

Feline babesiosis: signalment, clinical pathology and concurrent infections

T Schoeman^a, R G Lobetti^a, L S Jacobson^a and B L Penzhorn^b

ABSTRACT

Fifty-six cats with naturally occurring *Babesia felis* infection were studied. No breed or sex predilection could be identified, but there was an apparent predilection for young adult cats less than 3 years of age. Macrocytic, hypochromic, regenerative anaemia was present in 57 % of the cats and in-saline agglutination tests were positive in 16 %. No characteristic changes were observed in total or differential leukocyte counts. Thrombocyte counts were variable and thrombocytopenia was an inconsistent finding. Hepatic cytosol enzyme activity and total bilirubin concentrations were elevated in the majority of cats. Serum protein values were mostly normal, but increased values were occasionally observed and polyclonal gammopathies were observed in all cats with increased total globulin concentrations. No remarkable changes in renal parameters were observed. A variety of electrolyte abnormalities occurred in a number of cats, but no consistent pattern of change could be identified. A close correlation was evident between peripheral and central parasite counts. Concurrent infections with *Haemobartonella felis*, feline immunodeficiency virus and/or feline leukemia virus were identified in a number of cats.

Key words: *Babesia felis*, cats, feline babesiosis, *Haemobartonella felis*, immune-mediated haemolytic anaemia, feline leukemia virus, feline immunodeficiency virus

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INTRODUCTION

To date, 6 piroplasms of the cat family have been reported: *Babesia felis* in an African wild cat in Sudan (*Felis ocreata*, syn. *Felis sylvestris*)⁵⁶ in 1929⁸; *Babesiella felis* in a puma (*Felis concolor*) in 1934⁵; *Nuttallia felis* var. *domestica* in a domestic cat in 1937²¹; *B. cati* in an Indian wild cat (*Felis catus*) in 1950⁴⁰; *B. herpailuri* in a jaguarundi (*Herpailurus yaguarundi*) in South America in 1967⁹; and *B. pantherae* in a leopard (*Panthera pardus*) in Kenya in 1972¹⁰.

Confusion surrounding this nomenclature led to a proposal by Dennig and Brocklesby¹⁰ that all feline piroplasms should be divided into either of 2 small (*B. felis* and *B. cati*) or 2 large *Babesia* spp. (*B. herpailuri* and *B. pantherae*). Of the small *Babesia* spp., *B. felis* has been reported to occur most commonly in domestic cats, but is believed to have a wide host range within the cat family²⁶. However, a small piroplasm recently isolated from lions (*Panthera leo*) in the Kruger National

Park, South Africa, was found to be morphologically similar to, but serologically distinct from *B. felis* found in domestic cats in South Africa³⁰. In addition, the original *B. felis* parasite isolated from an African wild cat was found to be transmissible to domestic cats, but did not cause clinical illness⁹. By contrast, the *B. felis* parasite found in domestic cats in South Africa appears to be morphologically similar, but it causes a distinct clinical illness that is potentially fatal^{13,47}. This raises the question whether *B. felis* of domestic and wild felids is, in fact, a single species.

Babesiosis of domestic cats has been reported sporadically from other countries, including France^{2,25}, Germany³⁹, Thailand²⁴ and Zimbabwe⁵¹, but it does not seem to be a regularly occurring significant clinical disease in any country other than South Africa. In a recent survey in South Africa²³, feline babesiosis was found to be endemic along most of the South African coast, from KwaZulu-Natal to the south-western Cape. Clinical cases are occasionally seen in non-endemic parts of the country. These usually involve pets that have returned home after coastal holidays with their owners^{23,47}. An isolated focus of naturally occurring feline

babesiosis has, however, recently been identified at Kaapschehoop, a village on the escarpment west of Nelspruit, Mpumalanga⁴⁵.

In 1980, Futter and Belonje published a series of articles on feline babesiosis^{12–15}. The first dealt with the history and classification of the disease¹², while the second article described clinical observations in 20 experimentally-infected and 70 naturally-infected cats¹³. Lethargy, anorexia and anaemia were consistent findings in both study groups and icterus was occasionally observed. Pyrexia was not a feature of the disease. The third article described the haematological findings in the same study groups¹⁴. Cases showed a rapid fall in haematocrit, haemoglobin and erythrocyte count, and the erythrocytes were often macrocytic and hypochromic. No significant changes were seen in total leukocyte counts. The fourth article described the chemical pathology and macroscopic and microscopic *post mortem* findings¹⁵. Total serum protein concentration was unchanged, but there was an increase in γ globulin concentration and a decrease in α and β globulin concentration. Moderate elevation in hepatic alanine transaminase (ALT) levels was recorded in a number of cases. Renal function was unaffected and venous blood pH remained within the normal range throughout the study. *Post mortem* findings included extreme pallor of the viscera, thin watery blood and yellow to orange rectal faeces. Bile stasis and hepatic necrosis were evident in some cases, while marked icterus was seen in only 2 cats¹⁵.

