CLINICAL AND CLINICO-PATHOLOGICAL CHANGES IN FELINE BABESIOSIS

by

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Résumé

Feline babesiosis is caused by the small intra-erythrocytic haemoprotozoan parasite, *Babesia felis*. Only in South Africa does it cause a significant clinical disease among domestic cats. A study was undertaken to further describe signalment and clinico-pathological changes and to identify concurrent infections. Fifty-six cats with naturally occurring *B. felis* infection, presented to private veterinarians for examination and treatment, were studied.

An age predisposition seemed to be present, as most affected cats were young adults of less than three years of age. Although no breed or sex predisposition was evident, Siamese cats seemed to be over-represented amongst purebred cats. Typical clinical signs included anorexia, listlessness and anaemia. Less common signs included icterus, weakness, weight loss, constipation and pica. Feline babesiosis was diagnosed by identification of parasites on stained, thin blood smears. Parasitaemias were variable and ranged between very low (0.3%) and extremely high (42.3%). A strong correlation existed between central and peripheral parasitaemias, indicating that sequestration does not occur.

Macrocytic, hypochromic, regenerative anaemia was the most consistent haematological finding; this became quite severe in advanced cases of disease. The anaemia was further classified as haemolytic, presumably resulting from both intravascular and extravascular erythrocyte destruction. Almost half the cats (43%) were not anaemic. No characteristic changes were seen in total or differential leukocyte counts; when abnormal values were present they were often accompanied by concurrent illness or infection. Thrombocyte counts were variable and thrombocytopaenia was an inconsistent finding. In-saline agglutination tests were positive in a number of cases, indicating that secondary immune-mediated haemolytic anaemia could also be a feature of this disease.

The most remarkable clinico-pathological changes were elevation of hepatic cytosol enzyme activities and total bilirubin concentrations. Serum alanine transaminase was significantly elevated in the majority of cases, whereas alkaline phosphatase and gamma glutamyltransferase were generally within normal limits. This provides evidence of primary hepatocellular injury or inflammation in feline babesiosis. The hyperbilirubinaemia was most likely a result of haemolysis, but secondary hepatocellular injury was probably an additional contributing factor.

No characteristic changes in renal parameters were observed and serum urea and creatinine levels were mostly within normal limits, indicating that gross renal damage was not a consistent feature of the disease. No characteristic pattern of changes in serum electrolytes (sodium and potassium) was seen, although a variety of electrolyte disturbances occurred in a number of cases. Serum protein values were mostly normal, but elevations were seen in some cases. Hyperalbuminaemia was considered indicative of patient dehydration. Polyclonal gammopathies were observed in all cats with hyperglobulinaemia and were ascribed to a combination of acute- and chronic-phase proteins produced in response to the *Babesia* antigens.

Concurrent infections with *Haemobartonella felis*, feline leukemia virus and/or feline immunodeficiency virus were identified in a number of cats in this study and seemed to have profound effects on response to treatment and outcome of the disease.

Samevatting

Babesiose van katte word veroorsaak deur die klein intra-eritrositiese parasiet, *Babesia felis.* Dit veroorsaak slegs in Suid-Afrika ‘n belangrike kliniese siekte in huiskatte. ‘n Studie is onderneem om die aanbieding en kliniese-patologiese veranderinge te beskryf en om vas te stel of ander infeksies terselfdertyd kan voorkom. Ses en vyftig katte met natuurlike *B. felis* infeksie, wat by privaat veeartse aangebied is vir ondersoek en behandeling, is in die studie gebruik.

‘n Voorkeur vir katte van ‘n sekere ouderdom was klaarblyklik teenwoordig, aangesien die meeste siek katte jong volwassenes van minder as drie jaar oud was. Nieteenstaande die feit dat geen voorkeur vir ras of geslag geïdentifiseer kon word nie, het Siamese katte die oorgrote meerderheid van die groep opregte katte wat aangetas is, verteenwoordig. Tipiese kliniese tekens het anoreksie, lusteloosheid en anemie ingesluit. Minder algemene kliniese tekens het geelsug, swakheid, gewigsverlies, konstipasie en pika ingesluit. Die diagnose is bevestig deur die identifikasie van die parasiete op dun, gekleurde bloedsmere. Parasiettellings het gewissel tussen baie laag (0.3%) en geweldig hoog (42.3%). ‘n Sterk korrelasie tussen sentrale en periferele parasiettellings was teenwoordig; dit het aangedui dat sekwestrasie van rooibloedselle nie voorkom tydens hierdie siekte in katte nie.

Makrositiese, hipochromiese, regeneratiewe anemie was die mees algemene hematologiese bevinding. Erge anemie het ontwikkel in gevorderde gevalle. Die anemie is verder geklassifiseer as hemolities en was waarskynlik ‘n gevolg van beide intra- en ekstravaskulêre afbraak van rooibloedselle. Byna die helfte (43%) van die katte was nie anemies nie. Geen kenmerkende veranderinge in totale of differensiële witseltellings is waargeneem nie en die meeste abnormale witselwaardes wat wel waargeneem is, kon toegeskryf word aan ander toestande wat terselfdertyd teenwoordig was. Trombosiettellings het gewissel en trombositopenie was nie ‘n algemene bevinding nie. Agglutinasietoetse van rooibloedselle was positief in ‘n

aantal gevalle; dit het aangedui dat sekondêre immuunbemiddelde hemolitiese anemie ook ‘n kenmerk van babesiose in katte kan wees.

Die mees opvallende klinies-patologiese veranderinge was ‘n verhoging van hepatiese sitosol ensiem aktiwiteite en totale bilirubien konsentrasies. Serum alanien transaminase vlakke was merkwaardig verhoog in die meeste gevalle, maar serum alkaliese fosfatase en gamma glutamieltransferase vlakke was meestal normaal. Dit het aangedui dat primêre lewersel skade of inflammasie voorkom tydens meeste gevalle van babesiose in katte. Die verhoogde bilirubien konsentrasies was waarskynlik ‘n gevolg van hemolise, maar sekondêre lewersel skade het waarskynlik ook daartoe bygedra.

Geen kenmerkende veranderinge in nierparameters is waargeneem nie en serum urea en kreatinien konsentrasies was meestal binne normale perke. Dit dui aan dat erge nierskade ‘n onwaarskynlike bevinding is van babesiose in katte. Alhoewel ‘n verskeidenheid elektrolietabnormaliteite in ‘n aantal gevalle voorgekom het, is geen kenmerkende veranderinge in serum elektroliete (natrium en kalium) waargeneem nie. Serum proteïenwaardes was meestal normaal, maar verhoogde waardes het in ‘n aantal gevalle voorgekom. Hiperalbuminemie was mees waarskynlik ‘n aanduiding van dehidrasie. ‘n Poliklonale verspreiding van die globulienfraksies is waargeneem in alle katte met hiperglobulinemie, en is toegeskryf aan ‘n kombinasie van akute- en chroniese-fase proteïene wat geproduseer is in reaksie op die *Babesia* antigene.

Bykomende infeksies met *Haemobartonella felis*, leukemie virus (FeLV) en/of immunogebrek virus (FIV) het terselfdertyd voorgekom in ‘n aantal katte in hierdie studie en dit het ‘n belangrike invloed gehad op die respons op behandeling en prognose van die katte wat geaffekteer was.

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CHAPTER 1

1. LITERATURE REVIEW

1.1 Background:

Very little is known about babesiosis in cats and the existing literature concerning the piroplasms of cats is very confusing. Six different piroplasms of the cat family have been named in published literature:

1. *Babesia felis* in an African wild cat in Sudan *(Felis ocreata* syn *Felis sylvestris)*[84] by Davis in 1929[13]
2. *Babesiella felis* in a puma *(Felis concolor)* by Carpano in 1934[9]
3. *Nuttallia felis* var. *domestica* in a domestic cat by Jackson and Dunning in 1937[30]
4. *Babesia cati* in an Indian wild cat *(Felis catus)* by Mudaliar, Achary and Alwar in 1950[56]
5. *Babesia herpailuri* in a jaguarundi *(Herpailurus yaguarundi)* in South America by Dennig in 1967[14]
6. *Babesia pantherae* in a leopard *(Panthera pardus)* in Kenya by Dennig and Brocklesby in 1972[15]

Dennig and Brocklesby[15] suggested that *Babesia felis*, *Babesiella felis* and *Nuttallia felis* var *domestica* should all be considered to be a single species, named *Babesia felis.* They further proposed that all feline piroplasms should be divided into either of two small Babesias (*B. felis* and *B.cati*) or two large Babesias (*B.herpailuri* and *B. pantherae*).

Of the small Babesias, *Babesia felis* has been reported to occur most commonly in domestic cats, but it appears to have a wide host range within the cat family[37]. Recently, a small piroplasm was isolated from lions (*Panthera leo*) in the Kruger National Park in South Africa. This piroplasm was found to be morphologically

similar to, but serologically distinct from *B. felis*[43]. This implies that not all small piroplasms in felids are, in fact, *B. felis* and could also represent a new species[43]. Of added interest is the fact that the original *B. felis* parasite isolated from an African wild cat was found to be transmissible to domestic cats, but did not cause clinical illness[13]. In 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[18,65]. This again raises the question whether *B. felis* of domestic and wild felids is, in fact, the same species.

Babesiosis of domestic cats has been reported sporadically from other countries, including France[3,35], Germany[55], Thailand[33] and Zimbabwe[74], but it does not seem to be a regularly occurring clinical disease in any country other than South Africa. The parasites described in the cats from Zimbabwe and Germany, as well as one cat from France, were morphologically different from *B. felis* and more closely resembled *B. herpailuri*[3,55,74]. This may provide evidence of a large Babesia causing naturally occurring clinical disease in domestic cats, and may therefore also indicate that babesiosis of domestic cats could be caused by more than one Babesia species.

1.2 Feline babesiosis in South Africa:

In South Africa, *B. felis* causes a significant and potentially fatal clinical disease in domestic cats[65]. Babesiosis in the domestic cat in South Africa was first described by Jackson and Dunning in 1937 and concerned a clinical case in the Stellenbosch area[30]. In the same journal in 1937, McNeil published his views on piroplasmosis of the domestic cat[53]. His findings were based on clinical cases seen in the Cape Town area. His main clinical findings in these cases were anaemia and exercise intolerance. In 1963, Robinson published an article on babesiosis in cats seen in the Knysna district[69]. Anaemia was the main clinical finding.

In 1980, Futter and Belonje published a series of articles on feline babesiosis[17,18,19,20]. The first dealt with the history and classification of feline babesiosis[17]. The second article described clinical observations made in 20 experimentally infected and 70 naturally infected cats[18]. None of the experimentally infected cats was treated; they were studied until they either died or were euthanased in the terminal stage of the disease. The time period from inoculation with *B. felis* infected blood until death in these cats varied from 12 to 118 days. The naturally infected cases received various forms of treatment for the disease and were hospitalised and studied for at least eight days. Lethargy, anorexia and anaemia were consistent findings in both groups and icterus was occasionally seen. Pyrexia was not a feature of the disease. Blood smear examinations revealed evidence of a regenerative bone marrow response to the anaemia. Erythrophagocytosis by monocytic-type leukocytes was also observed[18].

