THE PATHOLOGY OF RIFT VALLEY FEVER. I. LESIONS OCCURRING IN NATURAL CASES IN NEW-BORN LAMBS

J. A. W. COETZER, Veterinary Research Institute, Onderstepoort, 0110

ABSTRACT


A widespread epizootic of Rift Valley fever occurred in the Republic of South Africa and South West Africa during 1974–75. This is a report on the gross pathology of 34 new-born lambs and the histopathology of 93 new-born lambs that died during this outbreak.

The liver was affected in every case and showed the most pronounced lesions. The organ was grossly enlarged in most cases, with scattered greyish-white necrotic foci 1–2 mm in diameter and haemorrhages of varying size throughout. Haemorrhages were also frequently seen in the mucosa of the abomasum.

The massive diffuse necrosis of hepatocytes (pannecrosis) associated with well-demarcated foci of primary coagulative necrosis, present in 100% of the cases examined, was characteristic of the histopathology of the new-born lamb. Bile thrombi were noticed in the livers of 31% of the lambs and intranuclear inclusions in 49% of the cases. The diagnostic significance of the microscopic liver lesions is discussed.

Focal necrosis and haemorrhages were frequently seen in the adrenal cortex while generalized destruction of lymphocytes in the lymph nodes and spleen occurred in many of the animals.

In addition, the following hitherto undescribed or previously not well-documented lesions are recorded: (i) mineralization of single or groups of necrotic hepatocytes in 62% of the livers; (ii) pyknosis and karyorrhexis of the cellular elements in the glomeruli and a hyalinized appearance of many of these affected glomeruli; and (iii) necrosis of the tips of the villi in the small intestine in some of the animals.

Résumé

PATOLÓGIE DE LA FIEVRE DE LA RIFT VALLEY. I. LÉSIONS PRODUITES CHEZ DES AGNEAUX NOUVEAU-NÉS DANS DES CAS NATURELS

En 1974–5 la République sud-africaine et le Sud-ouest africain ont connu une épidémie généralisée de fièvre de la Rift Valley. Le présent travail est un rapport sur la pathologie macroscopique de 34 agneaux nouveau-nés et l'histopathologie de 93 agneaux nouveau-nés, ayant tous succombé à la manifestation de cette épidémie.

Le foie était toujours atteint et montrait les lésions les plus accusées; il présentait une forte hyper trophy dans la plupart des cas avec une dispersion de foyers nécrotiques blanc-grisâtres de 1 à 2 mm de diamètre. On a trouvé partout des hémorragies d'ampleur variée; la maquette de la caillette présentait souvent aussi des hémorragies.

L'histopathologie de l'agneau nouveau-né se caractérise par la pan-nécrose (nécrose diffuse et massive) des hépatocytes, associée à des foyers bien délimités de nécrose coagulante primaire: on l'a trouvée dans tous les cas examinés. Des caillots de bile ont été observés dans le foie chez 31% des agneaux examinés et des inclusions intra-nucléaires étaient présentes dans 49% des cas. On discute l'importance pour le diagnostic des lésions hépatiques au niveau microscopique.

Le cortex surrenal était souvent le siège de nécroses focales et d'hémorragies, tandis que de nombreux animaux présentaient une destruction généralisée des lymphocytes dans les nodules lymphatiques et la rate.

En outre, on a noté des lésions qui jusqu'alors n'avaient pas été décrites ou sur lesquelles on n'a que peu de documents: (i) la minéralisation d'hépatocytes isolés ou de groupes d'hépatocytes dans 62% des foies; (ii) la pyknose et la karyorrhexis des éléments cellulaires des glomérules ainsi que l'apparition hyalinisée d'un grand nombre de glomérules affectés; et (iii) la nécrose des extrémités des villosités de l'intestin grêle chez certains animaux.

INTRODUCTION

Rift Valley fever (RVF) was originally diagnosed in sheep and cattle in Kenya by Daubney, Hudson & Garnham (1931). The disease was subsequently reported in the Republic of South Africa in ruminants and in man (Mundel & Gear, 1951; Alexander, 1951; Schulz, 1951; Van der Linde, 1953; Weiss, 1957).

