Case Report

A haemolytic anaemia of unknown cause affecting young red deer

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Abstract

Three deer showing signs of haemolytic anaemia and haemoglobinuria were investigated In late March 1999 a four-month-old red deer weaner stag grazed with other deer on perennial ryegrass/white clover pasture and involved in a research programme at the Massey University Deer Research Unit was presented with haemoglobinuria The deer was mildly anaemic but recovered No leptospira were seen in urine Leptospiral serology was negative Urinalysis, haematology and serum biochemistry yielded changes consistent with clinical signs No cause could be diagnosed.

In late April a five-month-old male weaner in the same herd (case 2) was examined with depression, high respiratory rate and haemoglobinuria Mucus membranes were extremely pale. The haematocrit was 2%. The deer died a few hours after examination. Simultaneously another deer of the same age in an adjacent management mob was found dead. Both were necropsied with similar findings of haemoglobinuria and pale tissues, but with no lesions consistent with known causes of haemolysis and haemoglobinurea Serology and urine darkfield examination were negative for leptospirosis Other known causes of haemolytic anaemia of farmed ruminants were excluded

A blood smear from case 2 yielded a small number of bodies within red blood cells resembling a protozoal haemoparasite upon light microscopy Transmission electron microscopy confirmed a small number of intraerythrocytic structures that resembled a protozoal organism Ticks had been observed on the research unit during the preceding summer

To investigate the hypothesis that a protozoal species might have been the cause of the disease, a transmission study was conducted in association with the MAF-BA and the National Centre for Disease Investigation Two seven-month-old red deer were purchased from a farm known not to have ticks and were splenectomised After recovery, one received a blood transfusion from the first (recovered) case, while the second was transfused with a pooled sample from five in-contact deer. The two recipients were clinically examined twice daily and were blood sampled two or three times weekly for examination of smears and for later testing for a protozoal haemoparasite A transient elevation temperature occurred in one animal days 11 and 12, concurrent with a mild mucopurulent eye discharge On day 25 deer were returned to pasture No other clinical abnormalities were observed

A *Theileria* spp haemoparasite was considered the most likely organism based on morphology Blood samples were submitted to a theileria reference laboratory in Kenya, Africa, for DCR testing for theileria. Generic and specific tests were negative for theileria Further evaluation samples were under consideration at the time of writing.

Key words· deer, haemoglobinuria, anaemia, theileria, protozoa

Introduction

There are few reported cases of haemolytic anaemia and haemoglobinuria in farmed deer in New Zealand Redwater associated with *Leptospira pomona* has been confirmed (Anon, 1980; Fairley *et al.* 1986) While there are few other infectious causes of haemolytic anaemia and haemoglobinuria potentially affected deer in New Zealand, there are a number of potential non-infectious causes including chemical and plant toxins, various immunological, genetic and metabolic disorders (Jain, 1993). There are, however, a number of haemoparasites not yet identified in New Zealand that could potentially cause anaemia in deer, including *Anaplasma* spp , *Babesia* spp., and *Theiliera* spp.

This paper presents the clinical occurrence in a deer research herd of haemolytic anaemia and haemoglobinuria for which known causes were excluded. It describes an investigation into the hypothesis based on appearance of intraerythrocyte protozoan-like bodies, that the cause may have been a *Theileria* organism

Clinical observations and investigations

Case 1

On March 23 1999 a four-month-old male red deer (No 837) at the Massey University Deer Research Unit was observed with red urine It grazed perennial ryegrass/white clover pasture with 10 similarly aged deer from which faecal samples were being collected regularly for experimental purposes. While some members of the group had received dexamethasone to suppress immunity, the affected animal had not received treatment The temperature was normal There was a mild increase in heart and respiration rates and mucus membranes were slightly pale Haematological, blood biochemical, leptospiral serology and urinalysis results are presented in Table 1

Table I. Test results for Case 1 (deer no. 837) during the initial clinical investigation of haemoglobinuria

Presenting signs	Clinical haemoglobinuria ,temp normal, RR and HR ↑, mm pale	
Blood – Haemogram	RBC (x10^{12}/L)	8 8 (L)
	Haemoglobin (g/L)	123
	PCV (L/L)	0 34
	MCH (pg)	14 0
	MCHC (g/L)	362
	MCV (fL)	39
	WBC (x10^{9}/L)	7.0
Blood – Leptospiral serology	Negative *L pomona. L. hardjo, L copenhageni*	
Blood - Biochemistry	Bilirubin 12 µmol/L (haemolysed sample)	
Urinalysis	Colour	Brown
	Turbidity	Cloudy
	Spec Gravity	1.030
	Glucose	Negative
	Bilirubin	Negative
	Ketones	Negative
	Blood	4+
	PH	8.5
	Protein	4+
Urine dark field microscopy	No organisms resembling leptospira	