The third article described the haematological findings in feline babesiosis and the same study group as described above was used[19]. Cases showed a rapid drop in haematocrit, haemoglobin and erythrocyte count. 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 findings[20]. Total serum protein concentrations were unchanged, but there was an increase in gamma globulin and a decrease in alpha and beta globulin concentrations. The increase in gamma globulins was ascribed to the antibody response of the reticulo-endothelial system of the patient to *Babesia* antigens. Moderate elevations in hepatic alanine transaminase (ALT) levels were recorded in a number of cases. Serum alkaline phosphatase (ALP) and gamma glutamyltransferase (GGT) levels were not measured[20]. Renal function was unaffected and venous blood pH remained within the normal range of 7,25 to 7,40 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 two cases[20].

A recent survey of feline babesiosis, which was conducted in 1999, has helped to establish the distribution of feline babesiosis in South Africa[32]. This survey confirmed that feline babesiosis is endemic along most of the South African coast, from KwaZulu-Natal to the Western Cape. This is a similar distribution to what has previously been described[65]. The survey showed that the distribution of the disease is largely coastal, but that some cases occur relatively far inland in endemic areas, particularly KwaZulu-Natal. Clinical cases are occasionally seen in non-endemic parts of the country – these are usually pets that had returned home after coastal holidays with their owners[32,65]. An isolated focus of feline babesiosis was, however, recently identified at Kaapschehoop, a village on the escarpment west of Nelspruit, Mpumalanga, South Africa[62]. By means of indirect fluorescent antibody tests, the piroplasms present in domestic cats at Kaapschehoop were identified as *B. felis* parasites[62]. As none of these cats had been away from the area prior to the development of the disease, one can assume that the natural vector of this disease can also be found in the area surrounding Kaapschehoop[62].

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

1.3 Feline immunodeficiency virus / Feline leukemia virus and feline babesiosis:

Feline immunodeficiency virus (FIV) infection causes alterations in the immune system of both naturally and experimentally infected cats[36]. Various immune parameters are impaired with FIV infection, which primarily involve the cell-mediated immune responses. Loss of adequate immune response in long-term FIV infection is paralleled by a significant decrease in the numbers of $CD4^+$ T-helper lymphocytes in peripheral blood that gradually develops over time[36]. The mechanism of $CD4^+$ T-cell depletion by FIV is thought to involve triggering of an apoptotic pathway, which is similar to the destruction of $CD4^+$ T-cells by HIV[83]. This impairment of helper T-cell function is considered to be the primary immunological lesion in the FIV-infected cat[83].

Infection of cats with FIV also significantly alters various parameters of macrophage function, including decreased interleukin-1 (IL-1) production[38]. As IL-1 is required for the appropriate responses of T-cells to antigen, the continued decrease of IL-1 production may lead to decreased antimicrobial activity of macrophages and ultimately an increased susceptibility to opportunistic infections in chronically infected animals[38]. Concomitant infections with protozoal parasites like *Toxoplasma gondii* and mycoplasmal parasites like *Haemobartonella felis* have been well described in cats with FIV[23,38]. Co-infection with *B. felis* could therefore also be a potential complication of FIV infection in cats. No research in this field has been done.

Like FIV, infection with feline leukemia virus (FeLV) also causes progressive suppression of immunity in cats[85]. This may result from several causes: kittens may develop thymic atrophy and resultant lymphoid (T-cell) depletion that could have fatal consequences. Cats of any age may also develop granulocytopaenia because of FeLV-induced myelosuppression, myelodysplasia or myelophthisis. A viral envelope protein, p15e, also plays a role in immunosuppression as it

suppresses in vitro lymphocyte blastogenesis. Circulating immune complexes are also produced in some cases; these immune complexes are likely to have an immunosuppressant effect by interfering with normal macrophage function, helper T-cell activation and the subsequent production of interleukin-2. In some cases, immunosuppression can also be attributed to a mutant strain of FeLV with defective and tissue-selective replication[59].

FeLV-induced acquired immunodeficiency syndrome (FAIDS) is the most common cause of death in naturally occurring FeLV infection of domestic cats. As mentioned, FAIDS is characterised by progressive depletion of $CD4^+$ cells, suppression of T-cell-dependent antibody response to antigen and opportunistic infections in the terminal stages of infection[85]. Concomitant protozoal infections with *Toxoplasma gondii* and mycoplasmal infections with *Haemobartonella felis* have also been documented[12,59]. The role of FeLV infection in cases with *B. felis* is currently unknown.

Recent research in mice has shown that $CD4^+$ T-cells and the production of gamma interferon play a crucial role in defence or protective immunity against infection with *Babesia microti*[25,27]. It is likely that this mechanism of protection is also true for cats and that functional $CD4^+$ T-cells are probably essential in protective immunity against infection with *B. felis.* As depletion of $CD4^+$ T-cells is a major immunological feature of both FeLV and FIV infections, this could potentially explain a link between FeLV and/or FIV infections and the development of feline babesiosis.

CHAPTER 2

2. BENEFITS

2.1. The information gained contributed towards the expansion of our current knowledge of feline babesiosis and a better understanding of the disease.

2.2. The research was undertaken in partial fulfilment of the requirements for the degree MMedVet (Med).

CHAPTER 3

3. OBJECTIVES

3.1 To review the typical signalment of naturally occurring cases of feline babesiosis.

3.2 To review the haematological and clinico-pathological changes in naturally occurring cases of feline babesiosis.

3.3 To evaluate the presence of concurrent infections with other haematological parasites, feline immunodeficiency virus and feline leukemia virus in cats naturally infected with *Babesia felis.*

CHAPTER 4

4. RESEARCH QUESTIONS

The following questions were addressed:

4.1 Signalment - Is there a specific predilection for infection with *B. felis* in cats with regard to:

a) age;
b) breed; and
c) sex?

4.2 What are the haematological changes seen in cats infected with *B. felis*, as reflected by changes in the:

a) erythrocyte;
b) leukocyte; and
c) thrombocyte parameters?

4.3 What are the clinico-pathological changes seen in cats infected with *B. felis,* as reflected by changes in:

a) serum proteins;
b) hepatic parameters;
c) renal parameters; and
d) serum electrolytes?

4.4 What is the prevalence of concurrent infection with feline immunodeficiency virus among cats that are infected with *B. felis*?

4.5 What is the prevalence of concurrent infection with feline leukemia virus among cats that are infected with *B. felis*?

4.6 What is the parasitaemia found on peripheral blood smears and how does it compare to the parasitaemia found on central venous smears?

4.7 Do cats infected with *B. felis* show positive in-saline agglutination of red blood cells?

4.8 Are any other haematological parasites present on peripheral and/or central venous blood smears?

CHAPTER 5

5. MATERIALS AND METHODS

5.1 Model system:

Cats with naturally occurring *B. felis* infection, presented to private veterinarians for examination and treatment over a study period of two months, were used in this study. The cats remained in the care of their owners. The study area was concentrated around George and surrounding towns in the Western Cape, South Africa.

5.2 Experimental design:

5.2.1 Inclusion criteria:

Cats were included in the study population if Dr. T. Schoeman positively identified *B. felis* on a stained peripheral blood smear that she had prepared herself. Owner's written consent was also required for inclusion of cats in the study.

5.2.2 Study population:

Sequential cases of feline babesiosis, presented to private veterinarians in the study area during the study period, were studied. The size of the study population was 56 cats.

5.3 Experimental procedures:

5.3.1 Sample collection:

Blood samples were drawn from the jugular vein after the skin had been aseptically prepared. Blood samples were collected using a 22G-venoject needle, a holder and EDTA and serum vacuum tubes[1]. Thin peripheral blood smears were prepared using a drop of capillary blood taken from the ear pinna. These blood smears were fixed and stained with Cam'sQuick[2]. Dr. T. Schoeman assessed body condition and habitus of all cats and recorded all relevant information on data capture sheets.

5.3.2 Clinico-pathological laboratory measurements:

The EDTA samples were stored at room temperature and were subjected to the following procedures within six hours of collection:

a) Thin central blood smears were prepared and subsequently fixed and stained with Cam'sQuick. Duplicate central blood smears were stored for later analysis.
b) In-saline agglutination tests were performed using the standard prescribed technique[79].
c) Full haematology was determined using a portable COULTER® A^{c}•T diff™ Haematology Analyzer Veterinary Applications counter[3] that was kindly lent to Dr. T. Schoeman for the duration of the study. The veterinary applications software was set for analysis of feline blood and the method of analysis was standard, as described in the operator's manual.

[1] Becton Dickinson Vacutainer Systems, Belliver Industrial Estate, Plymouth, United Kingdom
[2] CA Milsch (Pty) Ltd, Krugersdorp, South Africa
[3] Beckman Coulter S.A. (Pty) Ltd, Cape Town, South Africa

The serum samples were centrifuged[4] at 3 000 rpm for 10 minutes so as to separate cells from serum. The serum was pipetted into storage tubes and frozen at –20^{o}C for later use. The maximum storage time was 2 months.

On completion of the data collection phase of the trial the duplicate central blood smears and serum samples (frozen and on ice) were transported to the Section of Clinical Pathology, Department of Companion Animal Medicine, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, where the samples were processed.

The serum samples were allowed to thaw and reach room temperature. The following clinico-pathological parameters were measured:

a) Serum biochemistry (total serum proteins, albumin, urea, creatinine, alanine transaminase (ALT), alkaline phosphatase (ALP), gamma glutamyl-transferase (GGT) and total bilirubin).

   These were determined on a Technicon RA-1000 system[5]. The methods of analysis were standard as described in the Technicon RA Systems Methods Manual.

   Serum globulins were calculated by subtracting the albumin value from the total serum protein.

b) Serum electrolytes (sodium, potassium)

   These were done using a Rapidlab™ 348 pH / Blood Gas Analyzer[6]. The analysis methods were standard as described in the Rapidlab™ 348 pH / Blood Gas Analyzer Operator's Manual.

c) Serum protein electrophoresis

   This was performed on a standard Beckman Microzone® Electrophoresis System[7], using a cellulose acetate membrane and Barbital buffer (0.5 ionic

[4] Jouan centrifuge, Model B3.10, Hawksley and Sons, Ltd, Sussex, United Kingdom
[5] Technicon Instruments Corporation, Tarrytown, New York, USA
[6] Chiron Diagnostics Ltd, Halstead, Essex, United Kingdom
[7] Beckman Instruments Inc, Palo Alto, California, USA

strength and pH 8.6). This system identifies alpha (α1 and α2), beta (β1 and β2) and gamma (γ) peaks.

5.3.3 Differential counts:

Central blood smears were used to determine relative differential white blood cell counts. The stained smears were examined with oil magnification (50x objective field) and leukocytes were identified until 100 to 200 cells had been classified by type. Each of these cell types was expressed as a percentage. The percentage of each leukocyte (relative count) multiplied by the corrected total white blood cell count gave the absolute number of each leukocyte type per microlitre of blood[16].