Apart from the excellent original description of the pathology of RVF in the new-born lamb by Daubney et al. (1931) and later by Findlay (1932) and Easterday, McGavran, Rooney & Murphy (1962), virtually no other detailed reports exist in the literature on the lesions in the very young lamb. Easterday et al. (1962) pointed out that the liver lesions of RVF in young lambs are consistently present in both natural and experimental cases. Daubney et al. (1931) affirmed that the microscopic appearance of the lesion in the liver can be used to establish a diagnosis beyond any possibility of error. Schulz (1951) regarded the lesion in the liver to be pathognomonic for the disease.

The purpose of the present study was to confirm the findings of the earlier workers and, in addition, to document lesions not previously described in new-born lambs.

HISTORY

South Africa experienced a severe outbreak of RVF during 1974–75 when abnormably wet climatic conditions favoured the breeding of large numbers of insect vectors. The first reports of severe losses in new-born lambs were received at the end of March 1974 from Bulfontein in the Orange Free State. The disease then spread rapidly through the whole of the Orange Free State, the north-western Cape Province and the southern parts of South West Africa. Outbreaks were also reported in the drier Northern and far Northern Transvaal in both sheep and cattle. Isolated outbreaks occurred in Northern Zululand, the Natal South Coast, Griqualand East and in the Swellendam district of the Cape Province (Fig. 1).

According to reports received from veterinarians and farmers, neonatal lamb losses of 90–95% were recorded in many flocks while a mortality rate of 15–20% in adult sheep occurred. The abortion rate in

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sheep ranged between 35-40% and many pregnant ewes that aborted died as a result of secondary complications. Neonatal calf mortality which varied between 10-15% was not as high as in lambs. An abortion storm was frequently the only manifestation of RVF in cattle without any clinical signs or deaths. However, some farmers reported a 3-5% mortality in adult cattle.

**Material and Methods**

**Autopsies**

Complete autopsies were performed on 34 lambs, 8 of which were sacrificed in extremis. The lambs originated from outbreaks on 7 different farms and each outbreak was confirmed as RVF by virus isolation (vide infra). A wide range of organ specimens was collected from these lambs in 10% buffered formalin.

**Routine histopathological specimens**

Formalin-fixed organ material from a further 59 cases was received from veterinarians in areas where active outbreaks occurred. Twenty-three of the above cases, representing different field outbreaks and where specimens on ice were also submitted, proved positive for RVF on virus isolation. While from some of these outbreaks a single virus isolation only was done, formalin-fixed specimens from a number of lambs were received in many instances.

The other cases examined histopathologically in this study, which were not confirmed as RVF by virus isolation, were included because they did not differ in any way microscopically from the confirmed cases. A history indicating RVF was received in all of these unconfirmed cases.

**Histopathological techniques**

Formalin-fixed organ specimens were processed in a routine manner and embedded in paraffin wax. Sections were made at 3-4 μm and stained with haematoxylin and eosin (HE). In addition, special staining techniques were applied to various tissues (Table 1).

**Virus isolation**

Liver and spleen specimens were ground and made up to a 10% suspension in buffer lactose peptone (BLP)* and injected into suckling albino mice by the intracerebral route. The brains of sick mice were harvested and suspended in BLP and saline. The virus was then identified by means of a serum neutralization test, using serial virus dilution constant serum (Cunningham, 1960).

**Results**

**Gross pathology**

At autopsy the lambs frequently revealed widespread haemorrhages in the subcutaneous tissues and serous surfaces. Slight icterus occurred in about 10% of the lambs.

