Diagnostic Exercise

From The Davis-Thompson Foundation*

Case #: **177** Month: **November** Year**: 2021**
Answer sheet

Title: *Rabbit hemorrhagic disease virus 2 in a backyard rabbitry*

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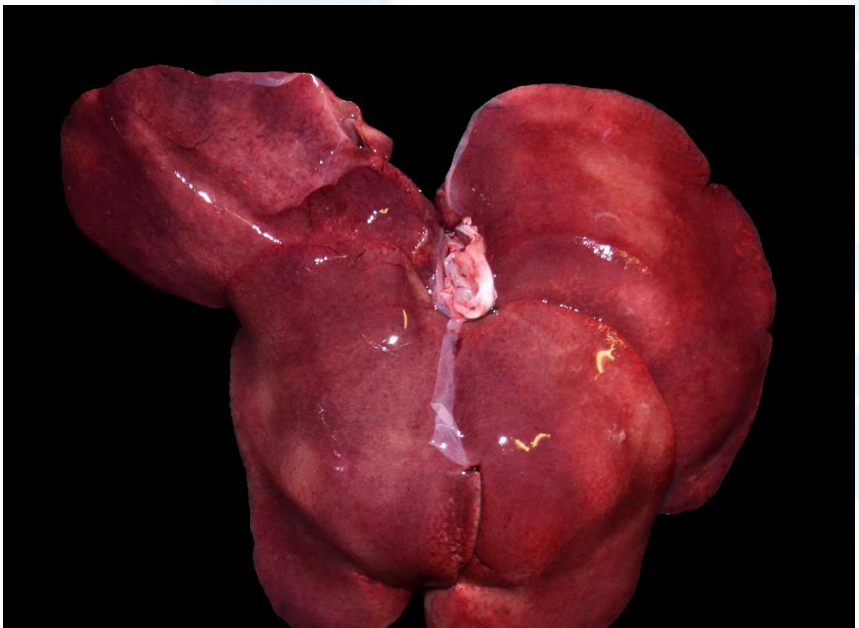
Clinical History: Nine out of 22 Standard Rex meat rabbits (*Oryctolagus cuniculus*) died within 72 hours in a backyard rabbitry of southern California. Animals were kept outdoors in individual cages. Some rabbits died suddenly without any clinical sign, whereas a few of them had dull eyes and abnormal position 4-8 hours prior to death. One carcass had blood coming through the nares.

Necropsy Findings: Four carcasses were examined and they all were in good nutritional condition. Lungs were swollen, wet, and there were occasional petechiae. Tracheal mucosa was variably congested. Livers were pale, occasionally with rounded borders, and there was a mild to moderate reticular/lobular pattern (Fig 1).



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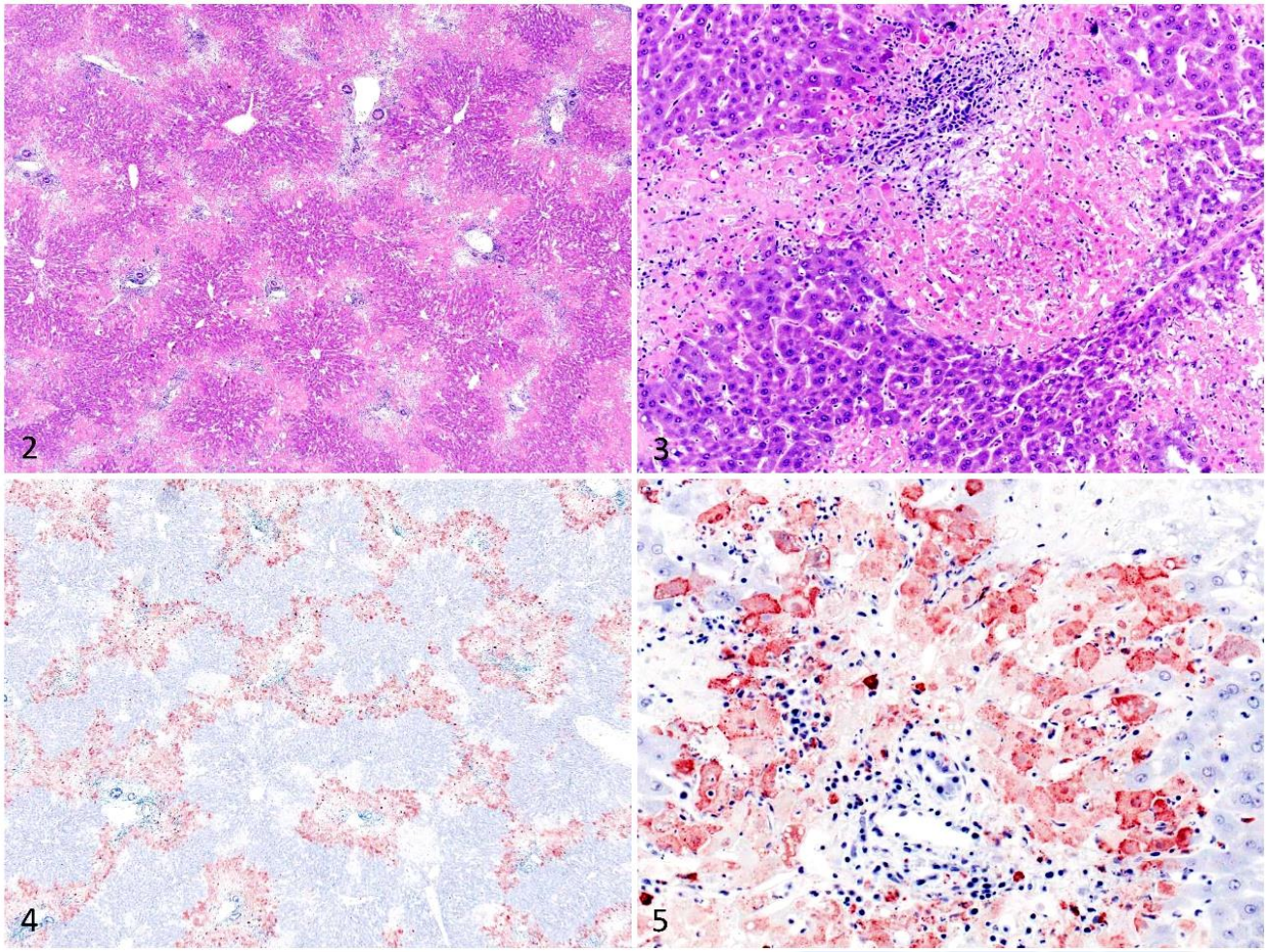