Nucleated erythrocytes were identified by examination of stained central blood smears. The number of nucleated erythrocytes (nRBC) per 100 leukocytes was recorded. These nRBC counts were used to give an indication of the degree of regeneration of the red blood cells and to correct the original total white blood cell count using the formula[16]:

Corrected WBC count = (initial WBC count x 100) / (100 + nRBC)

Stained central blood smears were also used to evaluate platelets and determine a manual platelet score in order to verify the automatic platelet count. The platelet number was recorded as being normal, increased or decreased based on observation of the stained blood smear[16]. Eight to ten platelets per 100x objective field were considered normal. Less than three to four platelets per 100x objective field indicated a significant thrombocytopaenia. The presence of platelet clumps on the stained smear was also noted. Counts recorded as low by automatic cell counters because of platelet aggregation in the blood sample were correctly reported as normal or increased following observation of the platelet clumps on the stained blood smear.

All of the above procedures were consistently performed by the same experienced laboratory technician at the Section of Clinical Pathology, Department of Companion Animal Medicine, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa.

5.3.4 Parasitaemias:

Dr. T. Schoeman examined the red cell area of both peripheral and central venous blood smears for each cat and calculated the percentage parasitised erythrocytes for each, according to the method used at the Parasitology Section, Onderstepoort Veterinary Institute, Onderstepoort, South Africa. The number of parasitised erythrocytes in 10 microscope fields (using a 100x oil immersion lens) were counted and the percentage of parasitised erythrocytes was calculated by dividing the total number of parasitised erythrocytes by 40 (400 erythrocytes per field, 10 fields examined).

Dr. T. Schoeman also recorded the presence of co-infection with other haematological parasites, as identified on peripheral and/or central venous blood smears.

5.3.5 Serology:

Serum samples were used to determine the presence of concurrent infections with feline immunodeficiency virus (FIV) and / or feline leukemia virus (FeLV). By means of enzyme-linked immunosorbent assay (ELISA), the commercially available test kits[8] simultaneously detected the presence of FeLV group-specific viral core antigen (p27) and/or specific antibodies directed to FIVgag or FIVenv(gp40) proteins. The sensitivity of this test kit for the detection of each of the diseases has been shown to be 100% and the specificity 99%[24,44]. Instavet,

[8] SNAP Combo Plus Feline Leukemia Virus Antigen / Feline Immunodeficiency Virus Antibody Test Kit, IDEXX Corp., Portland, USA

South Africa kindly sponsored the majority of these test kits and Dr. T. Schoeman performed all test procedures according to the manufacturers' recommendations.

The presence of FIV antibodies was indicated by the development of a blue colour in the positive control well, a definite blue colour in the FIV test well and no colour development in the negative control well.

FeLV antigenaemia was indicated by the development of a blue colour in the positive control well, a definite blue colour in the FeLV test well and no colour development in the negative control well. A very faint blue colour in the test well was reported as an equivocal positive result. Where possible, a further serum sample was collected for re-testing of all equivocal and clear-cut FeLV positive cases 12 weeks after the original samples to determine whether FeLV antigenaemia was persistent or transient. Cats that were positive for FIV infection only were not retested.

5.4 Data analysis:

Statistical analysis of the data was performed on a commercial statistical software package[9]. 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 serum ALT and haematocrit, serum ALT and ALP, serum ALT and total bilirubin, total bilirubin and haematocrit, serum urea and creatinine, central and peripheral parasitaemias and central parasitaemia and haematocrit.

[9] SigmaStat™ v2.0.3, Jandel Scientific Software, USA

When the group of cats with clinical icterus was compared to the group of cats without clinical icterus, the Mann-Whitney Rank Sum Test was used. The Mann-Whitney Rank Sum Test was also used to compare the group of in-saline positive cats to the group of in-saline negative cats and to compare the group of cats with *H. felis* co-infection to the group of cats with *B. felis* infection alone. In the addendum, the Mann-Whitney Rank Sum Test was also used to compare the group of treated cats to the group of untreated cats.

For all tests, the probability value for significance was set at $P<0.05$.

5.5 Ethical considerations:

The protocol for this trial was approved by the Animal Use and Care and Research Committees of the Faculty of Veterinary Science on 1999-06-29, reference number 36-5-401. All blood samples collected from the study cats were done with the informed consent of the owners. Owners were requested to read and complete the informed consent form prior to inclusion of their pets in the study. Intervention was limited to the collection of two blood samples per cat.

CHAPTER 6

6. RESULTS

6.1 Signalment:

The signalment for each case is tabulated in Appendix 1.

6.1.1 Age:

Ages of affected cats ranged between 6 months and 13 years, with a median of 2 years. The majority (80%) of cats (45/56) were less than three years old at the time of presentation. Only 9% of the cases (5/56) were older than 10 years at the time of presentation and the remaining 11% of the cats (6/56) represented the age group between three and ten years old. Graphical representation of the age distribution is shown in Figure 1.

6.1.2 Breed:

The majority of cats did not represent a specific breed, with 77% (43/56) being either of the domestic shorthair (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), and the rest of the cats represented a mixture of Burmese (1/56), Oriental (1/56), Russian blue (2/56), Chinchilla (1/56) and Persian breeds (1/56).

6.1.3 Sex:

No marked sex predilection could be seen as the study population included 57% females (32/56) and 43% males (24/56). Of the female group, 69% of the cats (22/32) had been neutered and 67% of the male cats (16/24) had been neutered.

6.1.4 Owner complaints:

Primary owner complaints of anorexia, depression and listlessness were reported in 77% (43/56) of cases. Other owner complaints included anaemia, weight loss, icterus, dehydration, constipation and pica.

In 7% of cases (4/56), the cats were presented for examination of clinical signs that were not typically associated with babesiosis (tail fracture, femur fracture, chronic weight loss and pollakiuria respectively), but *B. felis* parasites were identified on peripheral blood smears.

A further 12,5% of the cats (7/56) did not show any noticeable clinical signs of babesiosis at the time of examination, but these cats were known to have been previously infected with *B. felis* and were on chronic extended therapy for the disease. They were re-examined at regular intervals by the private veterinarians and parasites were still identified on peripheral blood smears.

A small group of 3,5% of the study cats (2/56) were presented to the private veterinarian for reasons other than disease. They were described as clinically healthy and normal cats, but on examination of peripheral blood smears, *B.felis* parasites were identified in these asymptomatic cats.

6.1.5 Clinical assessment:

Affected cats were found to be in normal body condition in 50% of cases (28/56), a further 25% of cases (14/56) were considered to be fat and the other 25% were thin at presentation.
Around 61% of cases (34/56) appeared depressed at the time of presentation, with 20% (11/56) being severely depressed and 41% (23/56) moderately depressed. The remaining 39% of cases (22/56) were alert at the time of examination.
Clinical icterus was diagnosed in 21% of cases (12/56).

6.2 Haematology:

The proportions of normal and abnormal haematological findings of all cats are summarised in Table 1.

6.2.1 Red blood cell parameters:

The most significant finding regarding the red blood cell parameters was a macrocytic, hypochromic, regenerative anaemia. Details are presented below. The red blood cell parameters for all individual cats are tabulated in Appendix 2.

a) Red blood cell count:

Reference range: 5-10 x $10^{12}/\lambda$

The erythrocyte counts ranged between 0.8 and 8.1 x $10^{12}/\lambda$, with 59% of the cats (33/56) having erythrocyte counts below the normal range. Of these, 69% or 25/36 had severely low counts of 2,5 x $10^{12}/\lambda$ or less. The median red blood cell count was low at 3.0 x $10^{12}/\lambda$.

b) Haematocrit (Ht):

Reference range: 24-45 %

Reduction in Ht was present in 57% of the cats, with 32/56 cats having a Ht of less than 24%. Ht values ranged from 7.9% to 41.2%. Normal Ht values occurred in 43% (24/56) of the cats, while moderate anaemia (Ht between 15% and 24%) occurred in 23% (13/56) of the cats. Severe anaemia (Ht<15%) was seen in 34% (19/56) of cases. The median Ht value of 18.7% was also low. Graphical representation of the distribution of haematocrit values is shown in Figure 2.

c) Haemoglobin concentration (Hb):

Reference range: 80–150 g/λ

The Hb concentrations were closely associated with the haematocrit levels and 59% (33/56) of the cats had concentrations below the normal range. Haemoglobin values for all cats ranged between 22 and 136 g/λ. The median Hb concentration was low at 59 g/λ.

d) Mean corpuscular volume (MCV):

Reference range: 39–55 fλ

The MCV was increased in 64% (36/56) of the cats with measurements in excess of 55 fλ. The MCV measurements varied between 41.8 and 109.3 fλ and 61% (22/36) of the cats with elevated MCV had measurements higher than 70 fλ. None of the cats had a MCV below the normal reference range. The median value for MCV was 60.9 fλ, which was higher than normal.

e) Mean corpuscular haemoglobin (MCH):

Reference range: 12.5-17.5 pg

Measurements of MCH showed that 71% (40/56) of the cats had levels higher than 17.5 pg. These cats were generally the same cats that showed an elevated MCV. MCH levels varied between 13.9 and 30.2 pg. None of the cases had MCH levels below the normal range. The median value for MCH was above normal at 19.9 pg.

f) Mean corpuscular haemoglobin concentration (MCHC):

Reference range: 300-360 g/λ

Only 29% (16/56) of the cats had abnormal MCHC values below 300 g/λ. Concentrations varied between 233 and 368 g/λ. The median value for MCHC was normal at 328 g/λ.

g) Nucleated red blood cell count (nRBC):

Nucleated red blood cells are not routinely present in circulation of normal animals. They were, however, present in 70% (39/56) of the cases. Counts varied between 1 and 814 nRBC / 100 white blood cells counted, with a median nRBC count of 10.5.

6.2.2 White blood cell parameters:

The white blood cell parameters for all cats are tabulated in Appendix 3 and the proportions of normal and abnormal findings are summarised in Table 1.

a) Total white blood cell counts (WBC) and corrected white blood cell counts (cWBC):

Reference range: 5.5-19.5 x 10^9/λ

Total white blood cell counts were recorded in 54 cases. The Coulter Analyzer was not able to give reliable total white cell count results for the remaining two cats, therefore those results were not used for further analysis.

