

RESEARCH COMMUNICATION

The diagnosis of Wesselsbron disease in a new-born lamb by immunohistochemical staining of viral antigen

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ABSTRACT

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Wesselsbron disease (WSL) was diagnosed in a 2-d-old lamb on a farm in the north-eastern Free State Province where a few abortions and neonatal deaths occurred in sheep in April 1994. The liver of the lamb was slightly swollen and orange-brown and, microscopically, it revealed single or small groups of necrotic hepatocytes that were randomly scattered throughout the lobules. Other histopathologic hepatic lesions included the presence of acidophilic bodies, intranuclear inclusion bodies in a few hepatocytes, neutrophils in the parenchyma, mild Kupffer-cell and bile-ductular proliferation and infiltration of mixed inflammatory cells in the portal tracts. Immunohistochemical staining of sections of formalin-fixed specimens of the liver with polyclonal antibody against WSL virus revealed positive staining in acidophilic bodies, cytoplasmic fragments of necrotic liver cells, the cytoplasm of degenerated hepatocytes and, rarely, in intranuclear inclusions. Positive staining was also obtained in liver sections from two new-born lambs experimentally infected with WSL virus.

The results of this investigation showed that the immunohistochemical staining of sections of formalin-fixed liver can be used to confirm the diagnosis of WSL in new-born lambs.

Keywords: Diagnosis, Wesselsbron disease, new-born lamb, immunohistochemical staining

INTRODUCTION

Wesselsbron disease (WSL) is a sporadic mosquito-borne flavivirus illness, particularly of new-born lambs

and kids, but adult sheep, cattle and goats may also become infected under field conditions. In new-born lambs and kids the disease is characterized by hepatomegaly, orange-yellow discoloration of the liver, severe icterus, and sometimes accumulation of chocolate-brown, partially digested blood in the abomasum. The mortality rate in new-born lambs and kids is about 20–30%. In adult animals, subclinical infections are common. Congenital malformations of the central nervous system, accompanied by arthrogryposis of ovine and bovine foetuses, have been reported following infection of the dam, during pregnancy, with WSL virus (Coetzer & Barnard 1977; Coetzer, Theodoridis & Van Heerden 1978; Coetzer, Theodoridis, Herr & Kritzinger

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1979; Swanepoel & Coetzer 1994a). Apart from its isolation from domestic ruminants, WSL virus has been isolated only from humans, a camel, a gerbil and arthropods (Swanepoel 1989), and recently from ostriches (D. Allwright, Regional Veterinary Laboratory, Stellenbosch, personal communication 1994).

WSL virus was originally isolated from an 8-d-old lamb on a farm in the Wesselsbron district of the Free State in 1955, where neonatal deaths and abortions occurred. Subsequently only a few isolates of the virus have been made from lambs and calves from different parts of South Africa (Swanepoel 1989; Swanepoel & Coetzer 1994a). Some outbreaks of WSL in sheep in South Africa have been complicated by other diseases such as Rift Valley fever (RVF), geel-dikkop (a hepatogenous photosensitivity caused by the plant *Tribulus terrestris*) and enzootic icterus (a form of chronic copper poisoning) (Swanepoel & Coetzer 1994a).

The epidemiology of WSL was reviewed by Swanepoel (1989). Floodwater-breeding *Aedes* spp. mosquitoes would appear to be the principal vectors of the virus in southern Africa during years of higher-than-normal rainfall. Serological surveys indicate that WSL virus is widely distributed in most parts of Africa, but firm evidence that it causes significant economic losses in livestock is lacking. In southern Africa, there is a high prevalence of antibody to the virus in domestic ruminants in the warmer and moister, largely cattle-farming areas of northern KwaZulu-Natal (South Africa), Mozambique and Zimbabwe. In contrast, antibody to the virus is less commonly found on the drier and more temperate inland plateau of South Africa which includes the southern Gauteng, Free State and Karoo scrublands where sheep farming predominates. For this reason the disease is more readily apparent in non-endemic than in endemic regions, and the drier conditions and winter frosts ensure that outbreaks in non-endemic areas are irregular and short-lived.