As no further studies on naturally-infected cases of feline babesiosis in South Africa had been performed since 1981, the purpose of this study was to review the signalment and clinico-pathological changes associated with naturally-occurring disease and to investigate certain parameters that were not studied previously, such as thrombocyte counts, ALP and GGT activities and serum electrolyte status. The study was also undertaken to identify concurrent infections and IMHA and to measure and compare peripheral and central parasitaemias, none of which had previously been investigated in feline babesiosis.

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MATERIALS AND METHODS

Experimental animals

Fifty-six cats with naturally-occurring *B. felis* infection, presented sequentially to private veterinarians over a 2-month period, were prospectively studied. The study area was concentrated around George and surrounding towns in the southwestern Cape Province. Cats were included, subject to the owners' written consent, if *B. felis* parasites were identified on a thin peripheral blood smear. Peripheral blood smears were prepared using a drop of capillary blood taken from the ear pinna and then fixed and stained with Cam'sQuick (CA Milsch). Blood samples were collected from the jugular vein into EDTA and serum vacuum tubes (Becton Dickinson Vacutainer Systems).

Clinico-pathological laboratory measurements

Within 6 hours of collection, EDTA samples were used to prepare thin central blood smears (as described above), to perform in-saline agglutination tests using a standard technique⁵⁵, and to determine full haematology using a portable COULTER® A^cT diff™ Haematology Analyzer Veterinary Applications counter (Beckman Coulter S.A.) The veterinary applications software was set for analysis of feline blood.

Serum was separated and frozen at -20 °C for a maximum of 2 months. On completion of the data collection phase, the serum samples were allowed to thaw and reach room temperature. Total serum protein, albumin, urea, creatinine, alanine transaminase (ALT), alkaline phosphatase (ALP), gamma glutamyl-transferase (GGT) and total bilirubin were determined on a Technicon RA-1000 system (Technicon Instruments Corporation). Serum globulin was calculated by subtracting albumin from total serum protein. Serum electrolytes (sodium, potassium) were measured using a Rapidlab™ 348 pH/Blood Gas Analyzer (Chiron Diagnostics). Serum protein electrophoresis was performed on a Beckman Microzone® Electrophoresis System (Beckman Instruments), using a cellulose acetate membrane and Barbitol buffer (0.5 ionic strength and pH 8.6). This system identifies alpha (α1 and α2), beta (β1 and β2) and gamma (γ) peaks.

Relative differential white blood cell counts, nucleated erythrocyte counts and platelet scores were determined manually using central blood smears under ×50 or ×100 oil magnification. Leukocytes were identified until 100 to 200 cells had been classified by type, and expressed as a percentage. The number of

nucleated erythrocytes (nRBC) per 100 leukocytes was recorded. The automated white cell count (WCC) was corrected for nRBC using the formula¹¹:

$$\text{Corrected WCC} = (\text{initial WCC} \times 100) / (100 + \text{nRBC})$$

Platelet scores were used to verify the automatic platelet count, as feline blood is notably prone to clotting during collection¹¹. Platelet numbers were scored as normal (8–10 per ×100 field), increased or decreased (<3–4 per ×100 field)¹¹. The presence of platelet clumps on stained smears were also noted and used to refine the score.

All blood smears, both peripheral and central, were prepared by the same investigator (TS), using a consistent technique. Percentage parasitaemia was estimated on central and peripheral blood smears. Parasitised red blood cells (pRBC) in 10 fields (x100 objective), each containing approximately 400 RBC, were counted and the percentage of pRBC calculated by dividing the total number of pRBC by 40 (400 RBC per field, 10 fields examined). The presence of any other haematological parasites was also recorded.

Serology

A commercial ELISA test kit (SNAP Combo Plus Feline Leukemia Virus Antigen/Feline Immunodeficiency Virus Antibody Test Kit, IDEXX Corp.) was used to determine the presence of concurrent infections with feline immunodeficiency virus (FIV) and/or feline leukemia virus (FeLV). The kit detects FeLV group-specific viral core antigen (p27) and/or specific antibodies directed to FIVgag or FIVenv(gp40) proteins¹⁸. A very faint colour reaction in the FeLV test well was

reported as an equivocal positive result. Where possible, cats with a positive (whether equivocal or clear-cut) FeLV test were retested after 12 weeks to determine whether FeLV antigenaemia was persistent or transient. Cats that were positive for FIV infection only were not retested.

Data analysis

Statistical analysis of the data was performed on a commercial statistical software package, SigmaStat™ v2.0.3 (Jandel Scientific Software). Normality of the data was tested using the Kolmogorov-Smirnov test and equal variance was tested using the Levene median test. Because the data obtained in most instances were either discrete variables or percentage data, or were not normally distributed, non-parametric methods of analysis were used. The Spearman rank order correlation was used to determine the strength of association between selected variables. The Mann-Whitney rank sum test was used for comparisons between 2 groups. For all tests, the probability value for significance was set at $P < 0.05$.