Erythrocyte concentration was slightly below the normal range, and haematocrit was at the low end of the normal range Urinalysis confirmed the presence of blood No leptospires were observed and leptospiral serology for serovars *pomona, hardjo* and *copenhageni* were negative The deer was isolated and treated with oxytetracycline and recovered uneventfully shortly afterwards

The history, clinical findings and laboratory tests thus failed to confirm any of the known causes of haemoglobinuria and intravascular haemolysis of red deer

Case 2

On April 25 a five-month-old male (No 828) from the same experimental management group as Case 1 was observed with extreme weakness and severe respiratory dyspnoea. Clinical examination revealed extremely pale mucus membranes, normal temperature and markedly elevated heart and respiration rates Blood was watery, and haematology (Table II) showed an acute haemoglobinaemia,

decreased haemtocrit, increased leukocyte count and high fibrinogen concentration This deer died shortly afterwards

Table II. Haemogram of Case 2 (No. 828) presented with haemoglobinuria and severe respiratory dyspnoea (April 25)

Haematocrit	0 02
RBC	0 44 x 10^{12}/L
Protein	90 g/L
Fibrinogen	7g/L
WBC	19 8 x 10^{9}/L
Neutrophils	49 5 "
- band	0.99 "
Lymphocytes	1 58 "
Monocytes	0 59 "

Postmortem revealed minimal body fat. The lungs had multiple small, dark, firm lesions which varied in size from 5-10 mm, consistent with lungworm (these deer had not been treated for internal parasites because they were used as donors for faecal egg and larvae cultures for research purposes) The abdominal contents were slightly yellow. No omental fat was present. A 10 x 10 mm lymph node attached to the right lateral rumen had black patches when examined in cross section. The rumen was full of stalky grass A 100 mm segment of the mid-jejunum was dilated and fluid-filled The contents of the small intestine were a golden-yellow colour and mucoid with a slight increase in fluid content There were few faecal pellets in the distal colon. Both kidneys were darker than normal, the medulla was a uniform dull red-brown in colour, the left kidney was slightly atrophied, measuring 30 mm from pelvis to cortex The spleen had a pale capsule and slightly haemorrhagic red parenchyma The bladder was half-full and the contents were a port red colour

Histopathology of kidney showed an acute haemoglobinuric nephrosis in which segments of cortical proximal tubules showed recent epithelial necrosis and extensive haemoglobin deposition. Many collecting tubules were also necrotic and contained sloughed epithelial cells, with pyknotic nuclei No leptospires were observed with silver stains.

In the liver there was a pronounced dissociation of centrilobular hepatocytes The lung showed severe alveolar oedema and accumulation of proteinacious fluid within alveoli in some areas. Pulmonary blood vessels often contained fibrinoid and leucocytic thrombi

Darkground examination of urine was negative for spirochaetes and culture of kidney was negative for leptospirosis. The diagnosis was of acute haemolytic anaemia of unknown cause

Case 3

At the same time a similar aged female (No 824) grazing in a separate mob of approximately 60 weaners on the deer research unit was found dead and delivered for postmortem. This mob had no direct contact with the experimental group containing cases 1 and 2, but these deer had been grazed together prior to weaning in early March and had grazed some of the same pastures in the previous eight weeks.

Gross pathological findings indicated the deer was thin and dehydrated There was no internal fat The liver was swollen with rounded edges, the kidney was dark brown in colour Small intestinal content was watery but mucosal lesions were not noted Mesenteric lymph nodes were enlarged and dark yellow in colour The urinary bladder contained about 250 ml of red-coloured urine.

Histopathology of kidney revealed a diffuse tubular nephrosis with cellular debris and large amounts of intraluminal proteinacious droplets and epithelial golden-brown material Medullary tubules were often dilated with swollen epithelial cells The lung showed a diffuse pulmonary oedema, small numbers of alveolar macrophages and small clumps of fibrin. Patchy alveolar necrosis associated with small patches of cocci and rods, some within alveolar macrophages, were observed Neutrophils were prominent in capillaries and very small numbers were present in the alveoli with some red blood cells.

