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PATHOLOGY AND ETIOLOGY OF NATURAL AND EXPERIMENTAL EUROPEAN BROWN HARE SYNDROME

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In recent years an increasing mortality has been recorded in the brown hare (*Lepus europaeus* P.) in various European countries (Gavier et al., 1988). In the majority of cases, the cause of death appeared to be liver failure due to a severe necrotizing hepatitis. This hepatic lesion seems to be a consistent finding, whereas lesions in other organs may be present in varying frequencies. Considering that a histologic lesion was the only common characteristic, and considering also that the cause was unknown, this disease has been designated provisionally as «European Brown Hare Syndrome» (EBHS). Both toxins (e. g. 00 rapeseed) and infectious agents (e. g. *Clostridium sordelli*) have been suggested as possible etiological factors. Nevertheless there is growing evidence that 00 rapeseed, although an important cause of death in the roe, probably is not involved in the brown hare syndrome. Preliminary experiments point to a possible viral etiology (Eskens and Volmer, 1989). The striking resemblance of the liver lesions in EBHS and those described in Viral Haemorrhagic Disease of rabbits (VHD) has led some authors to suggest a common etiology (Marcato et al., 1989). Unfortunately in this disease also the etiology is still a matter of considerable controversy. Recently Morisse (pers. commun.) has succeeded in reproducing symptoms and lesions characteristic of VHD in rabbits by the oronasal and intramuscular route in 6 rabbits using an inoculum obtained from the livers of 2 hares. He considers that both VHD and EBHS are caused by a calici-like virus present in bile and liver homogenate. Nevertheless so far no evidence has been produced of a direct association between a virus and the liver lesions in EBHS. In the present study the histologic lesions of both natural and experimental EBHS have been investigated in detail and ultrastructural evidence of a viral etiology is given.

Material and methods

Between september and december 1989, 62 hares have been autopsied, 44 of which were wild hares found dead or captured moribund. From the wild hares samples were taken systematically from the following organs for histological examination: liver, conjunctiva, cornea, retina, trachea, apical and diaphragmatic lung lobes. In selected cases samples also were taken from spleen, thymus, kidney, adrenals, cerebral cortex, cerebellum, brain stem, stomach fundus, duodenum, jejunum, ileum, caecum and colon. These samples were fixed in formalin and embedded in paraffin. Sections were made and stained with H&E or other staining techniques when indicated. For the experimental studies, 4 captive hares were obtained from an isolated farm, where no previous outbreaks of EBHS were recorded.

These animals were inoculated as described elsewhere (Nauwynck et al., in preparation). In short, a bacteria free suspension of conjunctivae, lungs and livers from wild and captive hares that died with lesions typical of EBHS, was used for the inoculations. At euthanasia (1 animal), or very shortly after natural death, samples for histological examination were taken as in the field cases. Samples for ultrastructural studies were taken from the liver, the trachea and the apical and diaphragmatic lung lobes. These samples were embedded in a Spurr-Epon mixture and contrasted with uranyl acetate and lead citrate. Samples of liver homogenate were examined by immunoelectron microscopy following negative staining with potassium phosphotungstate.

Results

Out of the 44 wild hares, 25 were classified as EBHS on the basis of positive histopathological criteria and absence of other possible causes of death. The other group of 19 animals contained a variety of different diagnoses, in which rodentiosis and coccidiosis were important.

The gross lesions in animals classified as EBHS were rather inconsistent. In the majority of cases icteric discoloration was seen of the sclera, the aorta intima, the subcutis and tendons. Internal organs in these animals usually were congested, but not haemorrhagic. The mucosa of the trachea in particular usually appeared dark red. Ten of these animals showed a catarrhal to necrotising conjunctivitis associated with an opacity and occasional ulceration of the cornea. Liver lesions usually were inconspicuous. Only in some cases an increased marking and pinpoint central redening of the lobules was noted.

At histological examination however, the liver lesions were marked. These lesions were used to classify an animal as EBHS positive. In all cases there was an obvious necrosis of hepatocytes, which was either diffuse or limited to the cells located at the periphery of the lobules. Non necrotic cells were swollen and showed an increased eosinophilia of the cytoplasm. Infiltrating lymphocytes usually were present near Kiernan's triangle. In some cases fatty degeneration of hepatocytes was seen. Bile duct proliferation also was observed in the majority of cases. In contrast to the lesions described in aflatoxicosis, fibrosis was not observed in any of the examined livers of hares. None of the other organs showed consistent histologic alterations, except for the lungs, where apical cytoplasmic droplets were seen pinching off from bronchiolar epithelial cells. The conjunctivitis lesions proved histologically to represent a variety of inflammatory lesions. In the cases where *Actinobacillus* was isolated on bacteriology, the lesions usually were frankly purulent.