Total white blood cell counts ranged between 2.8 and 58.9 x 10^9/λ. These counts were corrected for nRBC counts and the corrected white blood cell counts were obtained. The cWBC varied between 2.6 and 41.8 x 10^9/λ. Of these counts, 28% (15/54) were outside the normal reference range. Leukocytosis was recorded in 11% (6/54) and leukopaenia occurred in 17% (9/54) of the cats tested. These leukocyte changes were mostly caused by changes in absolute neutrophil, lymphocyte and monocyte counts. The abnormal leukograms were classified as

a combination of inflammatory, stress-induced and physiologic leukograms. The median cWBC was within normal range at 10.1 x $10^9/\lambda$. Graphical representation of the corrected total WBC counts is shown in Figure 3.

b) Differential white blood cell counts:

Absolute differential counts were recorded for 54 cats.

i. Mature neutrophils:

Reference range: 2,5-12,5 x $10^9/\lambda$

Thirty-one percent of the cats (17/54) had absolute mature neutrophil counts outside the normal reference range. Elevated counts were recorded in 11% (6/54) of the cats and 20% (11/54) had low counts. Counts varied between 1.0 and 39.3 x $10^9/\lambda$. The median absolute mature neutrophil count was within normal range at 5.3 x $10^9/\lambda$. Graphical representation of the absolute mature neutrophil counts is shown in Figure 3.

ii. Immature neutrophils:

Reference range: 0-0.3 x $10^9/\lambda$

Thirty-one percent of the cats (17/54) had elevated absolute immature neutrophil (band neutrophil) counts. Pronounced left shifts with immature neutrophil counts above 1.0 x $10^9/\lambda$ were seen in 13% (7/54) of the cats, while the rest of the cats had only mildly elevated counts. Counts varied between 0 and 5.0 x $10^9/\lambda$. The median absolute immature neutrophil count was normal at 0.2 x $10^9/\lambda$. Metamyelocytes were observed in 9% of the cats (5/54) during examination of blood smears, all of which had regenerative left shift neutrophilias. Of the 17 cats with left shift neutrophilias, 88% (15/17) were regenerative. Degenerative left shift neutrophilias were seen in only 2 cats (4%). Graphical representation of the absolute immature neutrophil counts is shown in Figure 4.

iii. Lymphocytes:

Reference range: 1.5-7.0 x $10^9/\lambda$

Absolute lymphocyte counts varied between 0.4 and 14.9 x $10^9/\lambda$. It was found that 45% of the cats (24/54) had absolute lymphocyte counts outside the normal reference range, with 15% (8/54) showing lymphocytosis and 30% (16/56) of the cats showing lymphopaenia. The median absolute lymphocyte count was normal at 2.8 x $10^9/\lambda$. Graphical representation of these lymphocyte counts is shown in Figure 3.

iv. Monocytes:

Reference range: 0-0.85 x $10^9/\lambda$

Absolute monocyte counts varied between 0 and 5.3 x $10^9/\lambda$. It was found that 20% of the cats (11/54) showed monocytosis with counts elevated above the normal reference range. The median absolute monocyte count was normal at 0.3 x $10^9/\lambda$. Graphical representation of the absolute monocyte counts is seen in Figure 4.

v. Eosinophils:

Reference range: 0-1.5 x $10^9/\lambda$

Absolute eosinophil counts varied between 0 and 1.7 x $10^9/\lambda$. Only one cat (2%) showed eosinophilia with a count elevated above the normal reference range. The median absolute eosinophil count was normal at 0.2 x $10^9/\lambda$. Graphical representation of these counts is shown in Figure 4.

vi. Basophils:

Reference range: 0-0.2 x $10^9/\lambda$

Absolute basophil counts of 0.1 x $10^9/\lambda$ were recorded in two cats (4%), both of which were within the normal reference values. No basophils were seen in 96% (52/54) of the cats.

6.2.3 Thrombocyte parameters:

Thrombocyte parameters for all cats are tabulated in Appendix 4 and the proportions of normal and abnormal findings are summarised in Table 1.

The automated platelet counts and manual platelet scores differed substantially (Figure 5). Only 27% (15/55) of the cats were categorised in the same way by both systems - 25% (14/55) were thrombocytopaenic and 2% (1/55) had a normal thrombocyte count. Of these cats, 33% (5/15) had platelet clots on examination of blood smears. Of the 73% (40/55) of cats that were not categorised in the same way by both systems, 73% (29/40) had evidence of platelet clots on examination of blood smears.

a) Platelet count:

Reference range: 300-800 x $10^9/\lambda$

Automated platelet counts were recorded for 55 cats, with no thrombocyte count given by the Coulter Analyzer for the other cat.

Generalised thrombocytopaenia was evident in 98% of the cats (54/55) where thrombocyte counts of less than 300 x $10^9/\lambda$ were obtained. Thrombocyte counts varied between 2 and 320 x $10^9/\lambda$ with a median count of 23 x $10^9/\lambda$.

b) Platelet score:

Using the manual platelet score, only 25% of the cats (14/56) were thrombocytopaenic, and 71% (40/56) were scored as normal. Two of the cats (4%) were thought to have thrombocytosis. Platelet clots were reported in 61% of the cats (34/56), 15% (5/34) of which were scored as thrombocytopaenic and the remaining 85% (29/34) had normal platelet scores.

6.3 Clinical pathology:

Clinico-pathological findings for all cats are tabulated in Appendix 5 and the proportions of normal and abnormal findings are summarised in Table 2.

6.3.1 Serum proteins:

a) Total serum proteins (TSP):

Reference range: 60-80 g/λ

Total serum proteins were elevated above 80 g/λ in 32% of the cats (18/56) while only 2% (1/56) had a TSP value less than 60 g/λ. TSP values ranged from 57.6 to 109.1 g/λ. The median TSP value was within normal limits at 77.3 g/λ.

b) Albumin:

Reference range: 25-35 g/λ

Albumin values were elevated above 35 g/λ in 45% (25/56) of the cases and only 2% (1/56) of cases had an albumin value less than 25 g/λ. Albumin values ranged from 23.5 to 49.2 g/λ. The median albumin value was top normal at 34.9 g/λ.

c) Globulins:

Reference range: 22-48 g/λ

Serum globulin levels were elevated above 48 g/λ in 23% (13/56) of cases. Globulin levels ranged from 29.3 to 70.7 g/λ and the median globulin value was within normal limits at 41.1 g/λ.

d) Globulin fractions (α, β and γ globulins):

Results of serum protein electrophoresis for all individual cats are tabulated in Appendix 6.

Reference ranges: *α globulins: 8-16 g/λ*
β globulins: 6-14 g/λ
γ globulins: 12-22 g/λ

Polyclonal gammopathies were observed in all cats with increased total globulin levels.

Alpha (α) globulins were elevated above 16 g/λ in 18% (10/56) of cases, below 8 g/λ in 3% (2/56) of cases and within normal limits in 79% (44/56) of cases. The median α globulin level was normal at 13.1 g/λ. Beta (β) globulins were elevated above 14 g/λ in 11% (6/56) of cases, within normal limits in 89% (50/56) and the median value was normal at 11.8 g/λ. Gamma (γ) globulin concentrations were elevated above 22 g/λ in 29% (16/56) of cases, below 12 g/λ in 5% (3/56) and within normal limits in 66% (37/56) of cases. The median γ globulin concentration was within normal limits at 18.6 g/λ.

Abnormal globulin fractions were recorded in 33% (14/43) of the 77% (43/56) of cats with normal total globulin levels. These included various combinations of abnormal α, β and γ globulin levels, but there was no consistent pattern of changes.

6.3.2 Hepatic parameters:

a) Alanine transaminase (ALT):

Reference range: <23 U/λ

Alanine transaminase levels were elevated above 23 U/λ in 89% (50/56) of cats and varied between 10 and 1 908 U/λ. The median ALT level was raised with a value of 54.5 U/λ. Severe elevations above 300 U/λ were seen in 20% (11/56) of cats. Graphical representation of the distribution of ALT is shown in Figure 6a.

A statistically significant, but weak, negative correlation was found between ALT levels and haematocrit (r_S=-0.497; P<0.05). Graphical representation of this negative correlation is shown in Figure 7.

Elevated ALT levels were observed in 100% (12/12) of the 21% (12/56) of cats with clinical icterus, while 86% (38/44) of the cats without clinical icterus had elevated ALT levels. When the ALT values for cats with clinical icterus were compared to those of cats without clinical icterus, a statistically significant difference was seen (T=588.00; P<0.001). The icteric cats had much higher ALT values. Graphical representation of this comparison is shown in Figure 8.

b) Alkaline phosphatase (ALP):

Reference range: <20 U/λ

ALP levels were elevated above 20 U/λ in 25% of the cases (14/56). The median ALP was within normal limits at 11.5 U/λ. Values ranged from 0 to 75 U/λ. Graphical representation of the distribution of ALP values is shown in Figure 6b.

Elevated ALP levels were seen in 42% (5/12) of the 21% (12/56) of cats with clinical icterus, while 20% (9/44) of cats without clinical icterus had elevated ALP values. When the ALP values of cats with clinical icterus were compared to those of cats without clinical icterus, no statistically significant difference was seen (T=396.00; P=0.285). Graphical representation of this comparison is shown in Figure 9.

No correlation between ALT and ALP levels could be found (r_S =0.002; P=0.985).

c) Gamma glutamyltransferase (GGT):

Reference range: <10 U/λ

Gamma glutamyltransferase levels were elevated above 10 U/λ in 2 cats (4%) only. Values ranged between 0 and 11 U/λ and the median GGT value was

normal at 0.0 U/λ. Graphical representation of the distribution of GGT values is shown in Figure 6b.

d) Total bilirubin:

Reference range: <6.8 μmol/λ

Total bilirubin levels were elevated above 6.8 μmol/λ in 86% of cases (48/56), with a median value of 12.9 μmol/λ. Values ranged between 5.5 and 372 μmol/λ.

Raised bilirubin levels were seen in 100% (12/12) of the 21% (12/56) of cats with clinical icterus, while 82% (36/44) of cats without clinical icterus had elevated bilirubin levels.

When ALT values were correlated with total bilirubin values, a significant positive correlation was seen (r_s=0.708; P<0.05). Graphical representation of the correlation between ALT and bilirubin is shown in Figure 10.

When total bilirubin levels were correlated with haematocrit, a statistically significant, but weak, negative correlation was seen (r_s=–0.553; P<0.05). Graphical representation of the correlation between total bilirubin and haematocrit is shown in Figure 11.

6.3.3 Renal parameters:

a) Serum urea:

Reference range: 7.1-10.7 mmol/λ

Serum urea measurements were elevated above 10.7 mmol/λ in 25% (14/56) of the cats and below 7.1 mmol/λ in 20% (11/56). Values ranged from 4.2 to 55.8 mmol/λ. The median value for urea was within normal limits at 8.6 mmol/λ. Of the 14 cats with elevated serum urea levels, 10 showed only mild or moderate elevations. Severe elevations were seen in 4 cats, with individual urea levels of 19.1 mmol/λ, 21.5 mmol/λ, 22.4 mmol/λ and 55.8 mmol/λ recorded. Of the 14

cats with elevated urea levels, only 6 had elevated creatinine levels concurrently, while the remaining 8 cats had normal creatinine levels.

b) Serum creatinine:

Reference range: <141 μmol/λ

Serum creatinine levels were elevated above 141 μmol/λ in 25% (14/56) of the cats. Values ranged between 72 and 388 μmol/λ and the median creatinine level was within normal limits at 128.5 μmol/λ. Of the 14 cats with elevated serum creatinine levels, 10 showed only mild elevations. Severe elevations in serum creatinine levels were seen in 4 cases with individual levels of 197 μmol/λ, 216 μmol/λ, 288 μmol/λ and 388 μmol/λ. Of the 14 cats with elevated creatinine levels, only 6 had concurrently high urea levels and the remaining 8 cats had normal urea levels.

When the urea and creatinine values were correlated, no statistically significant relationship was seen between these values (r_s=0.211; P=0.117). Graphical representation of this correlation is shown in Figure 12.

6.3.4 Serum electrolytes:

a) Sodium:

Reference range: 141-156 mmol/λ

Measurements of sodium revealed that 9% (5/56) of the cats had levels elevated above 156 mmol/λ, where 27% (15/56) had levels below 141 mmol/λ. The median sodium level was within normal limits at 147 mmol/λ and values ranged from 126 to 159 mmol/λ.

b) Potassium:

Reference range: 4.0-5.1 mmol/λ

Potassium levels were higher than 5.1 mmol/λ in 20% (11/56) of the cats and below 4.0 mmol/λ in 16% (9/11). Values ranged from 3.2 to 6.9 mmol/λ and the median potassium level was within normal limits at 4.6 mmol/λ.