This is the first report of the diagnosis of WSL in a new-born lamb by means of immunohistochemical demonstration of viral antigen in sections prepared from formalin-fixed specimens of the liver.

CASE HISTORY

In April 1994, abortions and neonatal deaths in lambs occurred on two farms between Frankfort and Cornelia in the north-eastern Free State Province. On one farm seven ewes aborted and five new-born lambs died in a flock of 220 sheep, while on an adjacent farm, four ewes aborted and one new-born lamb died in a flock of 250 ewes. Weakness was seen in a few affected lambs, but most were found dead within 2 d after birth without revealing any signs of illness. No clinical signs were noticed in the mature sheep.

A necropsy was performed on a 2-d-old lamb from the first property. The liver was slightly enlarged and orange-brown, and a presumptive diagnosis of RVF was made. The first frost occurred in the area approximately 2 weeks after the outbreak and no further losses were encountered. According to the farmers there were increased numbers of mosquitoes at the time of the outbreak, as compared to the previous 4–5 years, most probably as a result of higher-than-normal summer rainfall in the area. The average rainfall figures measured at Frankfort for the period July to June for the years 1988–1994 were:

- 793,8 mm (1988–1989)
- 500,1 mm (1989–1990)
- 404,6 mm (1990–1991)
- 368,4 mm (1991–1992)
- 581,8 mm (1992–1993)
- 851,9 mm (1993–1994)

(G. Swart, South African Weather Bureau, Pretoria, personal communication 1994).

MATERIALS AND METHODS

Specimens of the liver, spleen, kidney, lung and brain of the necropsied lamb were collected in 10% buffered formalin, routinely processed and stained with haematoxylin and eosin (HE) for histopathology. For immunohistochemical staining, sections of formalin-fixed liver of this lamb and of two new-born lambs that had been experimentally infected with WSL virus (Coetzer, Theodoridis & Van Heerden 1978) and that served as positive controls, were used. Sections ($\pm 6 \mu\text{m}$ thick) were mounted on slides coated with 3-aminopropyltriethoxysilane, deparaffinized and hydrated. Endogenous peroxidase activity was quenched with 3% H_2O_2 in methanol for 30 min, followed by a wash in phosphate-buffered saline (PBS) containing 0,1% bovine serum albumin. Slides were treated with 0,05% protease XIV in 10 mM PBS (pH 7,6) for 15 min at 37 °C and washed twice in PBS, each for 5 min. Blocking of non-specific staining was achieved with 10% normal rabbit serum for 20 min at room temperature. The blocking serum was drained and the sections were covered with polyclonal hyperimmune mouse ascitic fluid to WSL virus (1:500), and incubated for 2 h at room temperature. After they had been washed, sections were incubated with biotinylated anti-mouse antibody (Dako), diluted 1:500, for 30 min at room temperature. The sections were washed and incubated for 30 min at 37 °C with avidin-biotin complex peroxidase solution prepared according to the manufacturer's directions (Vector Laboratories). Following this, the sections were incubated with the chromagen, 3,3'-diaminobenzidine 4HCl (DAB) (0,03% in PBS with 0,006% H_2O_2) for 8 min and the tissues were counter-stained with Mayer's haematoxylin. As controls, liver sections were stained with polyclonal hyperimmune mouse ascitic fluid to RVF virus,

and non-immune rabbit serum, and the primary antibody was substituted with PBS.

RESULTS

Microscopic examination of the liver revealed widespread hepatocellular necrosis characterized by single or small groups of necrotic hepatocytes scattered randomly throughout the lobules. Necrotic liver cells were shrunken and usually angular in outline and their cytoplasm was stained deeply eosinophilic and appeared granular, while the nuclei were pyknotic or karyorrhectic. As a result of hepatocellular necrosis and apoptosis, the liver-cell cords were disrupted and several dissociated necrotic hepatocytes, resembling acidophilic bodies, were present in sinusoids. The remainder of the hepatocytes showed degeneration and their nuclei occasionally contained single, irregular, inclusion bodies of variable sizes that stained weakly eosinophilic with HE. Small groups of neutrophils were scattered throughout the parenchyma. Mild proliferation of Kupffer cells, particularly in periportal areas, was evident. The portal tracts appeared diffusely cellular owing to mild bile-ductular proliferation that extended into the periportal parenchyma. The triads also showed a mild infiltration of neutrophils, macrophages and lymphocytes. The lesions in the other organs were mild and non-specific and included nephrosis, and congestion and oedema of the brain.

