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Recent Fulminant Incursions of Rabbit Haemorrhagic Disease (RHD) in Saudi Arabia

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Abstract: Severe fulminant incursions of Rabbit Haemorrhagic Disease (RHD) swept Saudi Arabia during 2004-2006. The disease was reported in most of the rabbitries in the country. The clinical signs were recorded and the virus was identified using sandwich ELISA and the Haemagglutination Inhibition (HI) test. Epidemiology of the disease in Saudi Arabia was discussed in relation to the present outbreak.

Key words: Rabbit haemorrhagic disease, fulminant outbreaks, Saudi Arabia

INTRODUCTION

RHD is a peracute viral ailment of rabbits *(Oryctolagus cuniculus)*. The disease is highly contagious and fatal. Adult rabbits are the main target of the disease. Appearance of the disease is usually manifested by sudden death. Initially, the affected rabbits show depression and at the terminal stages they show excitement and other neurologic signs, such as incoordination and opisthotons. Also at the terminal stages, blood can be seen oozing from the rectum and nostrils. The morbidity rate can reach 100%, while the mortality rate is between 40-90 %. The liver is the most affected organ and richest with virus.

The first description of the disease was made in China in 1984, where it wiped-out millions of rabbits[1]. From China it was reported in many of the South Asian countries. The disease then swept Europe, Oceania and was reported in Africa, Arabia, the Middle East, North, Central and South America[2].

The causative agent is a member of the genus *Calicivirus* of the family *Caliciviridae*[1] It is persistent in the environment, thus repeated infections are encountered whenever a population of susceptible rabbits is available in the vicinity.The first description of RHD in Saudi Arabia was made in 1999[3]. In that instance it caused great losses in domestic rabbits. Since then no published data was available regarding the epidemiological situation of the disease in the country.

The present study describes a recent fulminant epizootic of the disease that covered most of the rabbitries in the kingdom.

MATERIALS AND METHODS

Field investigations: During 2004, 2005 and the first quarter of 2006, a severe epidemic of a highly fatal disease struck rabbitries in most regions of Saudi Arabia. Outbreaks were reported in Buriydah (Central region), Tibrak (Riyadh area), Taif (Western region) and the Eastern region. The approximate number of rabbits kept in the different rabbitries was noted.

Clinico-epidemiological observations were recorded. That included the age of the affected rabbits, the morbidity rate, the mortality rate, the clinical signs, the possible factors that aided in the spread of the disease and the sanitary measures adopted for its control.

The haemagglutination test (HA): The standard method of the OIE for the HA was followed[4]. Human type 'O' Red Blood Cells (RBCs) at a concentration of 0.75%, in Phosphate Buffered Saline (PBS) pH 7.4, were used. Livers from freshly dead or moribund rabbits were made into 10% suspension in PBS pH 7.4 and clarified by centrifugation at 2000 rpm for 15 min. The supernatant fluid was collected and used in the HA test. Two-fold dilution series were made in PBS pH 7.4, in microtitre plates. An equal volume of RBCs was added to each well. Incubation of the plates and reading of the results were as described by the OIE[4].

Pathological investigations: Moribund and freshly dead rabbits from the various farms were subjected to post mortem examination. Samples from the livers were collected and stored at-86° C until used in the virological investigations. Samples from the livers and kidneys were also collected in 10% formol saline, processed in paraffin,

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sectioned 4-6 μ thick, stained with haematoxylin and eosin (H and E) and examined for histopathological changes.

ELISA for detection of the RHD virus: Liver samples were made into 10% suspension (w/v) in Phosphate Buffered Saline (PBS) pH 7.4, containing 0.1% Tween 20 and 1% bovine albumin (PBS-T-BA). The suspension was centrifuged for 10 min at 2000 rpm. The supernatant fluid was collected and used for the detection of the RHD virus antigen employing the ELISA test as described below.

The ELISA reagents were obtained from Instituto Zooprofilattico Sperimentale Della Lombardia E Dell'melia, Brescia, Italy. The ELISA procedure used followed that prescribed by the OIE[2]. This included the standard methods of washing, incubations and volumes of reagents. In brief, the plates were coated with the RHD Virus specific IgG or negative rabbit IgG at the prescribed dilution in carbonate bicarbonate buffer pH 9.6 by incubation overnight at 4°C. Following washing, the liver extract was diluted 1/5 and 1/30 in PBS-T-BA pH 7.4 and added to the appropriate wells of the plate. Following incubation and washing, the conjugate was added, the plates incubated and washed. This was followed by the addition of the substrate, stopping the reaction after 5 min and reading the results at 492 nm. Interpretation of the results was as described in the instructions of the manufacturers.