6.4 Co-infection with feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV):

FIV and FeLV test results for all cats are tabulated in Appendix 7 and summarised in Table 3.

Eight cats (14%) tested positive for FIV infection. Of these, five cats (9%) also had concurrent FeLV infection.
Eighteen cats (32%) tested positive for FeLV infection. Five of these cats (9%) also had concurrent infection with FIV.
Concurrent infections with either FIV or FeLV or both viruses were thus diagnosed in 38% (21/56) of the cats.
In addition to this, a further 6 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 to still be alive after the 12-week period, while 3 were lost to follow-up and the remaining 7 had died. Four of these died by themselves while the other 3 were euthanased because 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 and the remaining 2 were dead after the 12-week period.

All of the 12 cats still alive 12 weeks after the original FeLV tests were retested for FeLV/FIV. Only two cats, both of which were clear-cut FeLV positives originally, tested positive for FeLV the second time round and one of these still

tested positive for both FeLV and FIV infections. Of the 5 cats that originally tested positive for both FeLV and FIV infections concurrently, this particular cat was the only one that was still alive after 12 weeks. This cat remained positive for both viruses and deteriorated slowly until it had to be euthanased 8 weeks after the second test. The only other cat that tested positive for FeLV again after the 12-week period, was still alive 6 months after the second test. Six of the original 18 cats that tested clear-cut positive for FeLV initially and 4 of the 6 cats that had equivocal results originally thus tested negative for FeLV after 12 weeks.

6.5 Parasitaemia:

The central and peripheral parasitaemia for each cat is tabulated in Appendix 8.

Peripheral parasitaemias ranged from 0.3% to 42.3% with a median of 5.9%. Peripheral parasitaemias were assessed in 55 cats only; the other one was not possible due to poor quality of the peripheral blood smear. A majority of 69% (38/55) of the cats had a peripheral parasitaemia of 0 to 10%, while 13% (7/55) had values of 10-20%, 4% (2/55) had values between 20-30%, 9% (5/55) had values between 30-40% and a further 5% of the cats (3/55) had peripheral parasitaemias between 40-50%.

Central parasitaemias were assessed in 56 cats and ranged from 0.2% to 41.4% with a median of 6.4%. A majority of 68% (38/56) of the cats had central parasitaemias of 0 to 10%, while 14% (8/56) had values between 10-20%, 4% (2/56) had values between 20-30%, 7% (4/56) had values between 30-40% and a further 7% (4/56) had central parasitaemias between 40-50%.

Of the cats with central parasitaemias less than 10%, 11% (6/56) had values between 0 to 1%, while 34% (19/56) had values between 1 to 5% and the remaining 23% (13/56) had values between 5 to 10%.

Central and peripheral parasitaemias showed a statistically significant strong positive correlation (r_s=0.997; P<0.05). Graphical representation of this correlation is shown in Figure 13.

When haematocrit and central parasitaemia values were correlated, a statistically significant, but weak, negative correlation was seen (r_s=-0.595; P<0.05). This negative correlation appeared to be most pronounced when central parasitaemias were higher than 20%, with 90% (9/10) of these cats showing a haematocrit of 15% or less. When central parasitaemias were less than 20%, haematocrit levels tended to be more randomly distributed. Graphical representation of this correlation is shown in Figure 14.

6.6 In-saline agglutination:

In-saline agglutination results for all cats are tabulated in Appendix 2.

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

Haematocrit values of in-saline positive cats were significantly lower than those of in-saline negative cats (T=137.00; P=0.008). Graphical representation of this comparison is shown in Figure 15.

6.7 Other haematological parasites:

The presence or absence of other parasites on the blood smears of all cats are tabulated in Appendix 7 and summarised in Table 3.

Co-infection with *Haemobartonella felis* was noted on both peripheral and central venous blood smears of 11% (6/56) of all the cats tested (Appendix 10). Of the 6 cats with *H. felis* co-infection, 50% (3/6) also tested positive for concurrent FeLV infection.
No other haematological parasites were seen on any blood smears.

Because *H. felis* is also known to cause anaemia and occasional icterus with elevation of hepatic ALT enzyme activity in cats[8], the group of cats with concurrent *H. felis* infection was compared to the group of cats with only *B. felis* infection to establish if *H. felis* co-infection caused worsening of those particular parameters.

When comparing the haematocrit values of the group of cats with *H.felis* co-infection to the haematocrit values of the group of cats without this co-infection, no statistically significant differences between the groups were observed (T=126.00; P=0.238). Graphical representation of this comparison is shown in Figure 16.

When comparing the ALT values of the group of cats with *H.felis* co-infection to the ALT values of the group of cats without this co-infection, no statistically significant differences between the groups were observed either (T=225.00; P=0.156). Graphical representation of this comparison is shown in Figure 17.

6.8 Tables:

Table 1: Proportions of normal and abnormal haematological values in 56 cats with babesiosis

Data are shown as the number of cats and percentage of total.

	Low		Normal		High	
	No	%	No	%	No	%
Haematocrit	32	57	24	43	0	0
Red blood cell count	36	64	20	36	0	0
Haemoglobin concentration	33	59	23	41	0	0
Mean corpuscular volume	0	0	20	36	36	64
Mean corpuscular haemoglobin	0	0	16	29	40	71
Mean corpuscular haemoglobin concentration	16	29	40	71	0	0
Corrected white blood cell count	9	17	39	72	6	11
Absolute neutrophils-mature	11	20	37	69	6	11
Absolute neutrophils-immature	0	0	37	69	17	31
Absolute lymphocyte count	16	30	30	55	8	15
Absolute monocyte count	0	0	43	80	11	20
Absolute eosinophil count	0	0	53	98	1	2
Absolute basophil count	0	0	54	100	0	0
Thrombocyte count	54	98	1	2	0	0
Thrombocyte score from smear	14	25	40	71	2	4

Table 2: Proportions of normal and abnormal clinico-pathological findings in 56 cats with babesiosis

Data are shown as the number of cats and percentage of total.

	Low		Normal		High	
	No	%	No	%	No	%
Total serum proteins	1	2	37	66	18	32
Albumin	1	2	30	54	25	45
Globulin	0	0	43	77	13	23
α globulin	2	3	44	79	10	18
β globulin	0	0	50	89	6	11
γ globulin	3	5	37	66	16	29
Alanine transaminase	0	0	6	11	50	89
Alkaline phosphatase	0	0	42	75	14	25
Gamma glutamyl transferase	0	0	54	96	2	4
Total bilirubin	0	0	8	14	48	86
Urea	11	20	31	55	14	25
Creatinine	0	0	42	75	14	25
Sodium	15	27	36	64	5	9
Potassium	9	16	36	64	11	20

Table 3: A summary of concurrent infections diagnosed in 56 cats with babesiosis

Data are shown as the number of cats and the percentage of total.

Concurrent infection	No	%
Haemobartonella felis	6	11
Feline leukemia virus (FeLV) -Clear-cut FeLV -Equivocal FeLV -Repeat FeLV	 18 6 2	 32 11 17
Feline immunodeficiency virus (FIV)	8	14
Combined FeLV and FIV	5	9
Either FeLV or FIV or both	21	38
Total concurrent infections (either *H. felis* or FeLV or FIV or combinations)	24	43