RESULTS

Signalment

Ages of affected cats ranged from 6 months to 13 years, with a median of 2 years (Fig. 1). Most cats (80 %; 45/56) were less than 3 years old, and most did not represent a specific breed, with 77 % (43/56) being either of the domestic short-hair (32/43) or the domestic longhair (11/43) type. Specific breeds were seen in 23 % (13/56) of cases. This included 12.5 % Siamese (7/56), with single representatives of several other breeds. No specific sex predilection was evident, with 57 %

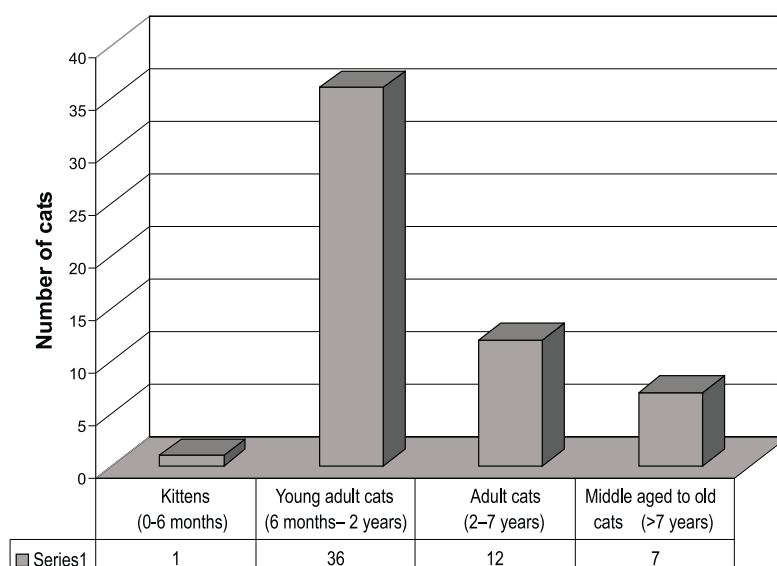


Fig. 1: Age distribution of 56 cats with *Babesia felis* infection.

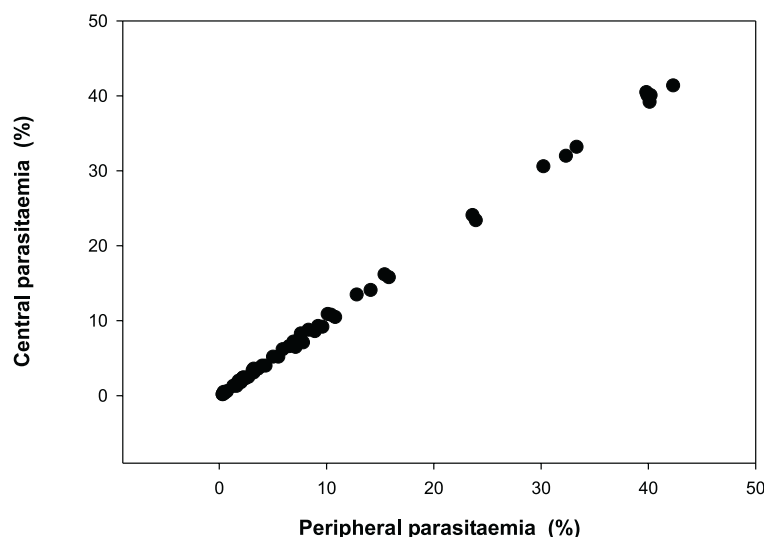


Fig. 2: Correlation of central and peripheral parasite counts in 56 cats with *Babesia felis* infection. A statistically significant strong positive correlation is evident, indicating that central and peripheral parasitaemias tend to increase together ($r_s = 0.997$, $P < 0.05$).

females (32/56) and 43 % males (24/56) being affected. Typical clinical signs attributable to feline babesiosis were observed in 77 % (43/56) of cats and consisted mainly of anorexia, depression and anaemia. Less common signs, such as weight loss, icterus, constipation and pica, were also observed in some cats. The other 23 % (13/56) of cats did not display typical clinical signs of the disease and

included 9 cats (16 %) that were examined for reasons other than illness, such as routine pre-surgical examinations. As these cats were reported to be healthy at the time of examination, they were likely to be subclinical carriers of the disease.

Parasitaemia

Peripheral parasitaemia was assessed in 55 cats (the other was not possible due to

poor quality of the peripheral blood smear) and ranged from 0.3 % to 42.3 %, with a median of 5.9 %. Central parasitaemia was assessed in 56 cats and ranged from 0.2 % to 41.4 %, with a median of 6.4 %. Central and peripheral parasitaemia showed a strong positive correlation ($r_s = 0.997$, $P < 0.05$) (Fig. 2). A statistically significant, but weak, negative correlation between central parasitaemia and haematocrit was also seen ($r_s = -0.595$, $P < 0.05$). This negative correlation appeared to be most pronounced when parasitaemia was higher than 20 %, with 90 % (9/10) of these cats having a haematocrit (Ht) of 15 % or less. When parasitaemia was less than 20 %, Ht levels tended to be more randomly distributed.

