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Intra-uterine Infection with *Babesia bovis* in a 2-day-old Calf

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With 2 tables

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Summary

Infection with *Babesia bovis* was diagnosed in a 2-day-old female calf apparently transmitted *in utero*. The calf was born as the second calving to a cross-bred beef cow permanently on pasture.Diagnosis was based upon identification of *B. bovis* in peripheral blood smears and clinical signs which included fever, jaundice, pale mucous membranes and convulsions. Anaemia, leucocytosis, thrombocytopenia and lymphocytosis were noted at the febrile acute stage of the disease. The blood smears revealed evidence of regeneration of toxic neutrophils with a left shift, severe spherocytosis and high degree of basophilic stippling. Elevated concentration of aspartate aminotransferase, lactate dehydrogenase, and creatine kinase were also noted, and were probably the result of haemolysis, dehydration and muscle damage because of recumbancy. Elevated total bilirubin concentration following haemolysis resulted in jaundice. The neurological symptoms observed were probably caused by sludging of parasitized erythrocytes in the brain capillaries. The calf recovered following treatment with diminazene aceturate and the recovery was followed up clinically, haematologically and biochemically.

Introduction

Intra-uterine transmission of *Babesia* parasites seems to be very rare, and accidental, probably requiring some pathological changes in the placenta. (Enigk, 1942; Neitz, 1956). The foetus may be infected with *Babesia* parasites at any stage of pregnancy (Erbsloh, 1976), and in enzootic regions it may cause abortions (Neitz, 1956).

Intra-uterine infections were reported in sheep infected with *Babesia ovis* (Neitz, 1956), in bovine with *B. bigemina* (Neitz, 1956) and *B. bovis* (Neitz, 1956; Klinger and Ben-Yossef, 1972), and in horses infected by *B. equi* and *B. caballi* (Konstantinov, 1936; Enigk, 1942; Neitz, 1956; Purchase, 1947).

In this report, an apparent intra-uterine infection with *B. bovis* is described in a 2-day-old female calf.

Materials and Methods

The calf was clinically examined and bled at the age of 2, 5 and 26 days. Blood for haematological and biochemical tests was obtained aseptically via jugular venupuncture in Ca-EDTA and plain tubes respectively. Capillary thin blood smears were prepared for detection of parasites. Haematological studies included a complete bolld count (CBC), performed by an automated cell counter calibrated for bovine blood (Technicon H_1; Miles, Inc., Tarrytown, NY, USA) and examination of air-dried, May–Grünwald–Giemsa-stained blood smears. The differential leucocyte count was performed manually by counting 400 white blood cells (WBC). Haematological parameters measured included: haematocrit [packed cell volume (PCV)], red blood cell count (RBC), haemoglobin (Hb), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), leucocyte count (WBC) and platelet count. Blood for biochemical studies was allowed to clot and the serum was separated, stored at 4°C and analysed within 24 h. Biochemical analysis was performed at 30°C by chemistry autoanalyser (Kone Corp., Vamtoa, Finland) and included: alkaline phosphatase (ALP), aspartate aminotransferase (AST), creatine kinase (CK), γ-glutamyltransferase (GGT), lactate dehydrogenase (LDH), creatinine, albumin, total protein, total bilirubin and urea. Globulin was calculated by subtracting the albumin from total protein.

The calf was treated by diminazene aceturate (Bernil, Hoechst AG, Frankfurt on Main, Germany) 5 mg/kg body weight, administered intramuscularly.

Results

A 2-day-old cross-bred female beef calf was presented with fever (40°C), weakness, dehydration, pale mucous membranes, jaundice and periodic convulsions. The calf was born as a second calving, and the dam kept permanently on pasture, has not been vaccinated against babesiosis, and had no previous history of tick fever. Examination of thin blood smears from the dam were negative for *Babesia* parasites. CBC revealed a moderate to severe normocytic normochromic anaemia, leucocytosis, lymphocytosis, a relative neutropenia and thrombocytopenia (Table 1). The differential leucocyte count is given in Table 1. Examination of jugular blood smears revealed moderate anisocytosis, mild macrocytosis, mild crenation, moderate polychromasia, severe spherocytosis (45%), marked basophilic stippling (37/1000 RBCs), erythrophagocytosis, Howell–Jolly bodies, rubricytosis (2/100 WBCs) and a marked left shift (neutrophilic bands $2.7 \times 10^3/mm^3$, 14%) with Dohle bodies. Toxic signs were more pronounced in neutrophilic bands, but were present in all neutrophilic granulocytes. Thrombocytopenia was confirmed in the blood smear. Examination of peripheral blood smears revealed a low-grade

Table 1. Haematology

Days	RBC (10^6)	PCV (%)	Hb (g/dl)	MCV (fl)	MCH (pg)	MCHC (g/dl)	WBC (10^3)	PLT (10^3)	NEUT (10^3)	LYMPH (10^3)	MON (10^3)	EEOS (10^3)	BAS (10^3)
2	4.63	18.8	6.2	40.5	15.4	33.1	16.2	492	4.1	11.9	0.1	0.06	0.4
5	4.5	18.7	6.4	41.2	14.1	34.2	19.5	872	5.2	14.1	0.2	0.04	–
26	8.6	22.8	8.3	26.6	9.7	36.4	13.5	1200	6.8	6.3	0.3	0.02	0.09
Normal value* (mean ± SD)	9.03 ± 0.95	37.9 ± 6.1	11.7 ± 1.16	41.8 ± 3.9	13 ± 0.5	31.4 ± 2.8	10.09 ± 3.53	1510 ± 727	3.08 ± 1.59	6.7 ± 2.15	0.16 ± 0.07	0.06 ± 0.05	0.09 ± 0.05

*Normal values for calves in beef cattle herds at the age up to 3 weeks.
RBC, red blood count; PCV, packed cell volume; Hb, haemoglobin; MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; WBC, white blood count; PLT, platelet count; NEUT, neutrophils; LYMPH, lymphocytes; MON, momophils; EEOS, eosmophils; BAS, basophils.