6.9 Figures:

Figure 1: Age distribution of 56 cats with babesiosis

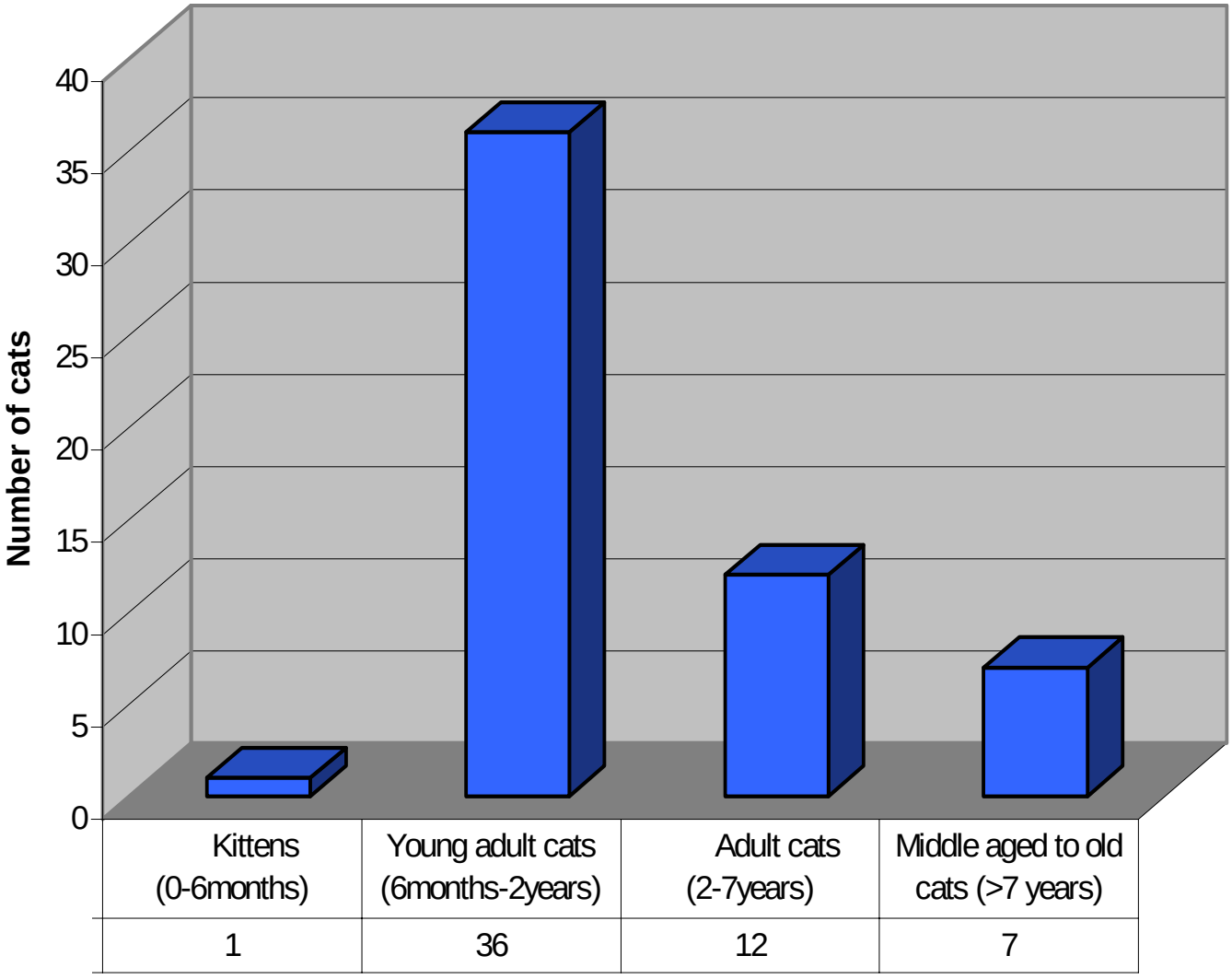


Figure 2: Distribution of haematocrit values in 56 cats with babesiosis

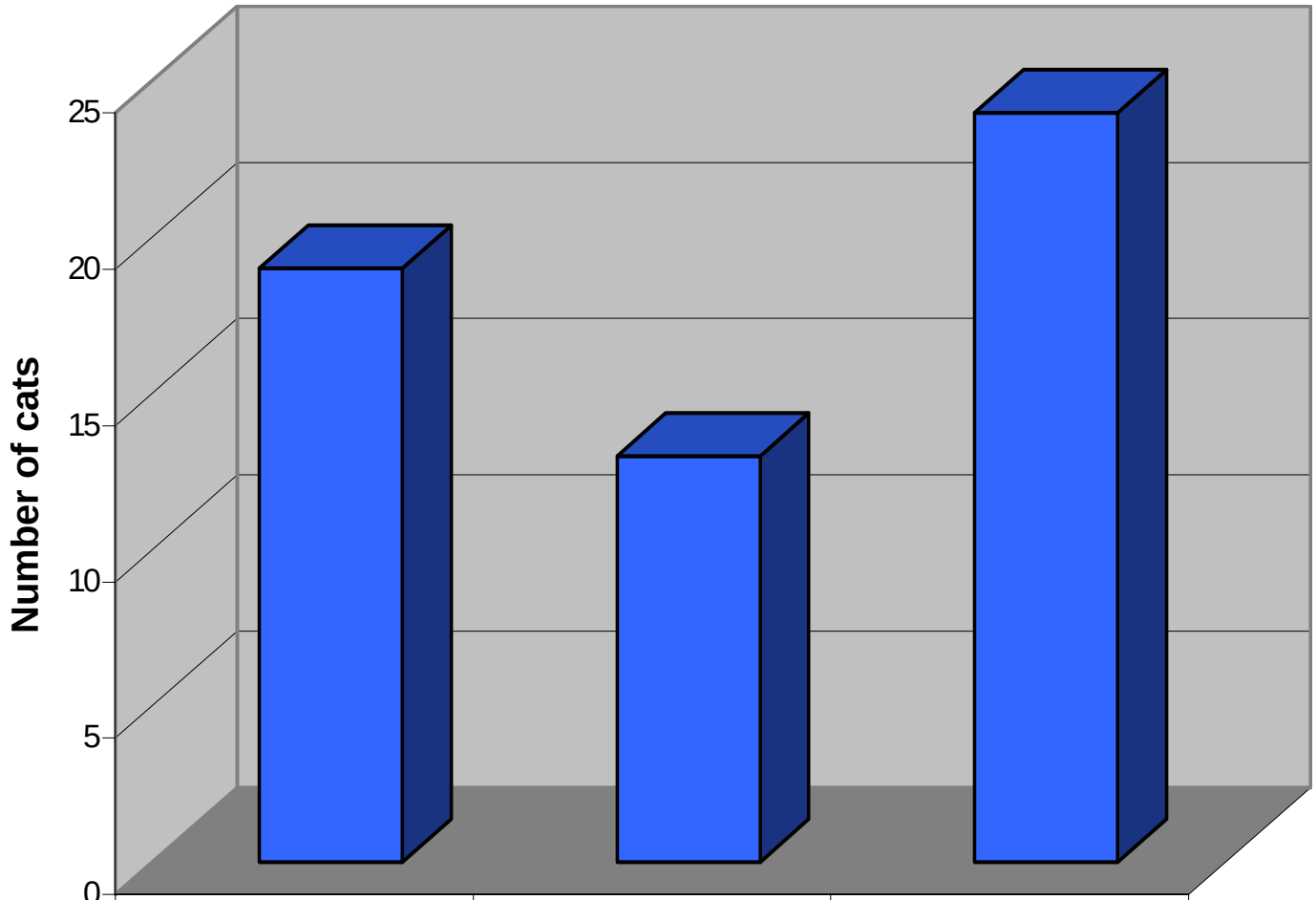


Figure 3: Distribution of corrected total white blood cell counts, mature neutrophils and lymphocytes in 56 cats with *B. felis* infection

The ends of the boxes indicate the 25th and 75th percentiles, with a line at the median. T-bars indicate the 10th and 90th percentiles. Circles represent outliers.

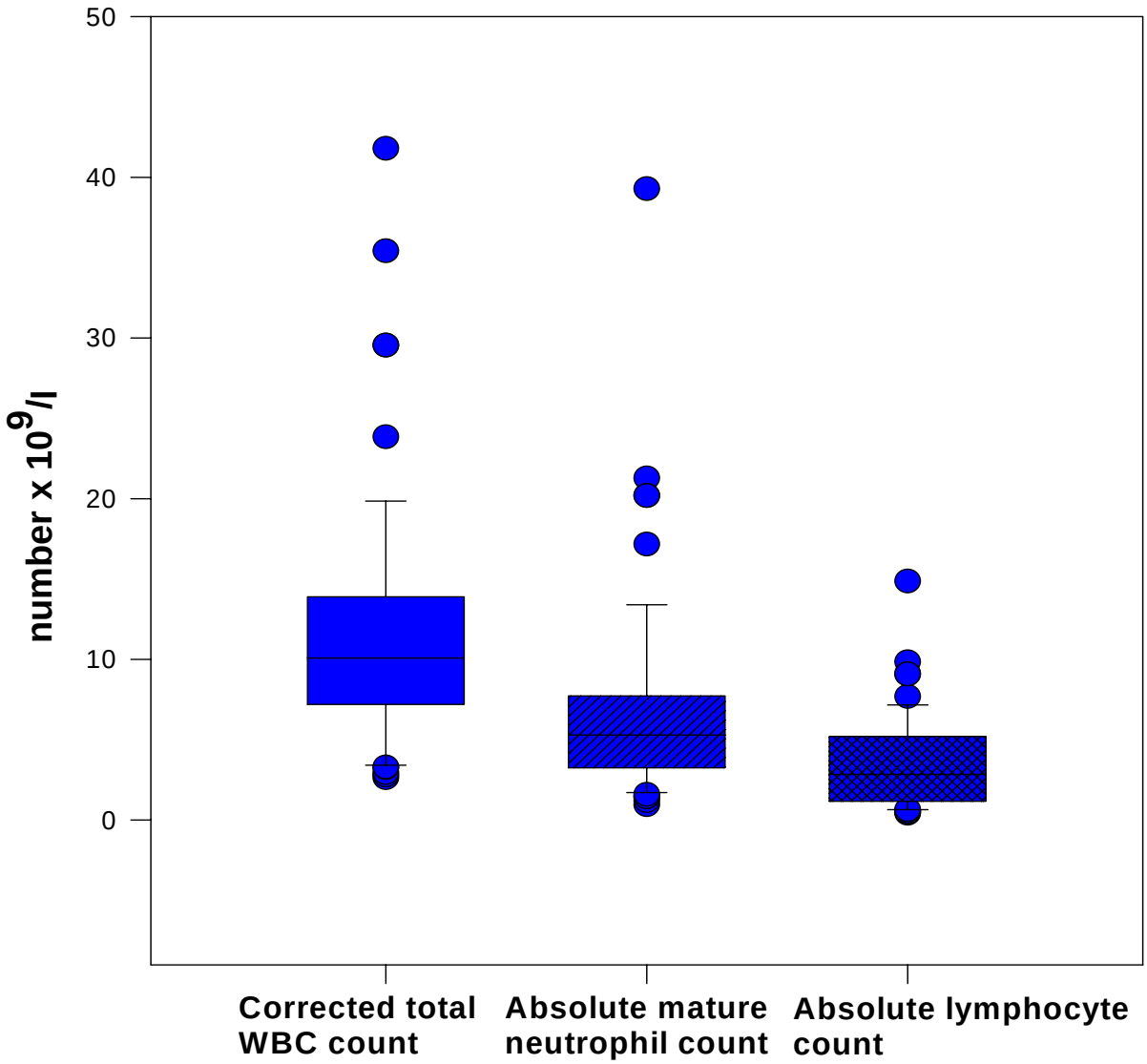


Figure 4: Distribution of absolute immature neutrophils, monocytes and eosinophils in 56 cats with *B. felis* infection

The ends of the boxes indicate the 25th and 75th percentiles, with a line at the median. T-bars indicate the 10th and 90th percentiles. Circles represent outliers.

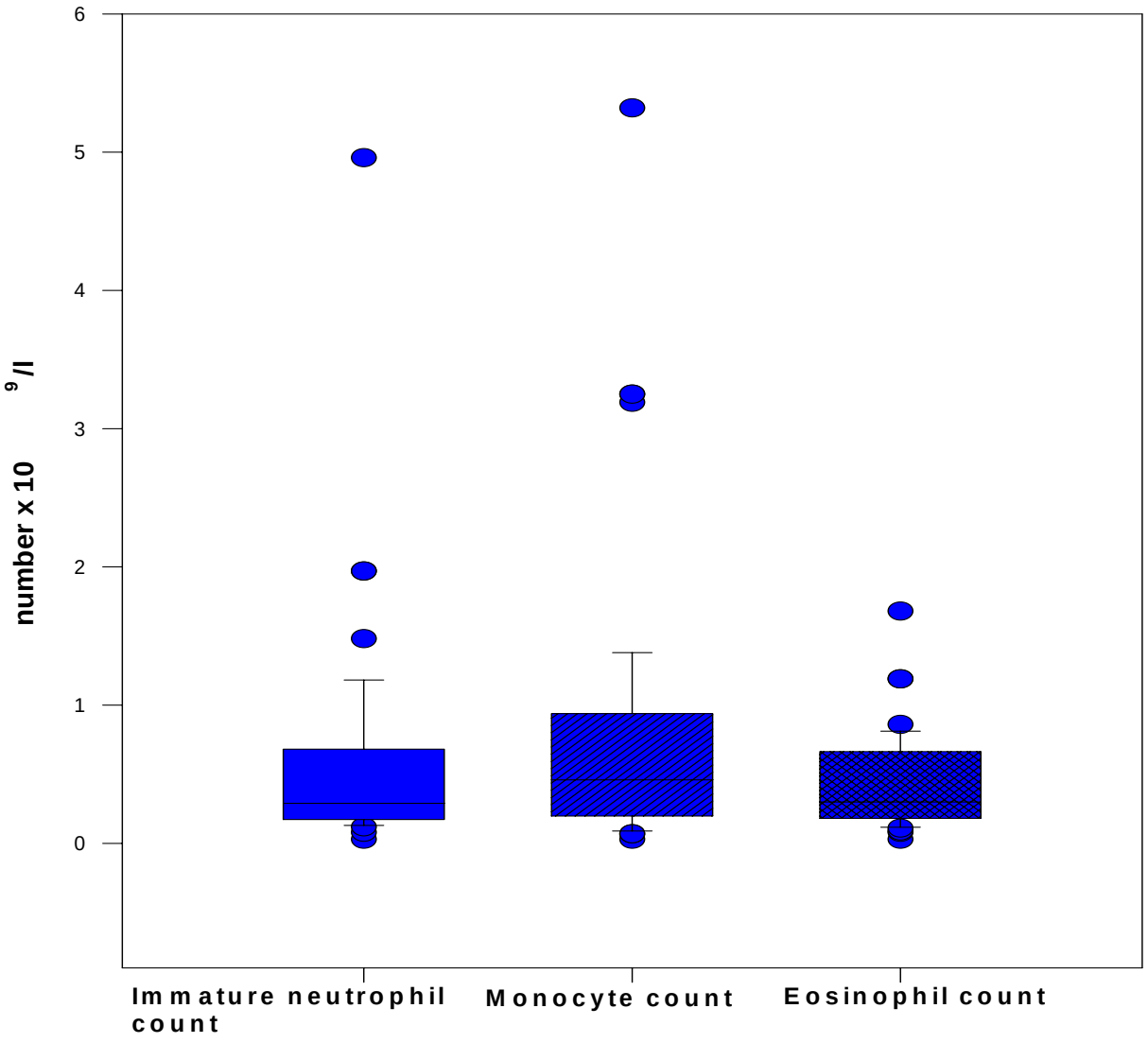


Figure 5: Thrombocyte parameters in 56 cats with babesiosis

The difference between the automated machine count (number x $10^9/\lambda$) and the manual platelet score is illustrated. The vertical line represents the lower end of the normal range for the automated machine count.