*BLP = Final concentration of 1% peptone and 5% lactose in 1/10 M phosphate buffer.
Gross changes were present in the livers of all the lambs autopsied. Most cases showed a slight to moderate enlargement of this organ (Fig. 2, 3 & 5). The livers were soft and friable and the colour ranged from a yellowish-brown (Fig. 2, 3 & 5) to a very dark red (Fig. 4). Greyish-white necrotic foci, 1–2 mm in diameter, scattered throughout the substance, were commonly observed (Fig. 4 & 5). However, the discoloration of the liver sometimes made it difficult to assess the focal disseminated necrosis (Fig. 5). Livers of normal size and showing only discoloration and inconspicuous focal necrosis were rarely encountered. Haemorrhages, varying in size from petechial to 2–3 cm in diameter, were often observed in the subcapsular region and throughout the substance of the liver (Fig. 2, 3, 5 & 7). One lamb had a subcapsular haematoma 1–2 cm in diameter. Focal congestion of the liver, manifested as large irregular red patches, was frequently seen (Fig. 3 & 5). The area surrounding the portal fissure usually was oedematous and the hepatic lymph nodes were enlarged and congested with petechial haemorrhages. The lambs petechiae were present in the cortex. In some of the lambs perirenal oedema was found. Infrequently small haemorrhages occurred in the urinary bladder. The mucosa of the abomasum (Fig. 9) and subcapsular and subcapsular petechiae were encountered. Subepi- and subendo-cardial haemorrhages were the only obvious macroscopic lesion in the heart although a few lambs also showed a slight hydropericardium.

Histopathology

Liver. The most important microscopic changes in the liver are summarized in Table 2.

<table>
<thead>
<tr>
<th>Lesion</th>
<th>% of cases positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary foci of necrosis</td>
<td>100</td>
</tr>
<tr>
<td>Pannecrosis</td>
<td>83</td>
</tr>
<tr>
<td>Bile stasis</td>
<td>31</td>
</tr>
<tr>
<td>Mineralization</td>
<td>62</td>
</tr>
<tr>
<td>Intracellular inclusions</td>
<td>49</td>
</tr>
<tr>
<td>Portal reaction</td>
<td>5</td>
</tr>
<tr>
<td>Pigmentation of hepatocytes and reticuloendothelial cells</td>
<td>32</td>
</tr>
</tbody>
</table>

Massive diffuse hepatic necrosis (pannecrosis), accompanied by the primary foci of coagulative necrosis, characterized the histopathology of the liver of the new-born lambs (Fig. 10, 11 & 12). Extreme pannecrosis was present in 83 % of the cases examined. Only the portal triads were preserved in these cases, while, in most of the remaining 17 % of the livers, small groups of surviving hepatocytes could be found close to the triads (Fig. 10). The surviving parenchymal cells frequently showed evidence of degenerative changes, including fatty metamorphosis. Necrotic hepatocytes still recognizable as such in the areas of massive necrosis revealed individualization, disintegration and eventually lysis. In some livers only the reticulo network remained (Fig. 11 & 12).
FIG. 2 Enlarged yellowish-brown liver with disseminated haemorrhages
FIG. 3 Focal congestion, manifested as large irregular patches, and scattered haemorrhages of the liver
FIG. 4 Well-circumscribed, greyish-white necrotic foci scattered through the substance. Oedema of the gall-bladder especially around the base
FIG. 5 Enlarged conspicuous yellowish-brown liver with greyish-white necrotic foci, haemorrhages and congestive areas
FIG. 6 Liver with a fibrinous perihepatitis
FIG. 7 Carcass showing a moderate icterus and an enlarged liver with haemorrhages. A blood coagulum present in the abdominal cavity can be seen in the lower right-hand corner
FIG. 8 Petechial and ecchymotic haemorrhages on the serosal surfaces of the gastrointestinal tract
FIG. 9 Haemorrhages in the mucosa of the abomasum. The abomasal content was very often dark brown in colour
FIG. 10 Liver with massive necrosis. Central vein in right-hand lower corner. Portal triad in upper left corner. HE × 75

FIG. 11 Massive hepatic necrosis, disintegration and lysis of the necrotic parenchymal cells. Central vein top centre and portal triad on the right. Four well-circumscribed primary foci of coagulative necrosis are visible. HE × 75

FIG. 12 Foci of coagulative necrosis. Severe lysis of surrounding hepatocytes. HE × 200

FIG. 13 Vacuolated hepatic nuclei with cigar-shaped eosinophilic inclusions. HP × 1 200

FIG. 14 Mineralization of single or small groups of necrotic hepatocytes. HE × 75