Figure 1. Gross aspect of the liver, Figures 2-3 (H&E), Figures 4-5 (IHC, Pan-*Lagovirus*[1])

Follow-up questions: Microscopic findings, morphologic diagnosis, cause, and associated lesions.

Microscopic findings: Liver. There is multifocal to coalescing, periportal to midzonal hypereosinophilia and dissociation of hepatocyte cords; individual hepatocytes are variably vacuolated, fragmented, and with pyknotic/karyorrhectic nuclei (necrosis).

Morphologic diagnosis: Liver. Necrosis, periportal to midzonal, bridging, acute, severe (Figs 2-3).

Cause: Rabbit hemorrhagic disease virus type 2 (RHDV2; *Lagovirus* GI.2) was identified by RT-qPCR (C_t values of 13.01 to 13.67) from liver tissue; Pan-*Lagovirus* immunohistochemistry was positive in the areas of necrosis (Figs 4-5).

Associated lesions: Hemorrhages in parenchymal organs (e.g., lungs) and serosae, fibrin microthrombi in small vessels (e.g., renal glomerular capillaries), lymphoid depletion in spleen, splenic red pulp necrosis.

Comments: Rabbit hemorrhagic disease (RHD) is a very contagious and almost invariably fatal disease of rabbits caused by a virus of the Family *Caliciviridae*, Genus *Lagovirus*. Classic RHD is caused by rabbit hemorrhagic disease virus (RHDV; *Lagovirus* GI.1), has a rapid disease course with very high mortality (70-90%), and affects only domestic and wild European rabbits (*Oryctolagus cuniculus*) (Abrantes *et al*, 2012). In 2010, a different variant, rabbit hemorrhagic disease virus type 2 (RHDV2; *Lagovirus* GI.2), was identified in France (Le Gall-Reculé *et al*, 2013). RHDV2 has a broader host range, which to date includes, in addition to domestic and wild European rabbits, several cottontail rabbit (*Sylvilagus* spp.) and hare (*Lepus* spp.) species (Asin *et al*, 2021; Lankton *et al*, 2021); this host range is currently increasing as the virus spreads through the American continent and native lagomorph species are exposed to it. The clinical course of RHDV2 may be more variable, and the mortality rate may be slightly lower (5-70%) than with classic RHDV, although there are recent descriptions of virulent RHDV2 strains with very high mortality and rapid clinical course similar to the classic form (OIE, 2021). Lesions of classic RHD are generally indistinguishable from those of the disease caused by RHDV2, and may often be very subtle due to the rapid course of the disease. Liver is often enlarged and pale, with a marked reticular/lobular pattern. There is usually pulmonary congestion and edema, and tracheal congestion. Spleen may be variably enlarged with rounded borders. There may be hemorrhages in multiple organs, including lungs, kidneys, and serosal surfaces. Jaundice may be detected, especially in animals with subacute to chronic forms. Histologically, periportal to midzonal and occasionally panlobular hepatic necrosis is a hallmark of the disease. Additionally, there may be splenic red pulp necrosis, lymphoid depletion in spleen and other lymphoid tissues, disseminated intravascular coagulation-associated lesions (especially fibrin microthrombi in small vessels such as the renal glomerular and pulmonary septal capillaries), and multisystemic hemorrhages and congestion (Abrantes *et al*, 2012). Confirmatory diagnosis is achieved by PCR, which is able to differentiate between RHDV and RHDV2, and is the most commonly used test (OIE, 2021). Other diagnostic tests include immunohistochemistry (Figs 4-5; Neimanis *et al*, 2018), electron microscopy, antigen ELISA, and in situ hybridization (OIE, 2021). Differential diagnoses include pasteurellosis and other septicemic bacterial infections, intoxications (e.g., with anticoagulant rodenticides), and heat exhaustion (OIE, 2019). These diseases may mimic RHD and, on top of that, the subtlety of the macroscopic lesions in some cases makes it difficult to establish a tentative diagnosis based on gross necropsy findings, only; however, an overnight formalin-fixed, hematoxylin and eosin-stained liver section has proven to be effective in providing rapid evidence of hepatic necrosis and supporting a presumptive diagnosis while the PCR results are still pending.

[1]Pan-*Lagovirus* antibody kindly provided by Drs. Lavazza and Capucci; OIE reference laboratory for RHD, Brescia (Italy).

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