An Outbreak of Sarcocystosis in Dairy Cattle

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Summary

Sixteen of 32 Friesian calves, 8 to 10 weeks old, died over 4 weeks. The calves were housed in pens previously used by dogs. Clinical signs included anorexia, pale mucous membranes, rapid weight loss, coughing and palpably enlarged superficial lymph nodes. At necropsy, calves were emaciated and had generalised enlargement of lymph nodes, pale mottling of skeletal muscles, excess peritoneal, thoracic and pericardial fluid and subpleural and subepicardial haemorrhages. Histologically there was a lymphadenitis, myositis, myocarditis, glomerulonephritis, interstitial pneumonia and encephalitis. Schizonts of a sporozoan parasite, presumably *Sarcocystis cruzi* were found in the endothelial cells of blood vessels in many organs. *(Aust vet. J. 63:22-24.*

Introduction

Cysts of the sporozoan parasite *Scarcocystis* are frequently seen on histological examination of cardiac and skeletal muscle of cattle. *Sarcocystis* sp were considered of doubtful pathogenicity until Fayer and Johnson (1973) demonstrated the life cycle and pathogenicity of *S. cruzi* in calves. Since then, outbreaks of sarcocystosis in cattle have been reported from Canada (Mead 1976), England (Clegg *et al* 1978), United States of America (Frelier *et al* 1977); Schmitz and Wolfe 1977; Giles *et al* 1980), Norway (Landsverk 1979) and Ireland (Collery and Weaver 1981). Although unrecognised at the time, Dalmeny disease (Corner *et al* 1963) was probably the first reported case of sarcocystosis in cattle.

This paper describes a field outbreak of sarcocystosis in dairy calves and appears to be the first description of the disease in Australia.

History

In October 1983 calves on a commercial dairy farm on the central tablelands of New South Wales experienced a disease

which resulted in the death of 16 of a group of 32 Friesian heifers. Calves 8 to 10 weeks of age had been dying for 4 weeks prior to veterinary investigation. Three calves were presented for necropsy over one week.

Calves were weaned at 3 to 4 days of age and placed in calf rearing pens where they were fed milk and crushed oats and had access to pasture. The 32 calves were the first group to be reared in these pens, which had been used for the previous 8 to 10 years to house greyhounds and consisted of fenced areas of pasture with a shed at one end for shelter. Meat from cows and calves that died on the farm has been fed intermittently to the dogs over this time. The greyhounds had been sold and the pens left vacant for 2 to 3 months prior to the calves being placed in them.

Calves became sick at 7 to 8 weeks of age and death occurred within 1 to 2 weeks of initial signs. Clinical signs included anorexia, pale mucous membranes, rapid weight loss, dry harsh coats, coughing associated with an increase in harsh respiratory sounds in the ventral thorax, mucopurulent nasal discharge, excessive salivation and palpably enlarged superficial lymph nodes. Terminally, calves exhibited muscular weakness and had difficulty rising. Affected calves that did not die had a protracted period of ill health. During the course of the outbreak calves were treated for internal and external parasites and were given systemic antibiotics with no apparent effect on the course of the illness.

Laboratory Findings

Haematological examination of 2 calves 24 h prior to their deaths showed a normocytic normochronic anaemia, with a marked reduction in the PCV (14-16%), RBC (3.2-4.0 x 10^6/ml) and haemoglobin (4.5-4.7g/dl) concentrations. Blood samples from 3 other clinically affected calves showed a similar, though less severe, anaemia.

Serology

Serum was collected from 5 calves during the acute stage of the disease (calves 1-5) and from 7 of the surviving calves 8 weeks later (calves 6-12). These serum were examined for antisarcocystis IgM and IgG in an enzyme-linked immuno-sorbent assay (ELISA) using the soluble antigen prepared

from *S. cruzi* bradyzoites as described by Gasbarre *et al* 1984). The indirect haemagglutination (IHA) titres of the serum were determined as described by Lunde and Fayer (1977) using the same antigen. Serological results (J. P. Dubey personal communication) are listed in Table 1. The ELISA results are based on comparisons of optical density readouts with known uninfected and experimentally infected calves.

TABLE 1
Serological results from serum of clinically affected and convalescent calves

Calf	ELISA		IHA
	IgM	IgG	
Clinically Affected			
1	+	−	160
2	+	−	80
3	+	−	40
4	+	−	640
5	−	−	< 16
Convalescing			
6	+	+	>1280
7	−	+	>1280
8	−	+	>1280
9	+	+	1280
10	−	+	640
11	+	+	1280
12	−	+	>16

(+): indicates ELISA values close to those obtained for experimentally infected calves.

