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CLINICAL AND PATHOLOGICAL STUDIES IN ADULT SHEEP AND GOATS EXPERIMENTALLY INFECTED WITH WESSELSBRON DISEASE VIRUS

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ABSTRACT

COETZER, J. A. W. & THEODORIDIS, A., 1982. Clinical and pathological studies in adult sheep and goats experimentally infected with Wesselsbron disease virus. *Onderstepoort Journal of Veterinary Research*, 49, 19–22 (1982).

The clinical symptoms and pathology in 33 adult sheep and 31 adult goats experimentally infected with Wesselsbron disease virus are described. There was moderate to severe hyperthermia in most animals, but no other clinical signs of disease or deaths were recorded.

Eleven sheep and 6 goats were sacrificed for pathological studies at various stages during the febrile response. The macroscopic and microscopic lesions in these cases are described. Microscopic studies revealed that the liver was consistently affected and showed small foci of necrosis. These were sparsely distributed and associated with a marked localized Kupffer cell response ("retothelial nodules"). In addition, acidophilic bodies and small groups of necrotic hepatocytes were evident in some lobules. Apart from the hepatic lesions, mild to moderate pyknosis and karyorrhexis of lymphocytes were seen in the spleen and lymph nodes.

This report also compares the microscopic lesions in the livers of adult sheep and goats with those of new-born lambs for Wesselsbron disease as well as with those reported for Rift Valley fever in adult sheep.

INTRODUCTION

Wesselsbron disease (WSL), originally described in the Wesselsbron district of the Orange Free State, South Africa, was considered responsible for deaths in new-born lambs and abortions in pregnant ewes (Weiss, Haig & Alexander, 1956). Since then only a few reports dealing with the disease in sheep and cattle (Belonje, 1958; Le Roux, 1959; Coetzer & Barnard, 1977; Coetzer, Theodoridis & Van Heerden, 1978; Coetzer, Theodoridis, Herr & Kritzinger, 1979; Blackburn & Swanepoel, 1980) and in man (Weiss *et al.*, 1956; Smithburn, Kokernot, Weinbren & De Meillon, 1957; Heymann, Kokernot & De Meillon, 1958; Kokernot, Smithburn, Gandara, McIntosh & Heymann, 1960; Rodhain, Ardoin, Metselaar, Salmon & Hannoun, 1975) have been published.

Wesselsbron disease virus is known to possess specific hepatotropic properties responsible for characteristic liver lesions in the new-born lamb (Coetzer *et al.*, 1978). In addition, its proven neurotropic property results in brain teratology in the sheep and bovine foetus (Coetzer & Barnard, 1977; Coetzer *et al.*, 1979).

Since the first description of the disease in sheep (Weiss *et al.*, 1956), no experimental work has been done in non-pregnant sheep to determine the effect of the virus in adult sheep. The purpose of this communication is to describe for the first time the clinical symptoms and pathology in adult sheep and goats which were infected experimentally with WSL virus.

MATERIALS AND METHODS

Animals

Fifteen full-month Merino ewes, 18 1-year-old Karakul ewes and wethers, and 31 full-grown goat ewes, none of which had previously been exposed to WSL virus, were used in the experiment. Blood from each animal was collected in sterile 10 mℓ vacuum tubes for serological assay to determine whether they were susceptible to WSL virus. They were then placed in an insect-free stable for WSL-related experiments.

Virus

The WSL virus used in this study was isolated from a new-born lamb in the Harrismith district (Coetzer *et al.*, 1978). It was injected intracerebrally into day-old albino mice, the brains of which were harvested, homogenized and made-up as a 10% suspension in buffer lactose pepton (BLP*). Ampoules with 0,5 mℓ of this suspension were lyophilized, sealed and the titre determined to be $4 \times 10^{5,8}$ MLD_{50}/mℓ.

This antigen was reconstituted to the original volume with distilled water and each animal was inoculated subcutaneously in the thigh with 1,0 mℓ of the suspension.

Temperature reactions and clinical symptoms

After the animals had been inoculated with WSL virus, their temperatures and clinical signs were recorded 3 times a day.

