**INTRODUCTION**

The rabbit hemorrhagic disease (RHD), also known as the viral hemorrhagic disease or hemorrhagic viral disease or rabbit calicivirus disease (2, 3), is an acute highly fatal viral disease of rabbits (1). The disease was first recorded in the People’s Republic of China (9). It spread to Korea (13), Italy (10), and continental Europe between 1988 and 1993 (2), reaching the United Kingdom in 1993 (5). The disease has recently been reported in Scotland (14), and the Republic of Ireland (5, 8). It has affected some countries of Northern Africa and the Mediterranean region (11, 15). In the Arabian Peninsula, it was reported for the first time in 1999 in Saudi Arabia, in Al-Hasa district (1). The onset of the disease was sudden, with a 100% mortality rate. The main clinical signs were bleeding from the mouth, nostrils, vagina and rectum, anorexia, listlessness, and dullness. Death occurred within 24 hours of the appearance of clinical signs. Only adult rabbits were affected. Antibiotic treatment was not effective (1).

In Bahrain, the disease appeared on May 16, 2001 among 800 adult domestic rabbits (*Oryctolagus cuniculus*) reared in a remote private farm and was highly contagious and fatal. Onset of the disease was sudden with a 100% mortality rate. Death could occur between a few hours and 24 hours after the appearance of the clinical signs of the disease. On the first day 80 rabbits were found dead. On the second day 60 rabbits died. The disease continued thereafter up to two weeks, 20-40 rabbits dying every day. Clinical signs of the disease were dullness, mouth and nostril bleeding, anorexia, gasping, and lateral paralysis. Antibiotic treatment was not effective.

**MATERIALS AND METHODS**

Twenty freshly dead rabbits were examined. Livers, lungs, and kidneys were aseptically collected in sterile containers for bacteriological, fungal and virological laboratory examination. Duplicate samples were also collected in 10% buffered formalin for histopathological examination. Samples for virological examination were preserved in water-based buffer. For molecular viral analysis, RNA was extracted from 100 µg of liver, kidney and lung tissues using RNA Agents Kit (Promega) following the manufacturer’s instructions. Primers for RT-PCR were designed from a known sequence based on the capsid protein VP60 (2). The authors sequenced 573 bp of the viral capsid gene (the primers used are described in Table I). First strand cDNA synthesis was performed using Superscript II reverse transcriptase (Invitrogen) with RHDV 4 primer using the procedure described previously (7). A nested PCR was then used to amplify DNA; the first reaction (RT-PCR) utilized primers RHDV1 and RHDV 4, and the second (nested PCR) utilized primers RHDV2 and RHDV3 to produce a DNA product of 573 bp. Thirty cycles of 94°C for 40 sec, 50°C for 40 sec and 72°C for 1 min were used for both sets of primers (12). These PCR products were gel purified and both strands sequenced using a PE Biosystems cycle sequencing kit, following the manufacturer’s instructions and using the primers RHDV2 and RHDV3 as described in Table I to give 573 nucleotides of sequence for analysis. Stomach and intestinal contents were examined for the presence of internal parasites using the Ovassay technique. To exclude bacterial contamination, a nested PCR was applied to the DNA products of the stomach and intestinal contents of the rabbits.

**Table 1**

| Primer sequences used to amplify and sequence a 573 bp region of the RHDV capsid protein |
|---------------------------------|---------------------------------|
| RHDV1 (forward) 5’ GGACTGCACACCACTACCTGG 3’ |
| RHDV2 (forward) 5’ TTTGAACTTGAATGGCAGCA 3’ |
| RHDV3 (reverse) 5’ CACCGGTGCGCTCGGAC 3’ |
| RHDV4 (reverse) 5’ CCAATTGTACTGGGAGTGG 3’ |
infection, cultures were made from the livers, lungs, and kidneys inoculation onto blood and MacConkey agar (Oxoid) and incubated at 37°C for 24 h. Duplicate samples were cultured onto Sabouraud dextrose agar (Oxoid) to exclude fungal infection.

■ RESULTS

Coprological examination of the stomach and intestinal contents revealed insignificant mild coccidiosis. Bacteriological and fungal examinations revealed no bacterial or fungal growth. RT-PCR yielded a DNA product of 573 nucleotides, which, in comparative alignments with the corresponding capsid region of other recognized European virulent strains of RHDV, showed more than 97% nucleotide identity. The only gross pathological lesions seen were severe hemorraghes and congestion in the liver, lungs, and kidneys. In some of the rabbits the urinary bladder was distended with urine.

Histopathological examination of the liver revealed severe necrosis of the hepatocytes, karyopyknosis, karyorrhexis and karyolysis, and severe congestion and hemorrhages (Figure 1). The kidneys showed severe necrosis of the renal tubules and renal glomeruli, karyopyknosis, karyorrhexis and karyolysis, intertubular and glomerular hemorrhages (Figure 2), and congestion of the blood vessels. Severe hemorrhages, congestion, edema and compensatory emphysema were also seen in the lungs.

■ DISCUSSION

Reports on the rabbit hemorrhagic disease are scarce, especially on the gross and histopathological lesions. The present paper reports the disease for the first time in Bahrain. The source of the viral infection and its introduction could not be traced back. The absence of bacterial and fungal infection coupled with the positive RT-PCR tests of the liver, kidneys, and lung, showed that the encountered clinical signs, gross and histopathological lesions were due to the infection of rabbits with the rabbit viral hemorrhagic disease.

The presence of the virus in the lungs and kidneys was confirmed for the first time by the RT-PCR test. Gross and histopathological lesions were also reported for the first time.

REFERENCES


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**Résumé**

Abubakr M.I., Gould E.A., Fadlalla M.E., Abuobeida S.A.
Maladie hémorragique virale du lapin

Cette étude rapporte la maladie hémorragique virale des lapins pour la première fois au Bahreïn et pour la deuxième fois dans la péninsule Arabe. Elle traite en particulier des signes cliniques, pathologiques et des lésions histopathologiques de cette maladie, et de l’isolation du virus.


**Resumen**

Abubakr M.I., Gould E.A., Fadlalla M.E., Abuobeida S.A.
Enfermedad hemorrágica del conejo en Bahrein

Este artículo constituye el primer reporte de la enfermedad hemorrágica del conejo en Bahrein y el segundo reporte en la Península arábica. Se refiere a signos clínicos, lesiones patológicas e histopatológicas y aislamiento del virus.

*Palabras clave: * Conejo – Virosis – Inspección postmortem – Bahrein.