Polyclonal hyperimmune mouse ascitic fluid to WSL virus, stained viral antigen in the liver sections of the necropsied lamb and in those of the two lambs that were experimentally infected with WSL virus and used as positive controls. Positive staining was characterized by a deep brown reaction: it was most striking in acidophilic bodies and in cytoplasmic fragments of necrotic hepatocytes (Fig. 1). Antigen was also detected in the cytoplasm and, less commonly, in the nucleus of degenerated hepatocytes and, rarely, in intranuclear inclusion bodies. Degenerated liver cells that stained positive, revealed a focal or diffuse, fine to coarse granular pattern in their cytoplasm and an indistinct pale brown, finely granular reaction in their nuclei. Granular cytoplasmic staining of cells, probably fragments of necrotic hepatocytes, occurred in the lumens of a few portal blood vessels and central veins. No staining of cells in the liver sections was obtained with the antibody to RVF virus, non-immune rabbit serum or when the primary antibody was replaced with PBS.

DISCUSSION

The epidemiologies of WSL and RVF are very similar and both diseases usually manifest themselves after periods of increased rainfall and a subsequent

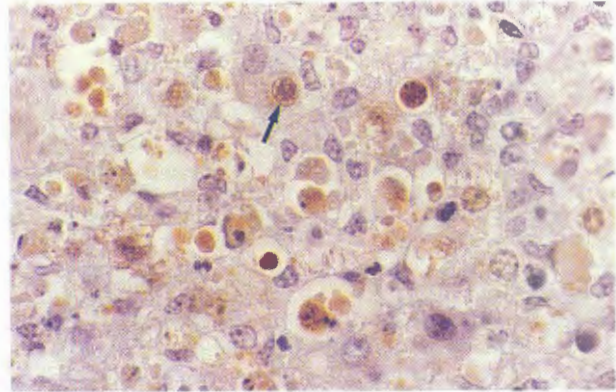


FIG. 1 Liver of new-born lamb. Wesselsbron-disease viral antigen is present in acidophilic bodies, fragments of necrotic hepatocytes, and in the cytoplasm and nuclei (arrow) of degenerated liver cells. Avidin-biotin peroxidase complex method. Mayer's haematoxylin counterstain $\times 600$

increase in the mosquito population (Swanepoel & Coetzer 1994a, 1994b). The definitive diagnoses of both WSL and RVF are currently based on virological and serological tests, and, when only formalin-fixed samples are available, on histopathological examination of the liver. Lesions in the present case of WSL in a new-born lamb were similar to those reported previously (Coetzer, Theodoridis & Van Heerden 1978). In the new-born lamb, WSL and RVF can be differentiated from each other on the ground of distinctive histopathological hepatic lesions. Despite the widespread hepatocellular necrosis that may be present in WSL, neither the well-defined primary foci of coagulative necrosis of hepatocytes nor the parenchymal-haemorrhage characteristic of RVF in lambs, are evident in WSL. In addition, lesions in WSL, in comparison to RVF, are generally less extensive and less acute, as evidenced by the presence of bile stasis, proliferation of Kupffer cells and bile-ductular epithelium, and portal infiltration of mononuclear cells (Coetzer 1977; Coetzer, Theodoridis & Van Heerden 1978).

The findings show that the detection of viral antigen in histological sections can be used to confirm a diagnosis of Wesselsbron-disease-virus infection in instances where specimens suitable for virological examination are not obtained. The method can also be applied in retrospective studies on formalin-fixed liver specimens.

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