Pathology

Eleven sheep and 6 goats were sacrificed for pathological studies at various stages of the temperature reaction (54–100 h post-inoculation). Two goats were necropsied 144 h and 147 h after infection with the virus. Tissues were collected in 10% buffered formalin and routinely processed and embedded in paraffin wax. Sections 4–6 μm thick were then cut and stained with haematoxylin and eosin (HE). In addition, special staining techniques such as the periodic acid-Schiff (PAS) without digestion, Masson's trichrome, Wilder's reticulin, Fontana's and Mallory's stains (Anon., 1968) were applied to liver sections.

RESULTS

Sheep and goats

Temperature reactions and clinical symptoms

Out of the 33 sheep and 31 goats inoculated with WSL virus, 26 sheep and 15 goats showed a moderate to marked temperature reaction, 6 sheep and 5 goats reacted mildly, while 1 sheep and 11 goats remained afebrile.

The temperature elevation usually occurred between 40–92 h (occasionally extending to 108 h) after inoculation, and body temperatures ranged from 40,2–41,6 °C.

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* BLP: Final concentration of 1% peptone and 5% lactose in 1/10 M phosphate buffer

A biphasic febrile reaction was observed in 7 sheep and 4 goats. The 1st peak in temperature occurred 40–50 h post-inoculation and lasted for approximately 5–10 h. This was followed by a 2nd peak of longer duration (32–48 h), during which higher temperatures were often recorded. Apart from the febrile response, no other clinical signs of illness were seen or deaths recorded in any of the animals.

Gross pathology

Despite the specific hepatotropism of WSL virus, the liver was only slightly swollen and discoloured a dull-brown in one sheep, while a moderate icterus occurred in another sheep. A slight hydropericardium and ascites, accompanied by lymphadenopathy and splenomegaly, were noted in some animals. No lesions were seen in any other organ.

Microscopic pathology

Liver: The microscopic liver lesions in all the animals were relatively mild and characterized by small, multifocal areas of necrosis with a pronounced localized Kupffer cell reaction (''retothelial nodules'') (Fig. 1). A few necrotic hepatocytes and sometimes neutrophils were dispersed among the macrophages in these foci (Fig. 2). In addition to reactive foci, acidophilic bodies and a few small groups of necrotic hepatocytes, associated with little or no inflammatory response, were seen in some lobules of most livers (Fig. 3). The acidophilic bodies were typified by a more eosinophilic, coagulated and shrunken cytoplasm which ofter contained a pyknotic or karyorrhectic nucleus. These bodies often detached and fragmented into smaller portions, lying free in the liver cell plates, Disse's spaces and sinusoids. Intranuclear eosinophilic, rod or avoid-shaped inclusions were occasionally seen in necrotic hepatocytes.

The hepatocytes in the rest of the lobule revealed mild degenerative changes such as cloudy swelling, hydropic degeneration and mild fatty metamorphosis. A few neutrophils were noted in the sinusoids while the Kupffer cells were slightly activated and sometimes contained yellow-brown pigment and phagocytosed cellular debris. A mild to moderate mononuclear cell infiltrate, comprised mostly of lymphocytes, was evident in the portal triads of a few animals (Fig. 4).

Other organs: Although the lymphoid tissues were not always affected, the majority of cases showed some degree of pyknosis and karyorrhexis of lymphocytes as well as lymphoid hyperplasia in the spleen and lymph nodes. An increased number of neutrophils was often present in the red pulp of the spleen. The only other noteworthy lesion occured in the heart which occasionally revealed hyalin degeneration and necrosis of individual myocardial fibres.

Discussion

Despite the widespread occurrence of WSL virus in sheep and cattle in southern Africa, the incidence of clinical disease and mortality in adult sheep and cattle seems to be very low (Weiss *et al.*, 1956; Coetzer *et al.*, 1978; Blackburn & Swanepoel, 1980). On the other hand, Coetzer *et al.* (1978) reported approximately 30% mortality among new-born lambs experimentally infected with WSL virus. The 33 sheep and 31 goats inoculated with WSL virus during this experiment clearly showed that adult sheep and goats are much less susceptible than new-born lambs. While some animals remained afebrile, the majority showed a mild to moderate temperature reaction. However, none of the adult sheep or goats became clinically ill or died. This finding confirms the report of Weiss *et al.* (1956), who found that deaths only occurred among new-born lambs and a few of the ewes that aborted during the first outbreak of WSL in South Africa. The wethers and yearlings in the same flock did not show any symptoms of the disease.