Production of hyperimmune serum against the isolated RHD virus: The identified RHD virus, which caused the present outbreaks, was inactivated with 1% formalin, emulsified in equal volume of light mineral oil adjuvant and inoculated intramuscularly (I/M) at a dose of 0.5 mL, per rabbit, into seronegative rabbits. After two weeks, another shot of the same inoculum and dose was also given I/M. The same procedure of inoculation was repeated a week later and the rabbits were exwangenated, the serum separated, inactivated by heating at 56°C, aliquoted and stored at-20°C.

The Haemagglutination Inhibition test (HI): The micro HI was performed, according to the standard OIE method[4], for identification of the RHD virus and to titrate the hyperimmune serum which was produced against the isolated RHD virus in rabbits.

RESULTS

Field investigations: The disease onset was prompt. Only adult rabbits were affected. Rabbits were found dead without showing salient clinical signs. However, with progress of the disease, it was observed that the affected rabbits showed dullness, anorexia and mild neurologic symptoms. Terminating rabbits showed epistaxis and rectal bleeding. The morbidity rate was above 80% while the mortality rate reached 100%.

Rabbitries in Saudi Arabia are distributed all over the country. The number of rabbits in each, varies from few hundreds to thousands. The keeping facilities also vary in their types and hygienic conditions. Some rabbitries are established as open systems others are sophisticated systems in both construction and hygiene. Although some rabbitries are strict, in allowing visitors and people to go in the premises, still many rabbitries are open for out-siders. Frequently, rabbits are procured from either local markets or from abroad.

VIRUS IDENTIFICATION

The ELISA test: According to the criteria set up by manufacturers of the ELISA kit, the tested liver samples, from the diseased or dead rabbits, were positive for presence of the RHD virus. The positive and negative controls also gave the expected results.

The HA and HI tests: The liver homogenates from the affected rabbits gave haemagglutination end-point titres that ranged between 1/2048 and above 1/4096. This activity was inhibited by the hyperimmune serum which was produced against the RHD virus, that was previously identified by the ELISA kit.

The HI end-point titre of the rabbit hyperimmune serum, which was produced against the identified RHD virus was 1/256.

PATHOLOGICAL INVESTIGATIONS

Postmortem examination: The P.M. picture was the same for all the examined rabbits. The livers, lungs and spleens were enlarged and showed haemorrhage. Necrosis was seen on the liver. The tracheal mucosa was congested. Petechial haemorrages were seen on the epicardium, endocardium, thymus and surface of the kidneys. The kidneys were dark in colour and enlarged.

Histopathological examination: The liver revealed severe hepatocellular necrosis or degeneration and haemorrhage throughout the liver. Closer examination showed different stages of cell death, with pyknosis nuclear dissolution and particularly karyorrhexis being prominent feature in the liver cells. Other parts of the parynchyma showed fatty change, vesiculation or foamy appearance of the hepatocytes. Small hyaline, globules, probably Councilman's bodies, were seen within some necrotic

hepatocytes. The lobular architecture was either disrupted or intact depending on the severity of damage in different areas, while the hepatic sinusoids and portal capillaries were diffusely and widely distended with blood. A black granular pigment, probably of hematinic origin, was noted focally between necrotic liver cells. There was no abnormality in the Kupffer cells lining the sinusoids.

The lesions were not accompanied by an inflammatory or cirrhotic process.

The marked features in the kidneys were massive , linear interistitial haemorrhages, coupled with tubular epithelial necrosis and vascular engorgement. Capillary intravascular clots, consisting of a homogenous coagulum and erythrocytes, were seen focally, accompanied by swelling of capillary endothelial lining. However, no evidence was seen of inflammatory cellular infiltration.

DISCUSSION

The clinico-pathological picture of the disease, as observed in the affected rabbits in the present outbreak, was highly indicative of RHD infection. However, the virological and serological investigations confirmed that the aetiological agent was actually, the RHD virus.

The severity and wide spread of the present outbreak, country, with the great losses encountered, could indicate that the herd immunity, in rabbits in the kingdom, at the time of the disease, was so low to the extent that gave way to the observed dramatic damage. This situation, along with the absence of vaccination in the country, could probably indicate absence of the RHD virus activity in the Saudi environment, for some time, before the present outbreak.

Although Saudi Arabia has not yet been declared endemic with the RHD virus infection, we are of the opinion that, with the persistent nature of this virus in the environment, it is high time for introduction of a vaccination programme using a vaccine to be prepared from the current field virus. Otherwise, the virus activity in rabbitries will be inevitable. At this stage, it worths mentioning that, a local experimental inactivated vaccine has been formulated from a local RHD field virus isolate which gave good protection in rabbits. This information will be published soon.

To understand the epidemiology of the RHD in Saudi Arabia, a nation wide study is needed so as to implement the best measures for its future control and possibly eradication. This is particularly important because rabbit industry is expanding in the kingdom and definitely, RHD may highly likely constitute a real scourge to it.

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