Gross Pathology

Necropsy findings in the 3 calves were similar. All calves were emaciated and subcutaneous petechial haemorrhages were seen in calf 3. Lymph nodes throughout the body were grossly enlarged and oedematous. Skeletal muscles had a pale mottling and in calf 3 the myocardium contained multiple, irregular, pale foci. There were increased amounts of peritoneal, thoracic and pericardial fluid. Subpleural and subepicardial haemorrhages were present in all calves. The lungs were oedematous and congested and in calves 2 and 3 the apical and cardiac lobes were consolidated. Purulent material exuded from the bronchi supplying these lobes. Livers were enlarged and pale, with rounded edges and the gall bladders were distended with thick dark mucoid bile. The colon and rectum of the 3 calves had a striped appearance due to petechial haemorrhages in the superficial mucosa.

Histopathology

Histological abnormalities were found in the lymph nodes, skeletal muscles, myocardium, lung, liver, kidneys and brain of the 3 calves. Developing stages of a sarcocystis parasite were identified in the endothelium of blood vessels in these organs and within myofibres of skeletal muscle and myocardium.

There were lymphoid hyperplasia in the cortex and paracortex of lymph nodes throughout the body. The medullary cords were distended with lymphocytes and plasma cells. Medullary sinuses were oedematous and filled with haemosiderin laden macrophages. Moderate numbers of schizonts were seen, mainly in the medulla of lymph nodes.

Degenerative myopathy was present in the skeletal muscles. The lesions varied in degree from muscle to muscle and between calves, being most severe in calf 3 and only mild in calf 2. Constant findings were a hypercellularity of muscles due to sarcolemma nuclear proliferation and infiltration with macrophages,, lymphocytes and plasma cells. These cells were primarily located in the interstitial space around myofibres and blood vessels. Individual myofibres were undergoing degeneration and necrosis. In calves 1 and 3, the myonecrosis was extensive. An intense cellular response was elicited by these degenerating and necrotic myofibres. Numerous immature sarcocysts, containing metrocytes, were found throughout skeletal muscles of calf 2. Small numbers of schizonts in the endothelium of blood vessels and a few immature sarcocysts were present in muscles of calf 3.

The myocardium of calves 2 and 3 showed a non-suppuratuve myocarditis characterised by an infiltration of mononuclear cells into interstitial and perivascular tissues. The heaviest infiltration occurred beneath the epicardium. These changes were only mild in calf 2. In calf 3 there was marked interstitial oedema, many haemorrhages and multifocal areas of necrosis characterised by loss of cross striation, hyalinisation of sarcoplasm, and pyknosis and karyorrhexis of nuclei. Many immature sarcocysts, containing metrocytes, were present in the myocardium of calf 2. In calf 3 a few immature sarcocysts were located in myofibres and schizonts were found in endothelial cells of blood vessels.

The lungs had a diffuse subacute interstitial pneumonitis with thickening of alveolar and interlobular septa due to interstitial oedema and mononuclear cell infiltration. There was mild to moderate alveolar septal congestion and multifocal haemorrhages. A few arterioles and capillaries contained fibrinous microthrombi. In calves 2 and 3 there was an overlying acute bronchopneumonia with pulmonary collapse and accumulation of polymorphonuclear cells in bronchi and some alveoli. A small number of schizonts were found in endothelial cells of blood vessels throughout the lungs.

Non-suppurative hepatitis characterised by disruption of hepatic cords and a generalised increase in mononuclear cells throughout the sinusoids was present in all calves. There was a mild to moderate mononuclear cell infiltration of the portal triads. All livers showed degenerative changes ranging from mild hydropic degeneration in calves 1 and 2 to centrilobular necrosis in calf 3. Schizonts were not identified in the livers.

A mild to moderate glomerulonephritis was present in the kidneys of the 3 calves. There were multiple foci of

mononuclear cell aggregations which were more prominent in perivascular and peri-glomerular sites in the cortex and between tubules in the renal medulla. Some glomeruli contained proteinaceous material within Bowman's space, while others were undergoing degeneration and necrosis. A few tubules contained proteinaceous material and desquamated epithelial cells. Small numbers of schizonts were found in glomeruli and in interstitial tissue of the renal medulla.

Brains from the 3 calves had similar changes, although these were most pronounced in calf 1. In addition to small foci of haemorrhage, often surrounding areas of necrosis, there were scattered aggregations of glial cells throughout the brain. A mild leptomeningitis characterised by infiltrations with low numbers of mononuclear cells was apparent. In calf 1 a few schizonts were found within endothelial cells of vessels, both in the brain and the meninges.

There was a moderate haemosiderosis of the spleen in calves 1 and 3. Moderate to heavy accumulations of iron were demonstrated in sections of spleen, lymph nodes and liver stained by Perl's method. In calf 1 there was a moderate infiltration of the lamina propria of the small intestinal mucosa with mononuclear cells and eosinophils, along with haemosiderin deposition which was most severe at the tips of the villi. Low numbers of schizonts were present in endothelial cells of blood vessels in the submucosa of the small intestines.