Haematology and chemical pathology

Laboratory data for the study population are summarised in Tables 1 and 2. The most frequent red blood cell parameter finding was macrocytic, hypochromic, regenerative anaemia, which occurred in 57 % (32/56) of the cats. Normal Ht values occurred in 43 % (24/56) of the cats, while moderate anaemia (Ht 15–24 %) occurred in 23 % (13/56). Severe anaemia (Ht < 15 %) was seen in 34 % (19/56) of cases. Nucleated red blood cells were present in 70 % (39/56) and counts ranged

Table 1: Laboratory data of 56 cats with *Babesia felis* infection. Summary data are shown as median and 25th and 75th percentiles.

	Normal values*	Median	25 %	75 %
Haematocrit (%)	24–45	18.7	12.9	28.2
Red blood cell count ($\times 10^{12}/l$)	5–10	3.0	1.6	5.5
Haemoglobin concentration (g/l)	8–15	5.9	3.8	9.6
Mean corpuscular volume (fl)	39–55	60.9	51.4	82.1
Mean corpuscular haemoglobin (pg)	12.5–17.5	19.9	17.2	23.7
Mean corpuscular haemoglobin concentration (g/l)	30–36	32.8	29.2	33.6
Nucleated red blood cell count (cells/100 WBC)	–	10.5	0.0	61.0
Corrected white blood cell count ($\times 10^9/l$)	5.5–19.5	10.1	7.2	13.9
Mature neutrophil count ($\times 10^9/l$)	2.5–12.5	5.3	3.3	7.7
Immature neutrophil count ($\times 10^9/l$)	0.0–0.3	0.2	0.0	0.5
Lymphocyte count ($\times 10^9/l$)	1.5–7.0	2.8	1.2	5.2
Monocyte count ($\times 10^9/l$)	0.0–0.85	0.3	0.1	0.8
Eosinophil count ($\times 10^9/l$)	0.0–1.5	0.2	0.0	0.4
Basophil count ($\times 10^9/l$)	0.0–0.2	0.0	0.0	0.0
Thrombocyte count ($\times 10^9/l$)	300–800	23.0	6.0	91.5
Central parasitaemia (%)	–	6.4	2.4	13.8
Total serum protein (g/l)	60–80	77.3	72.8	82.4
Albumin (g/l)	25–35	34.9	32.3	38.5
Total globulin (g/l)	22–48	41.1	37.5	48.0
α globulin (g/l)	8–16	13.1	10.5	15.3
β globulin (g/l)	6–14	11.8	10.3	12.8
γ globulin (g/l)	12–22	18.6	15.1	23.8
Alanine transaminase (U/l)	<23	54.5	36.0	106.0
Alkaline phosphatase (U/l)	<20	11.5	2.0	22.0
Gamma glutamyltransferase (U/l)	<10	0.0	0.0	2.0
Urea (mmol/l)	7.1–10.7	8.6	7.1	10.9
Creatinine (μ mol/l)	<141	128.5	113.0	145.0
Total bilirubin (μ mol/l)	<6.8	12.9	7.8	27.9
Sodium (mmol/l)	141–156	147.0	139.0	153.5
Potassium (mmol/l)	4.0–5.1	4.6	4.1	5.2

*Normal values are those used at the Onderstepoort Veterinary Academic Hospital.

Table 2: Proportions of normal and abnormal laboratory findings in 56 cats with *Babesia felis* infection. Summary data are shown as the percentage of total.

	Low (%)	Normal (%)	High (%)
Haematocrit	57	43	0
Corrected white blood cell count	17	72	11
Automated thrombocyte count	98	2	0
Thrombocyte score from smear	25	71	4
Total serum protein	2	66	32
Albumin	2	54	45
Globulin	0	77	23
α globulin	3	79	18
β globulin	0	89	11
γ globulin	5	66	29
Alanine transaminase	0	11	89
Alkaline phosphatase	0	75	25
Gamma glutamyl transferase	0	96	4
Total bilirubin	0	14	86
Urea	20	55	25
Creatinine	0	75	25
Sodium	27	64	9
Potassium	16	64	20

from 1 to 814 nRBC/100 white blood cells, with a median count of 10.5. Signs of bone marrow erythropoiesis were commonly evident on blood smear examinations and included the presence of reticulocytes, numerous nucleated red blood cells, marked anisocytosis, polychromasia, an increased number of Howell-Jolly bodies (in excess of 1 % of RBC) and basophilic stippling of some red blood cells.

In-saline agglutination tests showed positive agglutination of RBC in 16 % (9/56) of cats. On examination of blood smears, it was evident that the vast majority of agglutinating RBC were non-parasitised, and that not only mature RBC, but also reticulocytes showed evidence of agglutination.