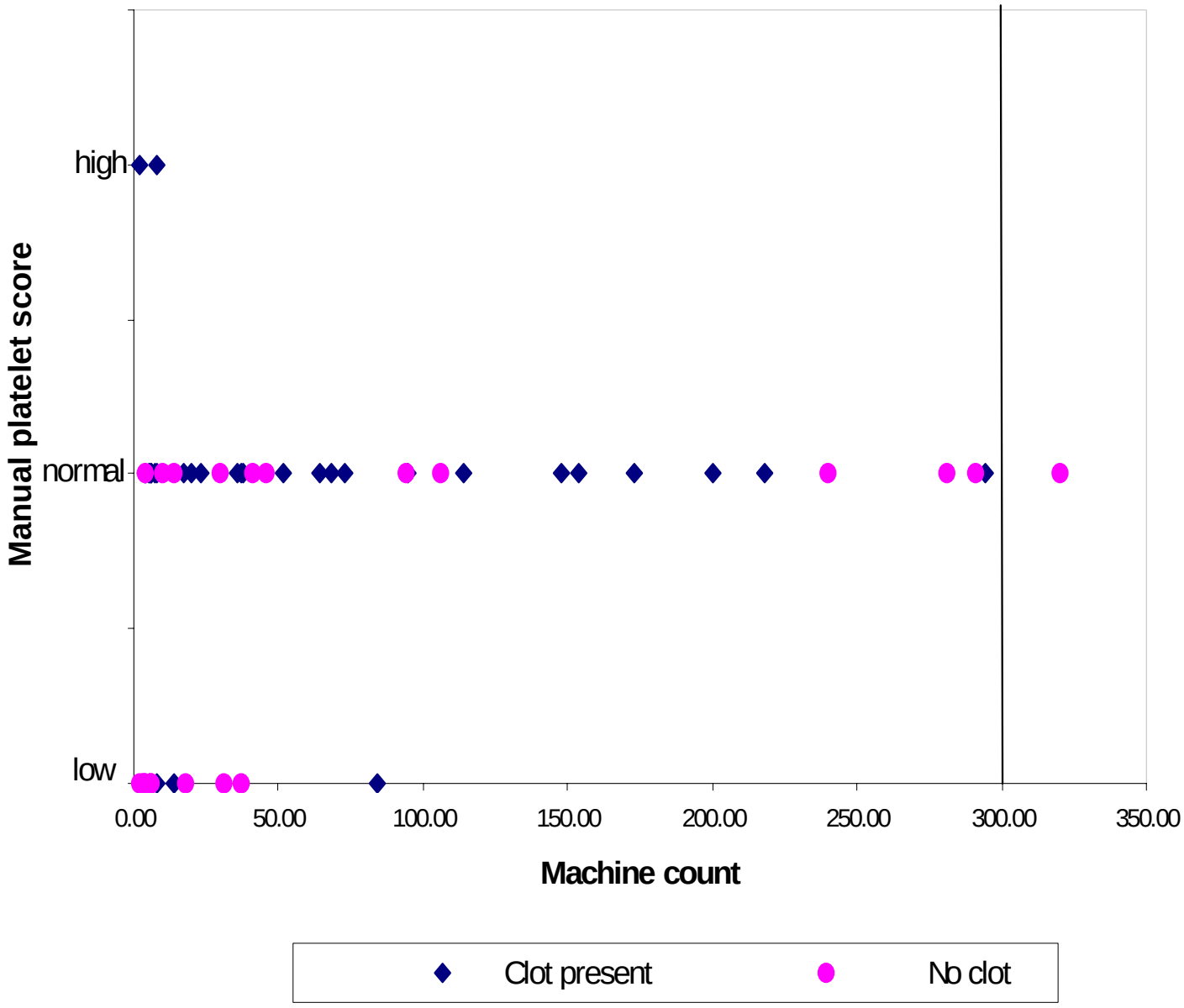


Figure 6a: Liver enzyme distribution in 56 cats with *B. felis* infection - ALT

The horizontal line indicates the upper limit of normal.

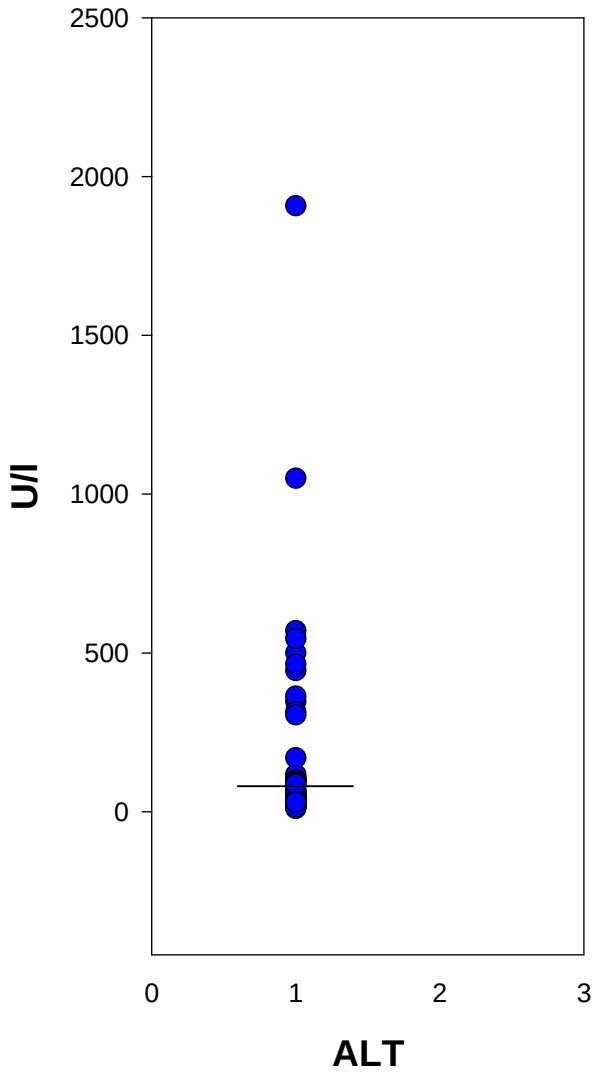


Figure 6b: Liver enzyme distribution in 56 cats with *B. felis* infection – ALP and GGT

The horizontal lines indicate the upper limit of normal.

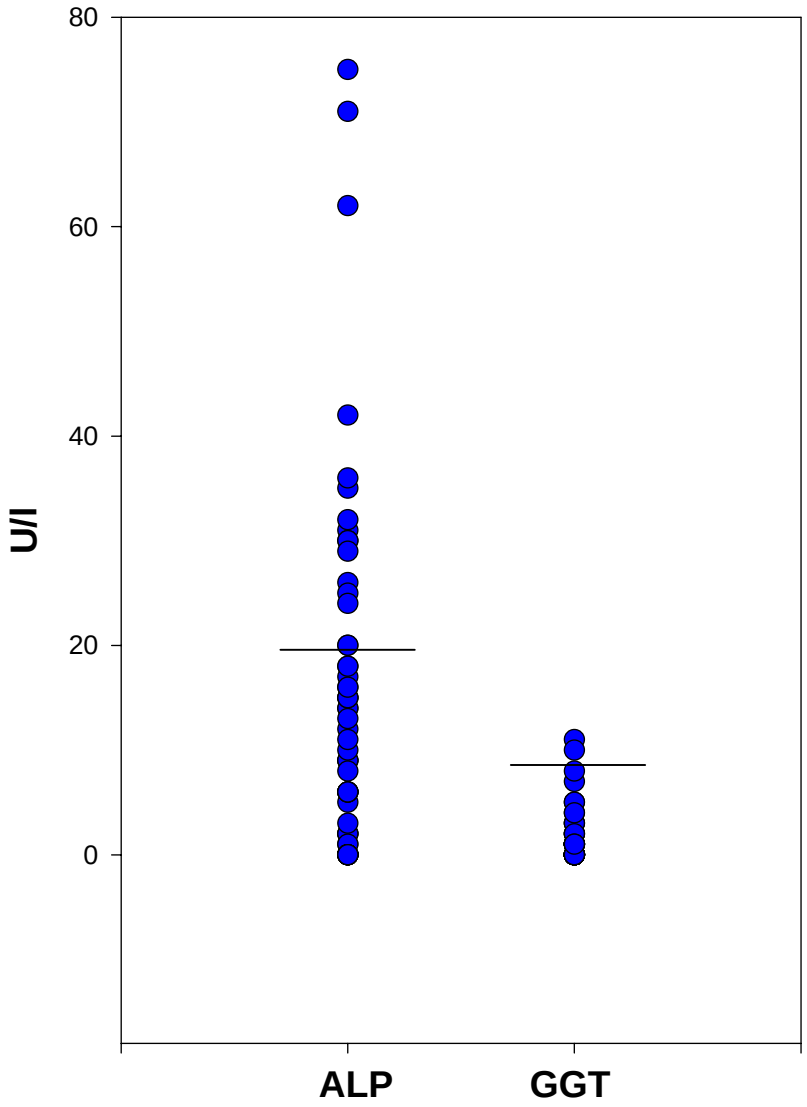


Figure 7: Correlation between ALT and haematocrit in 56 cats with *B. felis* infection

A statistically significant negative correlation is seen, indicating that ALT tends to increase while haematocrit decreases (r_s = –0.497; P<0.05).