Schizonts were present in endothelial cells of blood vessels or in close proximity to vessels in many tissues of the 3 calves. The schizonts were usually oval in shape, however, when located in small vessels they conformed to the shape of the vessel and were elongated. The size of schizonts varied from $35\mu m$ x $9\mu m$ to $13\mu m$ x $9\mu m$ with an aveage of $21\mu m$ x $14\mu m$ (n = 20) and were of 3 types. One, in the upper limit of the size range, was usually oval, protruded into the lumen of the vessel and had a coarsely granular, basophilic cytoplasm with no distinct nuclei or meronts. The other 2 types were smaller and were either densely packed with basophilic nuclei or the nuclei were arranged in a palisade pattern around the periphery, leaving a clear central zone.

Discussion

Sarcocystis species have an obligatory 2-host life cycle. Intermediate hosts acquire infection by ingesting sporocysts or oocysts that are shed in the faeces of the definitive hosts (carnivores). Sporozoites are released from sporocysts in the intestine of the intermediate hosts and invade many tissues. Schizogony (asexual reproduction) occurs in endothelial cells of blood vessels in most organs of the intermediate host preceding the development of typical muscle cysts. Definitive hosts become infected with *Sarcocystis* by ingesting the encysted form of the parasite in the musculature of the intermediate host. Bradyzoites are released from the cysts and penetrate the mucosa of the small intestine where they undergo gametogony (sexual

reproduction) with the formation of oocysts which are shed in the faeces (Dubey 1976).

The intermediate host may harbour more than one species of *Sarcocystis*. At least 3 species can occur in cattle, namely *S. cruzi* (definitive hosts: dog, coyote, wolf, red fox and racoon), *S. hirsuti* (definitive host: cat) and *S. hominis* (definitive hosts: man, rhesus monkey, baboon and chimpanzee) (Mehlhorn 1978; Levine and Tedros 1980; Dubey and Fayer 1983). Of the 3 species, *S. Cruzi* is the most pathogenic (Markus 1978; Dubey 1982).

The clinical signs, serology, gross and histopathology findings in this case agree with those previously described in natural infections (Frelier *et al* 1977; Giles *et al* 1980; Collery and Weaver 1981) and in experimental infections with *S. cruzi* (Fayer and Johnson 1973; Johnson *et al* 1975; Dubey *et al* 1982; Nakamura *et al* 1982). The pleomorphism of schizonts seen in this case is similar to that described by Corner *et al* (1963) and Clegg *et al* (1978) in cases of naturally occurring sarcocystosis in cattle and probably represent separate phases in the developmental cycle of the parasite.

Normocytic normochronic anaemia is seen in natural (Collery and Weaver 1981) and experimental (Fayer and Prasse 1981); Dubey *et al* 1982) cases of sarcocystosis in cattle. The anaemia of acute bovine sarcocystosis is primarily haemolytic (Frelier *et al* 1979; Fayer and Prasse 1981). In the present outbreak haemolysis was strongly implicated in the anaemia, as indicated by the presence of haemosiderin laden macrophages in lymph nodes and spleens and the demonstration of iron deposition in the spleen, lymph nodes and livers. Another factor contributing to the anaemia was excessive haemorrhage.

In experimental cases of bovine sarcocystosis (Johnson et al 1975), schizonts are found in capillaries of many organs between 20 to 33 days after infection, immature sarcocysts are found in muscle from day 33 and mature sarcocysts are present by day 75. In this report the presence of schizonts in endothelial cells of blood vessels in a number of organs, along with immature sarcocysts in skeletal and myocardium in 2 of the 3 calves, indicates that the calves has been infected for approximately 30-35 days. This conclusion is further supported by the serological results. IHA titres begin to rise 30 to 45 days after infection and reach peak levels at approximately 90 days, titres of 1:486 or less are considered to be nonsignificant (Lunde and Fayer 1977). Cattle develop anti-sarcocystis IgM responses, beginning 21 to 28 days after infection and IgG antibody response 35 to 42 days after infection (Gasbarre *et al* 1984). In this outbreak, serum from clinically ill calves had IHA, IgM and IgG titres consistent with an infection of 21-35 days duration.

S. cruzi is the only known sarcocyst which has dogs as a definitive host and cattle as the intermediate host. Approximately 20% of Australian dogs pass sporocysts of Sarcocystis in their faeces (Blake and Overend 1982); Collins *et al* 1983). Calves in this report were housed in pens heavily contaminated with the faeces of dogs that had been

fed meat from cattle strongly suggesting that *S. cruzi* was the sporozoan parasite involved in this disease outbreak.

Acknowledgments

References

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