the virus (with the lowest titer of 6-16 and the highest of over 2048). According to our experiments, the HA tests done on large-well plates gave preferable results due to easy recognition of the HA pattern. No strains of the virus had the ability to agglutinate the RBCs of animals such as the horse, cow, sheep, goat, pig, dog, chick, duck, goose, rat, guinea pig, nude mouse, brown mouse, white mouse, hamster or rabbit.

Hemagglutination-inhibition (HI) tests can be done with conventional procedures. Results show that the specific anti-VHDV serum against one standard strain of the virus can inhibit all strains of the virus from agglutinating HRBCs.

Serotype of VHDV. The serotype of the virus was identified with conventional cross-HI tests, immunoelectroscopical technique and cross protection in rabbits.

Results showed that a specific antibody to the standard strain of the virus could inhibit the hemagglutination of 18 strains, protect susceptible rabbits from challenge of 13 strains and agglutinate all strains of the virus under immunoelectroscope and vice versa. These results indicated that all strains of VHDV belonged to one identical serotype.

## Development and Application of Inactivated Tissue Vaccine Against VHD

In 1984 when VHD emerged and spread in China, it was very urgent to develop an effective vaccine to control the outbreak. Fortunately, inactivated tissue vaccine was successfully developed immediately after the agent of the disease was confirmed. From 1984 to 1985, 35 batches of vaccines (a total of 5 x $10^6$ dal) were produced and used all over the country (19).

Diseased rabbit liver tissues, which contain a large amount of the virus, are the best materials. The liver tissue is ground with a tissue homogenizer and diluted with a tenfold volume of 0.15 M saline. The suspension is filtered and inactivated with formaldehyde at 37° C for 48 hr. After a safety examination, the vaccine is ready for use in rabbits. The vaccine is easy to distribute, store and administer. It also can be used in 20-day-old suckling animals, with no effects on pregnant animals or meat quality.

Inoculating rabbits subcutaneously at a dose of 1 ml per animal can establish significant protection against natural infection and challenge of VHDV within one week. The protection rate of the vaccine is 95-100% and lasts as long as eight months. Therefore, the disease can be controlled effectively. The vaccine has been used for 7 years throughout the country and has proved to be excellent against the disease.

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