Ht values for in-saline-positive cats ranged from 8.6 % to 19.1 %, with a median of 13.2 %. Ht values for in-saline-negative cats ranged from 7.9 % to 41.2 %, with a median of 24 %. The difference in Ht values between in-saline-positive and in-saline-negative cats was statistically significant ($T = 137.00$, $P = 0.008$).

Corrected WCC were recorded in 54 cats and varied between 2.6 and $41.8 \times 10^9/l$. Of these counts, 72 % (39/54) were within the normal reference range. Leukocytosis was present in 11 % (6/54) and leukopaenia in 17 % (9/54). These leukocyte changes were caused by changes in absolute neutrophil, lymphocyte and monocyte counts, but no consistent pattern of changes was seen. The abnormal leukograms were classified as a combination of inflammatory, stress-induced and physiologic leukograms.

Automated platelet counts were recorded for 55 cats, with no thrombocyte count given by the Coulter Analyzer for 1 cat. From the automated counts, throm-

bocytopenia was evident in 98 % of the cats (54/55). Using the manual platelet score, only 25 % of the cats (14/56) were thrombocytopenic, and 71 % (40/56) were scored as normal. Two of the cats (4 %) were thought to have thrombocytosis. Platelet clots were reported in 61 % (34/56). Only 27 % (15/55) of the cats were categorised in the same way by both manual and automated systems. Of the other 40 samples, 73 % (40/55) had visible platelet clots on central smears.

Total serum protein values were elevated in 32 % (18/56) of cats. Hyperalbuminaemia was recorded in 45 % (25/56) of the cats, while hypoalbuminaemia was

recorded in only 1 cat. Hyperglobulinaemia was present in 23 % (13/56) of cats, in all cases due to polyclonal gammopathies. Of the 77 % (43/56) of cats with normal total globulin concentrations, abnormal globulin fractions were recorded in 33 % (14/43). These included various combinations of abnormal α , β and γ globulin concentrations, but no consistent pattern of changes was seen.

Increased alanine transaminase (ALT) activity was present in 89 % (50/56) of cats. However, increased alkaline phosphatase (ALP) and gamma glutamyltransferase (GGT) activities were recorded in only 25 % (14/56) and 4 % (2/56) of cats respectively. No correlation could be found between ALT and ALP ($r_s = 0.002$, $P = 0.985$). A statistically significant, but weak, negative correlation was found between ALT and Ht ($r_s = -0.497$, $P < 0.05$). Total bilirubin concentrations were elevated in 86 % (48/56) of cats, but clinically visible icterus was observed in only 12. There was a statistically significant difference in ALT values between cats with and without visible icterus ($T = 588.00$, $P < 0.001$), with much higher values in the icteric cats (Fig. 3). A significant positive correlation was found between ALT and total bilirubin concentrations ($r_s = 0.708$, $P < 0.05$).

Renal parameters were within normal limits in most cats. Serum urea concentrations were increased in 25 % (14/56) of cats and decreased in 20 % (11/56), while increased creatinine concentrations were recorded in 25 % (14/56). Of the 14 cats with increased urea concentrations, only 6 had concurrently increased creatinine

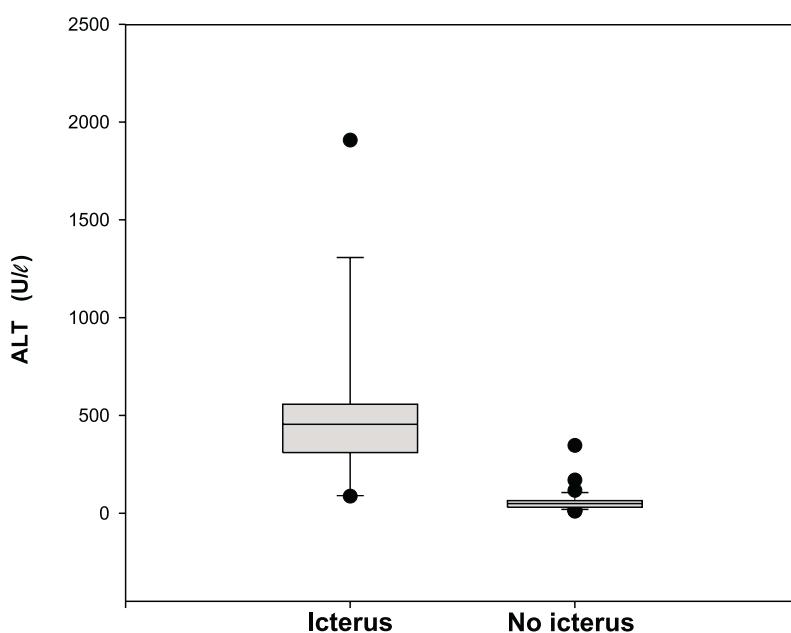


Fig. 3: Comparison of ALT values between icteric and non-icteric cats in 56 cats with *Babesia felis* infection. The ends of the boxes indicate the 25th and 75th percentiles, with a line at the median. Error bars indicate the 10th and 90th percentiles. Circles represent outliers. There is a statistically significant difference between the 2 groups ($T = 588.00$, $P < 0.001$).

concentrations, while creatinine concentrations were normal in the remaining 8 cats. No statistically significant relationship was found between urea and creatinine concentrations ($r_s = 0.211$, $P = 0.117$).