In sharp contrast to our findings and those reported for new-born lambs and adult sheep (Weiss *et al.*, 1956; Coetzer *et al.*, 1978; Blackburn & Swanepoel, 1980), Belonje (1958) described a relatively high mortality (up to 25%), with various clinical signs and lesions, including widespread haemorrhages, intense icterus, marked hepatomegaly and gastrointestinal stasis associated with field outbreaks of WSL in the Middelburg district in the Karoo. Le Roux (1959) reported on the histopathology of many fatal cases during these outbreaks. According to Le Roux (1959), the liver lesions of some atypical cases were of such a variable nature that interpretation was rather confusing and difficult. He stated that he could not differentiate some of the Karoo cases of WSL from enzootic icterus, a form of chronic copper poisoning prevalent in certain areas in the Karoo (De Kock, 1928; Bath, 1979). Furthermore, Bath (1979) mentioned that various stress factors such as drought, handling of animals, dosing procedures and viral infections could precipitate an outbreak of enzootic icterus. Thus, WSL virus, which has a specific tropism for the liver, might well have triggered enzootic icterus in some of the sheep, thus accounting for the relatively high mortality and atypical symptoms and pathology associated with WSL in the Karoo (Belonje, 1958; Le Roux, 1959).

Although there was moderate icterus in 1 sheep and a slightly swollen liver in another, macroscopic examinations of the sheep and goats were essentially negative. However, microscopic hepatic lesions were present in all the animals and were characterized by sparsely distributed foci of necrosis associated with a marked localized Kupffer cell response. In addition to these ''retothelial nodules'', acidophilic bodies and small groups of hepatocellular necrosis were evident in some of the lobules. A mild portal inflammation occasionally accompanied these intralobular changes. The hepatic lesions in adult sheep and goats were considerably milder and also somewhat different from those described for new-born lambs (Coetzer *et al.*, 1978). In general, the parenchyma, which showed individual or small groups of hepatocyte necrosis throughout the lobules, was more diffusely affected in the new-born lamb. These necrotic changes were frequently accompanied by moderate to marked Kupffer cell proliferation and pigmentation, bile stasis and portal inflammation. Well-circumscribed, reactive foci, characteristic of the hepatic lesions in adult sheep and goats, were not seen in neonate lambs infected with WSL virus.

The epizootiology of Rift Valley fever (RVF) is very similar to that of WSL (Weiss, 1957), and both diseases are responsible for losses in new-born lambs (Weiss *et al.*, 1956; Coetzer, 1977; Coetzer *et al.*, 1978). However, Coetzer *et al.* (1978) demonstrated a mortality rate of 90% and higher for new-born lambs infected with RVF virus. According to these authors, there are also distinct differences in the hepatic lesions induced by these 2 diseases in the new-born lamb.

This study indicated that, as in the neonate lamb, WSL is a much milder disease in adult sheep and goats than RVF. Although our cases did not represent the fatal stage of WSL, there were noteworthy differences between them and reported cases of terminal RVF in adult sheep. The liver lesions in WSL were mainly limited to small sparsely distributed ''retothelial nodules'' which consisted almost exclusively of proliferating Kupffer cells interspersed with a few necrotic hepatocytes and