FIG. 15 The cytoplasm of the necrotic hepatocytes, packed with small, purplish-blue granules. HE × 1 200
FIG. 16 Mineralized primary foci and single cells. Dahl's Alizarin method for calcium. × 75
FIG. 17 Lymph node showing follicular necrosis. HE × 75
FIG. 18 Pyknosis and karyorrhexis of the lymphocytes. HE × 1,200
FIG. 19 Pyknosis and karyorrhexis of the cellular elements in the glomeruli. HE × 500
FIG. 20 Necrosis and desquamation of the epithelial cells situated on the tips of the jejunal villi. HE × 75
FIG. 21 Higher magnification of a necrotic villus tip. Note dilatation of lymphatics. HE × 500
cells were usually observed throughout the liver. These Councilman-like hepatocyte nuclei and infiltrating polymorphonuclear hepatocytes became more eosinophilic and were evident. Reasonably well-demarcated foci of severe coagulative necrosis, present in every case examined (Table 2), were scattered throughout the liver (Fig. 11 & 12). Although these foci were predominantly centrilobular or midzonal, they had no definite distribution in the lobule and appeared identical with the primary foci of necrosis described by Easterday et al. (1962). Some of them involved the portal areas, causing partial necrosis of the triads. When located centrilobularly or paracentrally, the central vein was frequently occluded by the primary necrotic focus of necrosis. Thrombosis occurred in only 3 cases where part of the central vein wall was necrotic. Infiltration of histiocytes, lymphocytes and neutrophils occurred in the foci. Many of these infiltrating cells revealed marked pyknosis and karyorrhexis. Disintegration and lysis of all the necrotic hepatocytes in the lobule, to the extent that only the reticulum network and residues of the necrotic foci remained, were encountered in 6 cases (Fig. 11 & 12). In these cases, the severe destruction of the parenchyma rendered the liver tissue hardly recognizable as such.

The nuclei of the still recognizable hepatocytes were vesicular with margination and fragmentation of the chromatin, giving them a vacuolated appearance. In 49% of the cases (Table 2), intranuclear inclusion bodies (Fig. 13) were seen in these cells. In appearance the inclusions varied from cigar-shaped through oval to round or ring-shaped (Fig. 13). As a rule the inclusion bodies were found with some difficulty, particularly in livers showing marked necrosis. However, in 5% of the livers, 80-90% of nuclei contained inclusions. These were livers in which less advanced necrosis occurred and in which more hepatocyte nuclei were still identifiable. The inclusions were eosinophilic in HE sections and also stained with HP and Lendrum's, but were unstained with the PAS reaction.
While the glomeruli appeared normal in many cases, extensive pyknosis and karyorrhexis of nearly all the cellular elements were found in approximately 10% of the kidneys examined (Fig. 19). Glomeruli showing necrotic nuclei were usually swollen, enlarged and hyalinized and contained a proteinaceous PAS-positive material. In addition some kidneys showed only odd glomeruli with mild pyknosis and karyorrhexis.

Adrenals. Focal disseminated haemorrhages and necrosis occurred throughout the cortex in 30-40% of lambs. The necrosis was more pronounced in the zona fasciculata and zona reticularis and was frequently associated with single or groups of neutrophils in the cortex. Fatty and hydropic degenerative changes were inconsistent observations in the adrenal cortex.

In the medulla necrosis was confined to individual cells and in isolated cases eosinophilic intracytoplasmic hyalin droplets were present.

Gastrointestinal tract. Haemorrhages occurred in the submucosa between the gastric pits as well as in the tunica muscularis and serosa of the abomasum.

More severe changes were seen in the distal jejunum and ileum. Necrosis and desquamation of the epithelial cells situated on the tips of villi were seen in 4 of the 8 lambs killed for autopsy (Fig. 20 & 21). Pyknosis and karyorrhexis of the mononuclear cells in the submucosa were also present. Fibrin adhered to the tips of the necrotic villi while a moderate oedema and a neutrophil infiltration occurred in the submucosa. The lumen of the gut and crypts contained necrotic debris. These changes were only discernible in animals killed for autopsy. Because of autolysis and post-mortem decomposition, these lesions could no longer be appreciated in most of the cases presented for autopsy some time after death. The lymphocytes in the Peyer's patches showed varying degrees of pyknosis and karyorrhexis, corresponding in degree of intensity to the changes in other lymphoid tissues in the body.