Bacteria were also observed in the airways but the epithelium appeared largely normal. There were prominent peribronchiolar lymphoid foci. The spleen showed lymphoid lysis, large numbers of neutrophils, some degenerate enlarged macrophages. The spleen was congested with relatively little haemosiderin present There were megakaryocytes present and erythrophagocytosis was prominent The liver showed centrilobular hepatic coagulative necrosis Erythrophagocytosis was visible. Central vein and centrilobular sinusoids were distended with proteinacious material

Histology of lymph nodes showed the subcapsular sinus expanded by large numbers of neutrophils and macrophages Moderate numbers of eosinophils as well as lymphocytes and plasma cells were present Nuclear debris was frequent as were haemosiderin-laden macrophages and some erythrophagocytosis was evident Medullary sinuses were similar and showed oedema. There were moderate to large numbers of laminaproprial eosinophils in the small intestine. There was no evidence of leptospires in the kidney stained with the Warthin Starry stain.

The morphological diagnoses were

- acute renal nephrosis and haemosiderosis,
- acute necrotising pneumonia,
- moderate acute centrilobular hepatic necrosis;
- splenic congestion with erythrophagocytosis

No salmonella or yersinia were isolated from intestinal content

The diagnosis was haemolytic anaemia of unknown cause.

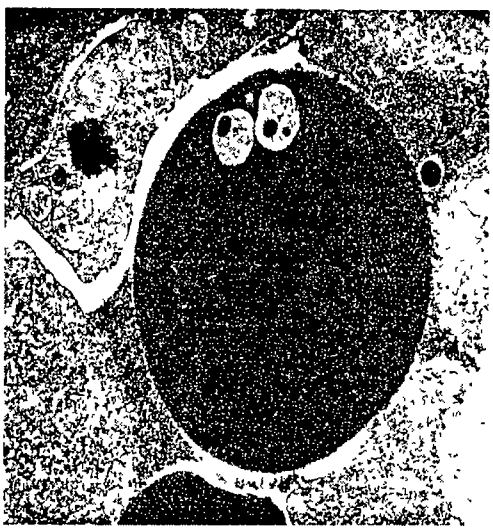
It was noted that the renal changes were likely due to haemoglobinuria and the hepatic necrosis due to hypoxia. Acute pneumonia was likely due to ante-mortem aspiration of rumen content. Further, erythrophagocytosis was common and may suggest an immune mediated component

Further investigations

Despite lack of gross pathological evidence of leptospirosis, blood samples were collected from six of the original experimental mob for serology early in May All were titre-negative for *L. hardjo* while titres to *L pomona* were 800 (n = 2), 1600 (n = 2) and negative (n = 2 including deer 837), the recovered animal from Case 1 above. Blood copper concentrations ranged from 12.9-25.9 µmol/L (mean 16 5 µmol/L) Gamma glutamyl transferase (GGT) ranged 29-41 iu/L in five deer, and in the sixth the GGT concentration was 624 iu/L, suggestive of some liver damage. It is notable that facial eczema had been prevalent in the district during that autumn, although no deer on the unit showed clinical signs.

Microscopic examination of a blood smear from Case 2 yielded a few cells with intra-erythrocytic bodies resembling a protozoal organism A sample of blood was prepared for electron microscopy using standard techniques (see Figure 1).

Figure 1. *Electron photomicrograph of an erythrocyte containing protozoal-like organisms.*



These bodies resembled an acomplexa protozoal haemoparasite There are few reports of this class of parasite in New Zealand *Theileria orientalis* has been reported in cattle in New Zealand (James *et al*, 1984). That case was diagnosed from routine haemological examinations of blood samples from 10 herds in the Northland region Clinical syndromes were illthrift and sub-optimal production sometimes associated with a mild regenerative anaemia. Of two cattle herds investigated the prevalence was 56% in one and less than 1% in the second *T orientalis* is a tick-borne protozoal parasite. The appearance of ticks on the Deer Research Unit had been confirmed for the first time during the summer immediately preceding this occurrence of haemoglobinuria.