Various serum electrolyte abnormalities were recorded, but no consistent pattern was present. Hyponatraemia was seen in 9 % (5/56) of the cats, and 27 % (15/56) were hyponatraemic. Hyperkalaemia was recorded in 20 % (11/56) of the cats and hypokalaemia in 16 % (9/56).

Concurrent infections

Eight cats (14 %) tested positive for FIV infection and 18 cats (32 %) tested clearly positive for FeLV infection. Of these, 5 cats (9 %) had concurrent FIV and FeLV infections. In addition to this, 6 further cats (11 %) had equivocal test results for FeLV. After a period of 12 weeks, an attempt was made to retest all 24 cats that were positive or equivocal for FeLV infection. Of the original 18 cats that were clear-cut FeLV positives, only 8 were known still to be alive after the 12-week period, while 3 were lost to follow-up and the remaining 7 had died. Four of these died naturally while the other 3 were euthanased on account of their moribund state at the time when babesiosis was diagnosed, or shortly thereafter. Of the 6 cats that had equivocal FeLV results, only 4 were still alive after the 12-week period. All 12 remaining cats were retested. Only 2, both of which were clear-cut FeLV positives originally, tested positive for FeLV the second time, while the remaining 10 were negative.

Concurrent infection with *Haemobartonella felis* was detected on both peripheral and central venous blood smears of 11 % (6/56) of cats. Of the 6 cats with *H. felis*, 50 % (3/6) also tested positive for concurrent FeLV infection.

DISCUSSION

The high prevalence of babesiosis in young cats is in agreement with previous findings¹³, and supports the possibility that cats in endemic areas contract the infection early in life and become subclinical carriers in a state of so-called premunity. The recommended treatment for feline babesiosis, primaquine phosphate (Primaquine, Kyron), does not sterilise the infection^{44,47}, and would thus promote development of premunity⁴³. It is possible that clinical infection in older cats in endemic areas would therefore occur either in situations where this premunity never developed or under certain conditions where the pre-existing protective immunity became suppressed. This phenomenon has been shown to exist in dogs³². In this study, most affected

cats older than 3 years had a concurrent illness or infection that could have influenced the cat's immune system. No breed or sex predisposition was evident, but Siamese cats seemed to be over-represented amongst purebred cats.

The close correlation between central and peripheral parasitaemia in this study indicates that *B. felis*-parasitised RBC do not sequester in capillary beds, which is different from what has been described in the dog⁵². The high parasitaemia observed in cats in this study is in agreement with previous reports^{13,21,49}. The chronicity of feline babesiosis, and its relatively low virulence, are probably related to both of the above phenomena. It has been suggested that *Plasmodium falciparum* malaria in man, which sequesters, is more pathogenic than *P. vivax*, which does not, owing to a combination of local tissue hypoxia caused by blockage of small blood vessels, and high local production of inflammatory cytokines near sequestered parasites⁷. The high parasitaemia in cats is probably due to a combination of the relatively low inherent virulence of the parasite, the fact that all parasitised RBC presumably circulate and are thus visible, and the cat's relative resistance to endotoxin⁴¹. Species that are refractory to endotoxin are also less susceptible to babesiosis and show symptoms of disease at a higher parasitaemia than endotoxin-sensitive species⁶.

Haemolytic anaemia (both intra- and extravascular) is a typical finding in canine babesiosis^{52,53}. Based on previous findings of haemoglobinuria¹⁵, splenomegaly²³ and erythrophagocytosis of both infected and non-infected cells by mononuclear cells¹³, it can be assumed that both intravascular and extravascular haemolysis also occur in feline babesiosis. The cats in this study with severe anaemia (<15 %) were moderately or severely depressed at presentation, but seemed to have an ability to adapt to the severe anaemia. This has been described previously in feline babesiosis¹³, as well as in other forms of anaemia in cats^{3,54}. A surprisingly large number of cats in this study presented without anaemia. It is possible that increased owner awareness of the disease in the area could have led to earlier recognition of signs of disease and subsequent presentation for treatment before the disease had progressed very far. This emphasises the importance of a blood smear examination during any routine clinical examination of cats, especially in endemic areas. This group also included subclinical carrier cats, in which babesiosis was diagnosed incidentally at the time of examination. As these cats were not suffering from clinical disease, normal

haematocrit values were to be expected.