Lungs. The lungs showed a slight to moderate congestion and alveolar and interstitial oedema with occasional focal haemorrhages. Other changes, not consistently present, were emphysema, single cell pyknosis and karyorrhexis in the alveolar septae and peribronchial lymphoid tissue. In a few cases a very mild neutrophil and macrophage infiltration into the interstitial tissue occurred. Thrombosis was seen in a small blood vessel in the lung in 1 lamb only.

Heart muscle. Except for subepi- and subendocardial haemorrhages no other histopathological changes were seen.

Brain and eyes. In some of the lambs the brain was slightly congested and oedematous. Encephalitis was not observed in any of the lambs, neither were any lesions seen in the eyes.

Other organs. Other organs, for example, the urinary bladder, testis, uterus, pancreas, thyroid gland, salivary glands, thymus, tongue and skeletal muscles were all normal.

DISCUSSION

The findings reported here concur fully with those of Daubney et al. (1931) and Schulz (1951) on the diagnostic significance of the microscopic lesions in the liver of the new-born lamb. This is substantiated by the fact that 100% of the cases examined in the present study showed primary foci of necrosis. In conjunction with this, a severe pan necrosis of the liver occurred in 83% of cases and in 49% of the lambs intranuclear inclusion bodies were noted in the hepatocytes. Histopathologically this lesion can hardly be confused with that of any other disease in the new-born lamb.

Both Daubney et al. (1931) and Schulz (1951) pointed out that the liver lesion in the new-born lamb differs from that in the older animal in that it is much more severe. In older animals the necrosis is as a rule confined to focal areas of the individual liver lobules while, in the new-born lamb, whole lobules throughout the liver are practically destroyed. According to Easterday et al. (1962), the initial liver lesion in experimentally-produced cases in lambs consisted of primary foci of necrosis. After these primary foci of necrosis were established, massive destruction of most of the remaining hepatocytes of the lobules ensued.

Neither Daubney et al. (1931) nor Schulz (1951) gave the total number of animals or the numbers of the various age groups examined by them. These workers also regarded the microscopic liver lesions in the older animal as highly characteristic. Recently Swanepoel (1976) reported a high degree of correlation between the histopathological diagnosis and the serum-virus neutralization test in RVF in bovine foetuses.

In addition to the typical liver lesions described by previous workers, a number of microscopic changes were observed which had not been described previously. Of these the most striking was the mineralization of single or groups of necrotic hepatocytes in 62% of the cases examined. The significance and pathogenesis of this phenomenon in RVF in the new-born lamb is not clear at this stage. A sudden pH change due to the rapid and extensive necrosis of hepatocytes may explain this dystrophic type of mineralization. Reynolds (1965) found accumulation of calcium in the mitochondria of rat liver cells 15-24 h after poisoning with carbon tetrachloride. He associated it with an increase in the amount of liver inorganic phosphate and the persistence of mitochondrial adenosine triphosphatase activity. Mineralization of focal necrotic hepatocytes has been described in an aborted bovine foetus due to infectious bovine rhinotracheitis (Kennedy & Richards, 1964).

Daubney et al. (1931) mentioned 'rhexis' of polymorphonuclear cells in the glomeruli of sheep which survived for 9 days after infection. According to these workers, the glomeruli also contained more nuclei than normal at this stage of the disease. In most of the cases reported here, varying degrees of pyknosis and karyorrhexis of nuclei in the glomeruli were seen. In addition many of the affected glomeruli contained a proteinaceous PAS-positive material, which gave them a hyalinized appearance. It was difficult to determine the exact origin of these necrotic cells. Three possibilities may be considered: circulating mononuclear or polymorphonuclear cells, intercapillary or mesangial cells and, finally, endothelial cells. According to Jubb & Kennedy (1970), the mesangial cells are the supporting connective tissue cells in the glomeruli and are surrounded by a scant matrix with a few collagen fibrils. They state that the mesangial cells and their matrix reflect much of the injury to the glomeruli. In view of this, the impression was gained that the mesangial cells were the main cells undergoing necrosis in the glomeruli.
and not polymorphonuclear cells as originally reported by Daubney et al. (1931). It should, however, be pointed out that the latter authors recorded this glomerular lesion in sheep recovering from the disease, while the cases reported here were new-born lambs, dying acutely shortly after the onset of the disease.