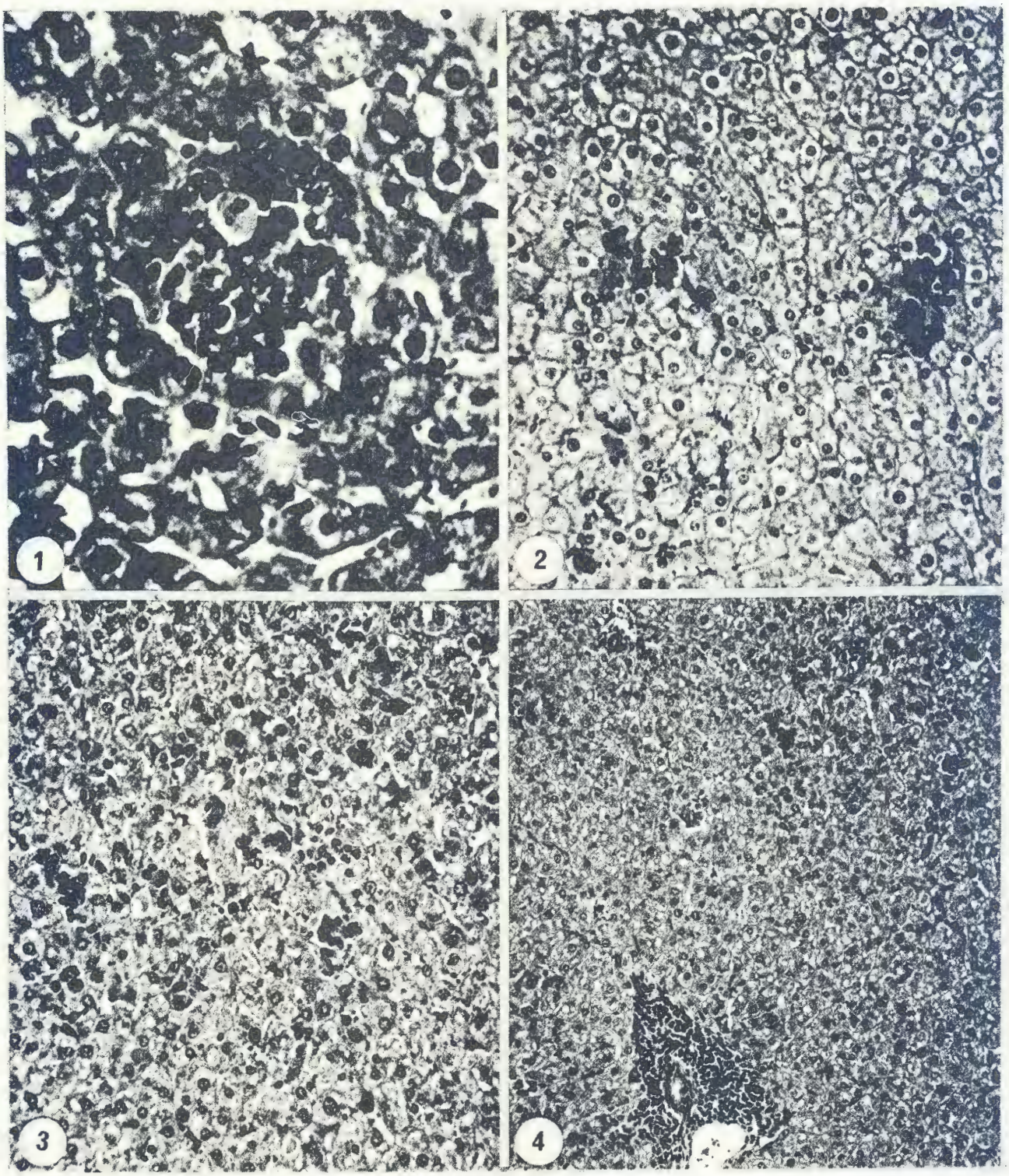

FIG. 1 Focal necrosis accompanied by marked localized Kupffer cell proliferation (''retothelial nodule''): HE × 250

FIG. 2. Small foci of necrosis and acidophilic bodies dispersed through the lobule: HE × 250

FIG. 3 Scattered groups of necrotic hepatocytes and acidophilic bodies with little or no inflammatory response: HE × 250

FIG. 4 Moderate lymphocytic infiltration in portal triad. Note single or small groups of hepatocellular necrosis throughout lobules: HE × 160

neutrophils. On the other hand, the hepatic lesions in RVF were characterized by widespread, larger, well-circumscribed foci of coagulative and eosinophilic necrosis, containing abundant cellular debris, karyorrhectic material, and a few neutrophils and macrophages.

Our results show that WSL virus is responsible for a very mild disease in adult sheep and goats and that primarily it is the liver and lymphoid tissues that are affected.

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