In the experimental animals, the autopsy lesions were inconspicuous. Icterus was present to a lesser or higher degree. No conjunctivitis was present. The histologic lesions in the livers were similar to those described in the field cases. In one animal that was euthanised at the onset of clinical signs, necrosis of hepatocytes was limited to the cells near Kiernan's triangle. In the other cases, hepatocyte necrosis was diffuse.

At the ultrastructural level, severe degenerative alterations were present. Low power magnification revealed a loss of structural integrity, with red blood cells scattered throughout, and hepatocytes rounded and in some instances even fragmented. Nuclei of hepatocytes mostly were pale, with large chromatin clumps and irregular indented outline. The nuclei of fragmented cells usually were dark staining. Endothelial lining of the capillaries was difficult to discern. In less damaged areas, focal thickening of endothelial cells with cytoplasmic accumulation of lysosomes was noted. At higher magnification the cytoplasm of the hepatocytes was seen to contain abundant annular membranous structures. These were thought to represent the fragmented endoplasmic reticulum. Some cells contained numerous lipid droplets. Multivesicular bodies also frequently were seen. On rare occasions, virus-like particles of approximately 25 nm in diameter were seen in a paracrystalline arrangement in the cytoplasm. 28 to 30 nm virus-like particles could also be observed in liver homogenates by immuno electron microscopy.

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IMMUNOMORPHOLOGIC CHANGES IN GOLDEN HAMSTERS CAUSED BY YERSINIA PESTIS EV

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The literature does not offer descriptions of immunomorphologic changes in animals and people caused by *Y. pestis* EV. However, Veljanov et al. (17) have reproduced chronic, non-lethal infection in golden hamsters aerosol-infected with vaccinal, plague strains *Y. pestis* EV. That is why our investigation were aimed at studying the morphological changes in golden hamsters infected by aerosol with *Yersinia pestis* EV with a view to explaining some immunogenic properties of this strain of the species *Yersinia*.

Material and methods

The experiments were carried out on golden hamsters at the age of 75 days with an average liveweight of 120 ± 10 at the beginning of the experiment. The test animals were aerosol infected with the bacterial strain *Yersinia pestis* EV-76 (Brubaker) tenfold passivated on white mice. The nutritive medium was sulfate agar with an additive of beef yeast extract - 0,5%. The inhalation immunization was carried out in an aerosol chamber of flow-and-dynamic type, at V/Q ratio = 1:1.4, described in a previous work (13). The bacterial suspension of 24-hour culture was spread in the working space of the chamber at a concentration of 5.0×10^9 microbial cells. The inhaled (Dinh) and efficient (Def) doses received by the test animals for 18 min. (exposure time), were as follows:

Discussion

From the results of this study it can be concluded that EBHS indeed is a separate disease entity of viral origin.

The lesions observed in the experimentally infected hares were similar to the field cases. Conjunctivitis was not reproduced. It is not clear whether this conjunctivitis is a symptom of the disease or merely an intercurrent phenomenon. The ultrastructural alterations in the hepatocytes suggest a primary disruption of the endoplasmic reticulum. The presence of virus-like particles in these cells may indicate that the hepatocyte necrosis would be a direct effect of the virus replication. The endothelial lesions observed may contribute to the often rapid course of the disease. The virus-like particles observed in ultra-thin sections of the livers of experimentally infected hares were similar to those observed by Peeters in rabbits exposed to the haemorrhagic disease virus (Peeters, pers. commun.).

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Dinh = 1.4×10^6 microbial cells Def = 3.8×10^6 microbial cells. The efficient (real) dose is 27% of the inhaled one.

The immunomorphologic changes were examined in their dynamics. To this end, five test animals were killed at different time intervals (on the 1st, 3rd, 7th, 15th and 30th day) after immunization. The materials designed for histological examination (lungs, pulmonary lymph nodes, tonsils, spleen, mesenterial lymph nodes and liver) were fixed in Baker's liquid, and treated according to the rapid histological technique.

All histocuts were stained with hematoxylin-eosin.

Results and discussion

By examination of the manifestation and development of the morphologic changes (Fig. 1) in the lungs, pulmonary lymph nodes, tonsils, spleen, mesenterial lymph nodes and liver, it has been found that the first changes occur on the 3rd day after the inhalation of *Y. pestis* EV; they come strongly expressed on the 7th up to the 15th day, and, then, these are gradually reduced, and after the 30th day they are attenuated, and this substantiates the assumption that the macroorganism has acquired immunity.

On the 3rd day after inhalation of *Y. pestis* EV in the lungs, the bronchi and alveoli are filled with serous cell exudate. On the 7th day small accumulations of leukocytes are found in the inflamed pulmonary tissue, that gradually increase, and towards the 15th day