No previous account of necrosis of the tips of the villi in the small intestine in RVF lambs could be found in the literature. This was seen only in fresh tissue with no evidence of autolysis. Owing to the rapid onset of autolytic changes in the epithelium of the intestine, this lesion was obscured in all the cases that were examined some time after death.

Although they were not constantly present, the percentage of hepatocytes containing intranuclear inclusions varied greatly from case to case. Forty-nine per cent of the livers examined contained intranuclear inclusions. No reference to the diagnostic significance of these inclusions was found. Easterday et al. (1962) reported that intranuclear inclusions in hepatocytes in new-born lambs appeared with 'some regularity'. Findlay (1933) reported on the nuclear changes and intranuclear inclusions in RVF in different animal species. According to him, 80-90% of the hepatocytes contained intranuclear inclusions at some stage of the disease in lambs and small rodents. He also mentioned that these inclusions were less numerous shortly before death when many hepatocyte nuclei showed karyorrhexis or had disappeared. The incidence of intranuclear inclusions recorded in the present study indicates that the mere presence of these structures is of limited diagnostic value in the new-born lamb.

According to Smetana (1963), Councilman bodies were named after Councilman who, in 1890, first described these structures in hepatocytes of patients that had died of yellow fever. Daubney & Hudson (1933) were the first to describe Councilman-like bodies in RVF in lambs. Subsequently, various other workers confirmed their finding: in sheep (Henning, 1952), mice (Mims, 1957) and in dogs and cats (Mitten, Remmde, Walker, Carter, Stephen & Klein, 1970). Mims (1957) stated that Councilman-like bodies were seen more commonly when inoculae, containing heat inactivated RVF virus or incomplete RVF virus due to successive passages, were injected into mice.

The term acidophilic body is sometimes preferred to describe these structures since a large group of agents may produce identical bodies. Proctor & Ruiz (1968) described acidophilic bodies in humans with Bolivian, Philippine and Thai haemorrhagic fevers, yellow fever and schistosomiasis. Histochemical and electron microscopic studies by them indicated that the acidophilic bodies were modified dead hepatocytes. They also suggested that some acidophilic bodies may be due to partial cytoplasmic degradation, resulting in death and expulsion of part of the cell. These bodies, lying free in the sinusoids, may be ingested by the Kupffer cells.

Ultrastructural studies on the livers of patients with hepatitis and hepatomas proved that these acidophilic bodies contained different organelles, pigment resembling lipofuscin, fat, components of bile, but no viral particles were seen (Biava & Mukhlova-Montiel, 1965).

Cholestasis was found in 31% of the livers examined histopathologically. In addition to bile pigments present in the bile canaliculi, other pigments were also encountered in hepatocytes, tubular epithelial cells in the kidney and RE cells in various organs. The latter pigments were identical histochemically with the lipoproteinaceous meconial bile pigment described by Basson, Morgenthal, Bilbrough, Marais, Kruger & Van der Merwe (1969). According to these authors, the pigment, which may be confused with bile pigments histologically and grossly, occurs in various lymph nodes, kidney, liver and other tissues of normal new-born lambs. They described a sequence in its development, distribution and histochemical nature.

Rift Valley fever in man is classified as one of the haemorrhagic fevers. Mims (1956) stated that infection with panprotropic RVF virus, which causes extensive liver necrosis, results in a prothrombin deficiency and subsequent delay in the blood clotting time in mice. However, according to him, mice infected with the neurotropic strains of RVF virus showed no prothrombin deficiency. A prothrombin deficiency may also play a role in the causation of widespread haemorrhages commonly seen in RVF infected domestic animals.

Daubney et al. (1931) reported that in some lambs the caecum and colon were impacted with a putty-like grey faeces resembling a canine stool. However, this was seen in only 2 lambs in this study. Perforation of the abomasum and caecum in lambs reported by Van der Linde (1953) was not recorded during this outbreak.

It is evident from the results presented here that the microscopic picture in the liver is characteristic for RVF in the new-born lamb. A highly accurate diagnosis of the disease can be based on the presence of these liver lesions.

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References