Upon consultation with staff at the National Centre for Disease Investigation and the Chief Veterinary Officer, it was agreed that an investigation into the possibility of this disease being a *Theileria* organism was warranted

Investigation of potential *Theileria* causation

On September 16 two nine-month-old male red deer (Nos 13 and 16) purchased from a property in the Manawatu with no evidence of tick infestation were splenectomised and isolated in the Massey University Large Animal Teaching Hospital On September 28, 50 ml of blood was collected from deer no 837 (Case 1 above) by sterile technique, into heparin anticoagulant. This was transfused into the jugular vein of the splenectomised recipient (No 16) Additionally, a pooled blood sample collected from five other animals from the experimental group affected and five from the group containing Case 3 were similarly collected and after pooling, transfused into splenectomised recipient no 13 No intraerythrocytic inclusion bodies were seen in smears of any donor animal

The splenectomised deer were held in isolation after blood transfusion. Twice-daily temperature and clinical signs were recorded, and jugular blood samples collected for haematology and microscopic examination at frequent intervals On days 11 and 12 post-transfusion, deer 13 developed a mild pyrexia (39 4-39.9°C), with a concurrent mucopurulent discharge from the right eye The temperature dropped after 48 hours and the ocular discharge resolved without treatment. No other clinical abnormalities were observed The deer were returned to pasture after 25 days.

Methanol fixed blood films from deer 828 containing erythrocytes with inclusion bodies, along with methanol fixed blood films and frozen whole blood from splenectomised deer 13 and 16 collected 6 days and 13 days after blood transfusion, along with a methanol fixed blood film and frozen whole blood from deer 837 (Case 1 recovered), were submitted to the International Livestock Research Institute Laboratory, Nairobi, Kenya, for polymerase chain reaction analysis for *Theileria* spp This analysis used SSU 16s rybozomal RNA gene sequences, which should amplify any *Theileria* spp., along with specific probes for *Theileria* spp. *orientalis* and *cervi*

PCR tests indicated no evidence of *Theileria* spp.

Discussion

The clinical occurrence, history, laboratory analyses, gross postmortem and histopathological examinations failed to determine a known cause (Jain, 1993) for the haemolysis and haemoglobinuria observed in these deer. The microscopic appearance of cellular bodies within erythrocites resembled a protozoal organism Morphologically the most likely organism was a *Theileria* spp

There are a large number of *Theileria* spp which affect a range of ruminants. The best known is East Coast Fever of cattle in Africa caused by *T parva* (Lawrence *et al.* 1994) *Theileria cervi* has been observed as a relatively benign infection in white-tailed deer in the USA (Kocan and Kocan, 1992; Waldrup *et al* 1989, 1992) It has also been found in Axis and Sika deer (Waldrup *et al* 1989; Takahashi *et al*, 1992), but there is a suggestion that fallow deer may be more resistant (Kocan *et al.* 1987) Intra-erythrocytic piroplasms of *Theileria* spp. were found in deer experimentally infected with the tick *Ambylomma americanum* (Barker *et al* 1973), with anaemia and deaths recorded in heavily tick-infested animals The latter was associated with anaemia, possibly of dual tick and *Theileria* causation

The incubation for *Theileria* is approximately 9-25 days Transmission is by sporozoites in the saliva of ticks. Many infections are inapparent and can persist in the animal, usually as intra-erythrocytic piroplasms Reduced immune competence could permit the asexual replication of merozoites normally present in macrophages to piroplasms which invade red blood cells in the host animal.

It is possible that an infection with *Theileria* could have been introduced to the Deer Research Unit in question on recently grazed cattle Alternatively, an inapparent infestation of ticks may have been on the unit from earlier years Adult ticks were observed on a range of deer classes on the unit during the preceding summer and were observed on deer in the mob which contained cases 1 and 2 This circumstantial evidence, together with the observation of the intraerythrocyte protozoal-like organisms justified the investigation into possible *Theileria* causation.

Transfusion of blood into splenectomised animals is a standard model for replication of *Theileria,* since transfusion into normal healthy animals is often not successful in replicating the organism or producing clinical disease This model was chosen for this investigation in an attempt to increase the potential number of protozoal organisms, to increase the sensitivity of laboratory testing, as well as to confirm the infectious nature of this condition Samples were tested with a generic probe for *Theileria* spp. and specific probes for *T orientalis* and *T. cervi. T. orientalis* has been found in cattle in New Zealand (James *et al* 1984), and the possibility of cross-species infection could not be discounted *Theileria cervi* has been observed in North America and potentially could have been introduced into New Zealand in elk imported via Canada several years ago *Theileria cervi* has been reported from elk (Chae *et al* 1999) However, the negative PCR results suggest *Theileria* was not the causative organism

Thus, at the time of writing the cause of this haemolysis and haemoglobinuria remains undiagnosed Further investigations into possible alternative protozoal causation are to be implemented

While many cases of intravascular haemolysis and haemoglobinuria of deer will be diagnosed by a cause, veterinarians investigating cases for which no known cause can be found and from farms which contain tick infestations should consider the possibility that a tick-borne protozoal organism may be the cause.

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