Auto-agglutination of red blood cells has not previously been described in feline babesiosis, and probably represents secondary immune-mediated haemolytic anaemia (IMHA). The other hallmarks of IMHA, spherocytosis and positive direct Coombs' test¹⁶, could not be identified, as spherocytes are difficult to identify in feline blood¹⁶ and the feline Coombs' test is not available in South Africa. Agglutination of RBC was differentiated from rouleaux formation by its failure to disappear on dilution with saline⁵⁵. Immune-mediated haemolytic anaemia in cats has rarely been documented, but has been described secondary to *Haemobartonella felis* or FeLV infections and certain anti-thyroidal medications^{4,19,46}. Six of the 9 cats with IMHA were FeLV-positive, while no infections other than babesiosis could be identified as a cause of IMHA in the other 3. Although cats with IMHA had lower Ht values than those without, the clinical significance of the agglutination is unclear, as all these cats responded predictably to antibabesial therapy without addition of immunosuppressive therapy.

Neutrophilia is a consistent feature of canine babesiosis⁴⁸, and a leukaemoid response has also been described²⁸. Neutrophilia was not a consistent finding in feline babesiosis. In fact, the leukocyte abnormalities in this study were more likely to be a result of concurrent problems or diseases than the babesiosis itself, as has been previously reported¹⁴.

Certain problems can occur when counting cat platelets electronically. Blood samples taken from cats have a tendency to form platelet clumps easily and this will potentially generate grossly inaccurate (low) counts when counted electronically¹¹. This study confirmed that automated platelet counts in cats should be treated with caution, and an additional manual platelet score should always be performed. Although thrombocytopaenia is a consistent feature of canine babesiosis^{20,53}, it was not a consistent finding in feline babesiosis.

Serum protein values tended to be either normal or increased in most cats. Hyperalbuminaemia, a relatively common finding in this study, was most likely due to dehydration, as it was mostly associated with concurrent hyperglobulinaemia and a normal albumin:globulin ratio¹¹. Apart from 1 cat, hypoalbuminaemia was not seen in the cats in this study, which is in contrast to what has been reported for canine babesiosis²⁹. A polyclonal gammopathy was identified in all cases with hyperglobulinaemia. Various combinations of abnormal α , β and γ globulin

concentrations were seen in these cases, but no consistent pattern of changes could be identified. Elevated α globulin concentrations are usually associated with an increase in acute-phase proteins, which are synthesised by the liver as part of the acute-phase response to tissue injury, infection or immunological disorders¹¹. The increase in α globulins that was seen in this study could therefore have been an indication of an acute-phase response to babesiosis, but the potential effect of concurrent diseases on α globulin concentrations must also be considered. Elevated β and γ globulin concentrations are usually indicative of increased production of immunoglobulins (chronic phase proteins)¹¹ and could be ascribed to the antibody response of the reticulo-endothelial system of the patient to *Babesia* antigens. The increase in γ globulin concentrations corresponds with previous findings¹⁵.

Marked increases in hepatic cytosolic enzyme activities were seen in most cats in this study, whereas hepatic membrane-associated enzymes were not commonly affected. Feline babesiosis therefore appears to be associated with significant primary hepatocellular involvement or damage¹¹. Repeat testing would have been necessary to determine whether this hepatic injury was reversible. In a previous study, ALT activities returned to normal during the recovery stage in most cases of feline babesiosis, but a few cases showed an increase in activity over time¹⁵. The hepatocellular damage is likely to result primarily from anaemia, as anaemia can lead to hepatocellular hypoxia, which in turn causes progressive hepatic centrilobular necrosis and hepatocellular cytosol leakage with increased enzyme activity¹¹. Centrilobular hepatic necrosis is a consistent pathological finding in both feline¹⁵ and canine babesiosis¹⁷. However, the somewhat inconsistent relationship between ALT and haematocrit, despite a statistically significant negative correlation, indicates that anaemia is probably not the only cause of hepatocellular injury and that other factors, such as inflammatory cytokines, could also play a role³⁸. Any primary hepatocellular injury can potentially be accompanied by cellular swelling that can compress bile canaliculi and cause some secondary cholestasis¹¹. This is likely to be the reason for cholestasis and resultant increases in ALP and GGT activity in a few cases in this study.

Even though hyperbilirubinaemia was seen in most cats in this study, clinical icterus was observed in only 12 cats. The hyperbilirubinaemia was most likely a result of haemolysis (both intra- and

extravascular), but secondary hepatocellular disease and intrahepatic cholestasis (as evidenced by increased ALP and GGT activities in some cases) were also likely to contribute. A combination of unconjugated and conjugated bilirubin would thus be expected in these cases¹¹. In a previous study of feline babesiosis, hyperbilirubinaemia was also described in most of the cats¹⁵. At total bilirubin concentrations of about 20 $\mu\text{mol/l}$ the unconjugated bilirubin values were higher than conjugated, whereas at higher total bilirubin concentrations the unconjugated and conjugated values were about equal¹⁵. Hyperbilirubinaemia has also been reported during acute disease caused by *B. canis* in dogs²⁰. As in cats, it seems to be a reflection of both erythrocyte destruction and intrahepatic cholestasis³⁵. The higher ALT values in icteric cats, compared to those in non-icteric cats, support the concept that clinical icterus requires hepatocellular damage to be present and is not merely a function of haemolysis⁵⁰. Similar to what has been described for dogs in general⁵⁰ and observed for cats with babesiosis in this study, it is also believed that haemolysis alone will not lead to clinical icterus in dogs with babesiosis³⁵. Instead, the degree of icterus observed in dogs with babesiosis will correlate with the degree of functional impairment, hepatocellular damage and bile stasis of the liver^{33,34}.