(0.001%) parasitaemia with *B. bovis*. Parasitized erythrocytes showed marked spherocytic changes. Serum biochemistry showed increased concentrations of AST, LDH, ALP, CK, GGT, total bilirubin, urea, total protein and globulin (Table 2).

Following treatment with diminazene aceturatae the calf showed clinical and clinico-pathological improvements, and by day 26 on the third check-up it recovered and all measured blood parameters were within the reference intervals (Tables 1 and 2).

Discussion

In the present case, the *in utero* infection of the calf might have resulted from a natural infection of the dam in pasture with babesiosis with a long-lasting premunition, despite the fact that no parasites were seen in its thin blood smears. Mahoney (1977) claimed that *B. bovis* parasites reach detectable levels in smears of peripheral blood between 8 and 16 days after tick attachment. Clinical babesiosis at the age of < 8 days, as in our case can be suspected as an intra-uterine infection. Sludging of parasitized erythrocytes in the brain capillaries during the acute phase of the disease is an important finding in *B. bovis* infections, leading to low parasitaemia in the peripheral blood (Wright, 1981), and neurological symptoms (Callow and McGavin, 1963). Such symptoms were also observed in this calf. The anaemia observed in acute bovine babesiosis is usually regenerative, as observed in this case (Schalm, 2000). In our case polychromasia, anisocytosis, macrocytosis, Howell-Jolly bodies, rubrycytosis, basophilic stippling were observed. Basophilic stippling is a common finding in anaemias in remission in cattle (Schalm, 2000).

Spherocytosis and erythrophagocytosis were supported by the elevated concentration of total bilirubin indicated haemolysis (Wright, 1981). This may explain the anaemia seen in this case. It seems that the immune response to the babesial antigen causes a significant lymphocytosis and an increase in the level of the globulin fraction as observed in this case. The relative neutropenia may be regarded as an absolute neutropenia when considering the age of the calf. In the first days following birth the bovine leucogram is characterized by an absolute neutrophilia (Schalm, 2000), rather than the relatively low number of neutrophils observed in the present case. Neutropenia in cases of *B. bovis* infection may have been the result of endotoxin release (Wright, 1981), and this mechanism is supported further by the presence of neutrophil left shift. Thrombocytopenia seen in the present case, is a common phenomenon in *B. bovis* infection and may lead to disturbances in the coagulation cascade (Dalgliesh et al., 1976; Wright, 1979).

Clinico-pathological studies showed that marked increase of AST and LDH was accompanied by the clinical symptoms (fever, decreased PCV, etc.). The AST and LDH increase could be due to red cell lysis and release of the intracellular enzymes. The high concentration in the GGT at the age of 2 days seemingly caused followed colostral intake by the calf (Bogin et al., 1993), and probably also as a result of haemolysis. The good correlation between GGT and total serum protein was reported in a few-day-old calves (Bogin et al., 1993). Increase in LDH, AST and CK concentrations is a result of muscular damage because of recumbancy and muscle tremor (Kaneko et al., 1997). The decrease in ALP was also reported in *B. ovis* infection (Yeruham et al., 1998). Elevated urea concentration observed in this case, occurs, possibly due to catabolism of

Table 2. Proteins, metabolites and enzymes

Days	Proteins			Metabolites			Enzymes				
	Total protein (g/l)	Albumin (g/l)	Globulin (g/l)	Creatinine (mg/dl)	Total bilirubin (mg/dl)	Urea (mg/dl)	ALP (u/l)	AST (u/l)	CK (u/l)	GGT (u/l)	LDH (u/l)
2	8.5	3.6	4.9	1.1	4.92	96.5	492	218	284	1115.6	11820
5	7.6	3.3	4.3	0.83	3.34	74.6	263	64	48	–	2859
26	6.5	3.5	3.0	1.1	0.25	23.0	392	40	36.9	31.6	1322
Normal value* (mean ± SD)	6.4 ± 0.7	3.4 ± 0.3	3 ± 0.4	1.26 ± 0.28	0.39 ± 0.1	26.7 ± 8.8	826 ± 377	57 ± 17	131 ± 99	32.5 ± 20	1958 ± 410

*Normal values for calves in beef cattle herds at the age up to 3 weeks.
ALP, alkaline phosphatase; AST, aspartate aminotransferase; CK, creatine kinase; GGT, γ-glutamyltransferase lactate; LDH, dehydrogenase.

lysed erythrocytes (Reyers, 1992). Elevated total bilirubin level associated with haemolysis and jaundice have been reported in *B. bovis* infections (Rogers, 1971).

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