No remarkable changes in renal parameters occurred in most cats in this study, indicating that, as in the dog²², and as reported previously¹⁵, marked renal damage and/or renal failure is a rare event in feline babesiosis. Only 2 cats (10 and 3 years old) had severe azotaemia and concurrent hyperkalaemia, which were indicative of renal failure. Although babesiosis could have been the primary cause for renal failure in these cats, it is likely that underlying renal disease was present and that acute decompensation with subsequent renal failure was precipitated by the babesiosis, as has been described in dogs³⁶.

Although no consistent changes in serum electrolyte concentrations were identified in cats with babesiosis, a variety of electrolyte disturbances did occur in a substantial number of cases. It is therefore advisable to measure electrolytes in any cat affected with babesiosis, as some individual cases might require specific treatment.

A surprisingly high prevalence of FeLV and FIV was seen in this study population, but without background data it is not clear whether this reflects the epidemiology of these diseases in the study

area or increased susceptibility of positive cats to feline babesiosis. The overwhelming majority of cats that seroconvert to FIV following infection remain infected^{1,42}, and the cats that tested FIV-positive in this study were therefore considered to be persistently infected. However, only about 30 % of all cats that are exposed to FeLV will become persistently viraemic, while the other 70 % will seroconvert to a negative state^{27,42}. As a direct relationship exists between a sample's FeLV antigen concentration and the degree of colour change in the test well, equivocal FeLV test results probably reflect low antigen concentration³¹. The interpretation of an equivocal test result is difficult and current recommendations are that the cat should be retested after at least 1 month^{31,37}. As equivocal test results were obtained for 6 cats in this study, proper interpretation of those results was not possible unless the cats were retested. Because the FeLV ELISA test detects the presence of antigen as early as the primary viraemic stage, it can neither indicate nor predict whether the cat is transiently or persistently infected. To determine whether a cat is persistently infected, an IFA test can be done immediately (a positive IFA result is highly predictive of persistent infection), or the cat should be retested with ELISA after a minimum of 6–8 weeks⁴². A second positive test result is indicative of persistent infection. Most of the cats in this study that were retested after 12 weeks were negative for FeLV the second time. These cats had probably seroconverted to a negative FeLV state, but it is also possible that the initial positive FeLV results included some false positives. The fact that most of the cats in this study population were young might have artificially increased prevalence of FeLV (which is more prevalent in young cats¹) and decreased that of FIV (which is more prevalent in older cats^{1,37}). However, the mean ages of infected cats, 3.1 and 5.2 years for FeLV and FIV respectively, correspond with those documented^{1,37}.

The differentiation between feline babesiosis and haemobartonellosis is difficult, as both diseases cause clinical signs of anorexia, depression, regenerative anaemia, weakness, weight loss and occasional icterus during the acute phase of the disease^{4,13,19}. Also, in both diseases the total and differential white blood cell counts are quite variable and of limited diagnostic value. Autoagglutination of erythrocytes in cases with haemobartonellosis has been well described, but, as in cats with babesiosis in this study, the clinical significance is not clear^{4,19}. *H. felis* organisms can sometimes, but not

always, be diagnosed on stained blood smears and appear as small rings, cocci and rods that are attached to the erythrocytes. Differentiation of *H. felis* parasites from *B. felis* parasites, Howell-Jolly bodies and basophilic stippling on a blood smear is problematic, as all of these can have a very similar appearance¹⁹. Additionally, *H. felis* parasites only appear in the blood in a cyclical manner within discrete parasitaemic episodes¹⁹. Generally, these organisms are present in adequate numbers to be easily recognised on stained blood smears only about 50 % of the time during the acute phase of the disease, thus the absence of *H. felis* organisms from the blood does not definitively rule out a diagnosis of haemobartonellosis^{4,19}. It is therefore possible that more than 6 cats in this study had concurrent babesiosis and haemobartonellosis. In the light of the above, it may be advisable to treat for both diseases simultaneously whenever co-infection is suspected in a case of feline babesiosis.

In conclusion, this study indicated that feline babesiosis is typically a subacute to chronic disease, often of young adult cats, that results in haemolytic anaemia and hepatocellular damage. It is frequently associated with concurrent infections including FeLV, FIV and *H. felis*, and an attempt should be made to identify such concurrent infections when chronic carrier adult cats become symptomatic, when cats are pyrexia at presentation, or when cats have recurrent infections that do not respond predictably to therapy.

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