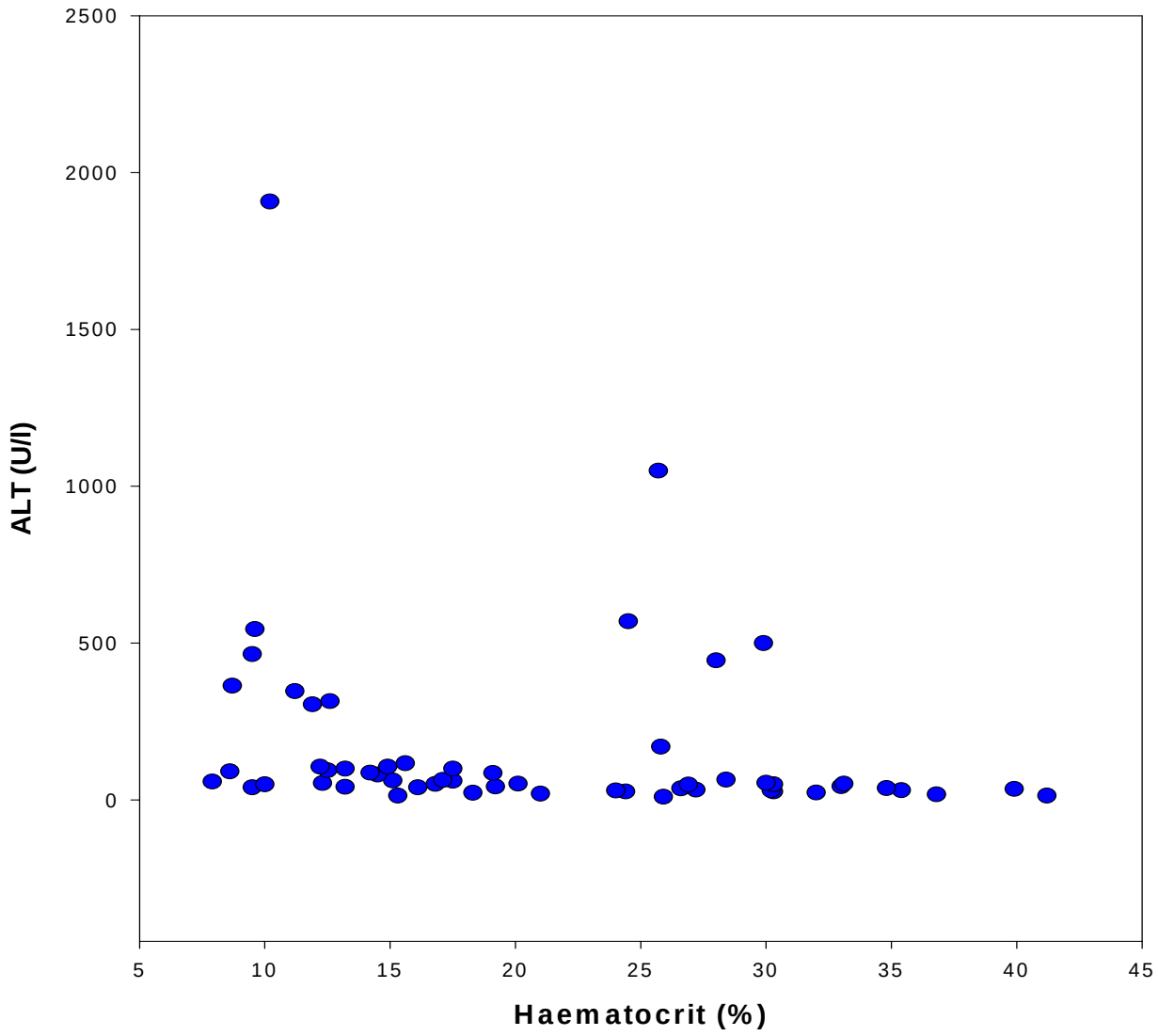


Figure 8: Clinical icterus and ALT in 56 cats with *B. felis* infection

The ends of the boxes indicate the 25th and 75th percentiles, with a line at the median. T-bars indicate the 10th and 90th percentiles. Circles represent outliers. There is a statistically significant difference between the two groups (*P*<0.001).

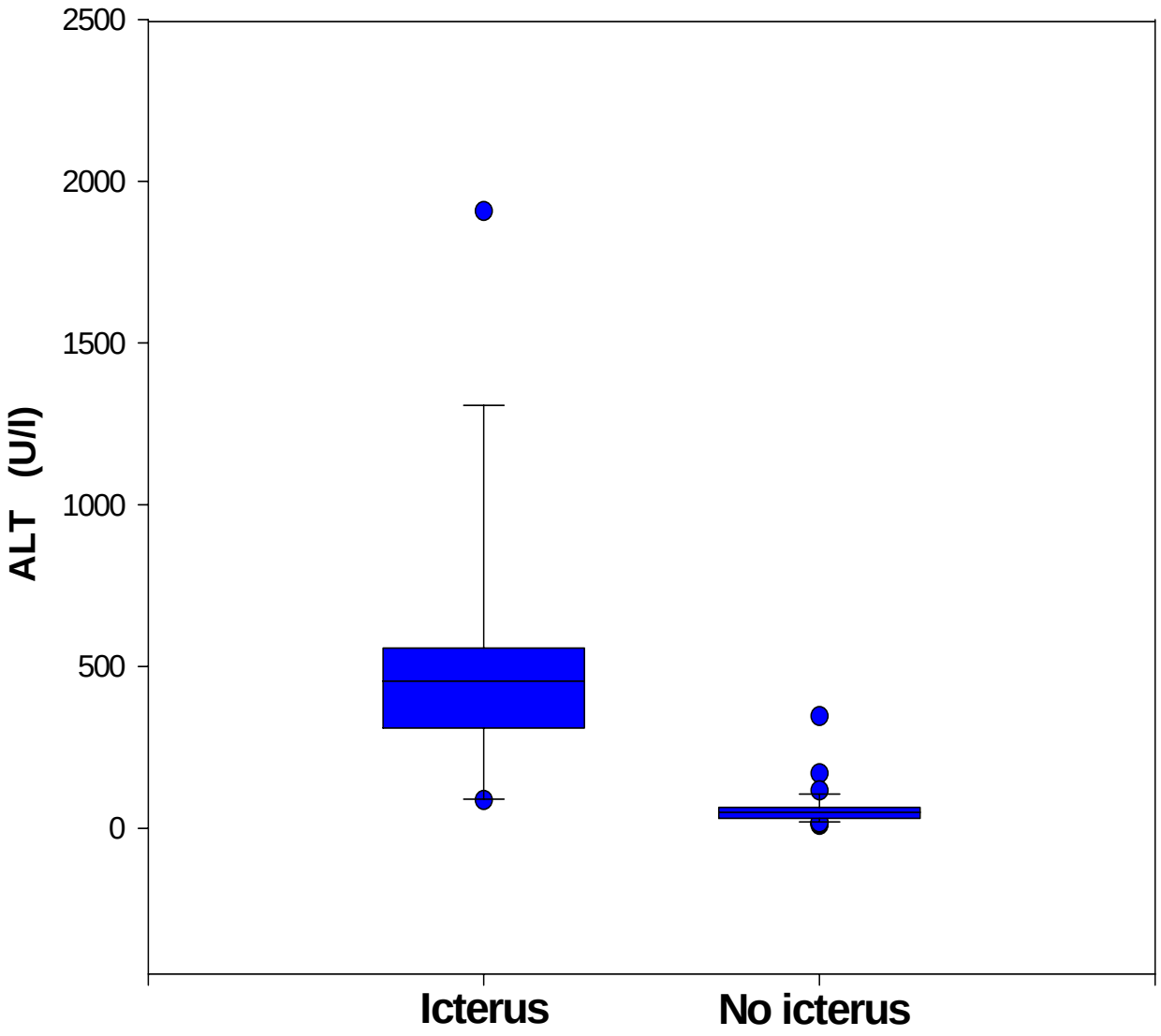


Figure 9: Clinical icterus and ALP in 56 cats with *B. felis* infection

The ends of the boxes indicate the 25th and 75th percentiles, with a line at the median. T-bars indicate the 10th and 90th percentiles. Circles represent outliers. There is no statistically significant difference between the two groups (*P*=0.285).

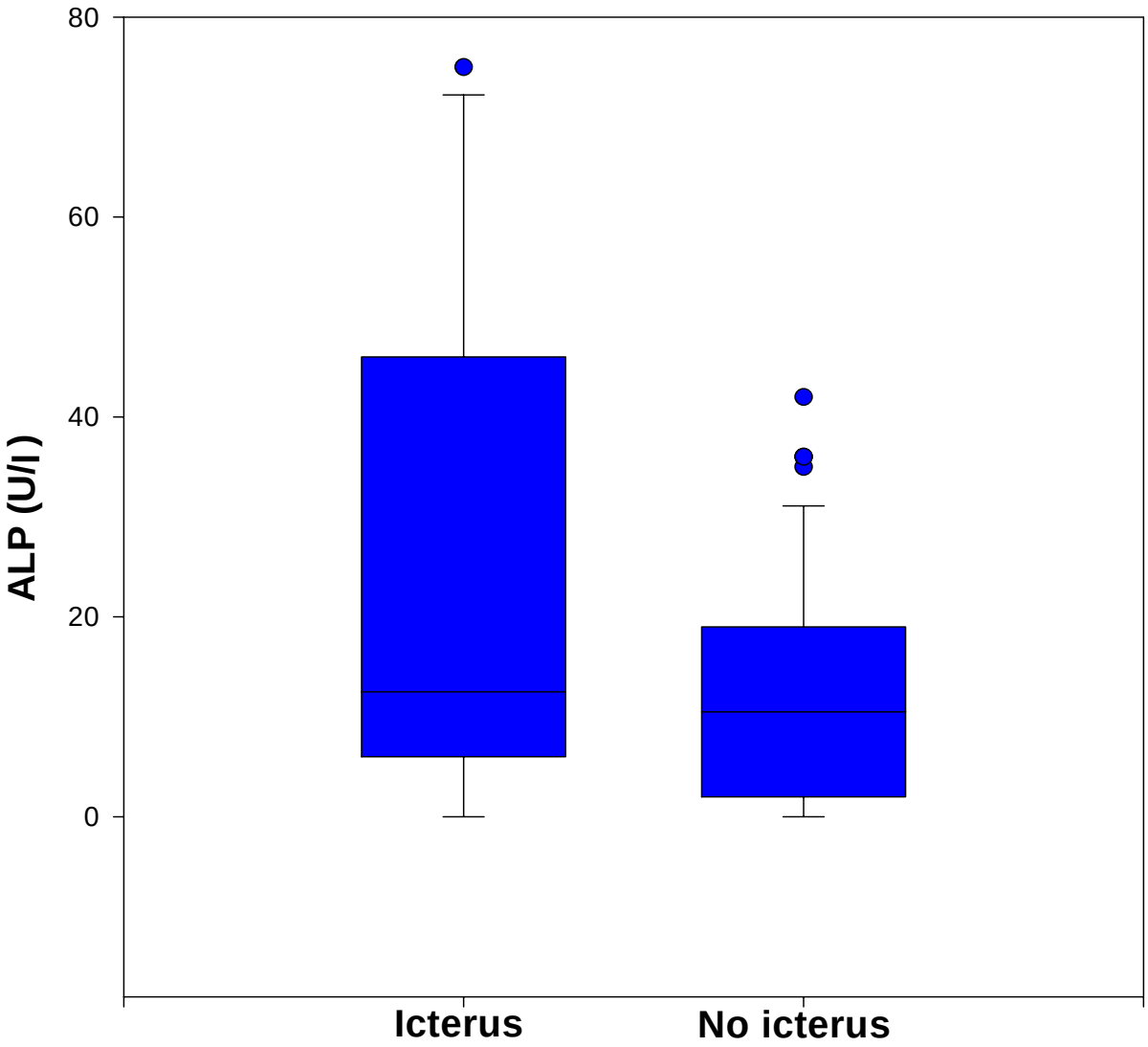


Figure 10: Correlation between ALT and total bilirubin in 56 cats with *B. felis* infection

A statistically significant positive correlation is seen, indicating that ALT and total bilirubin tend to increase together (r_s =0.708; P<0.05).

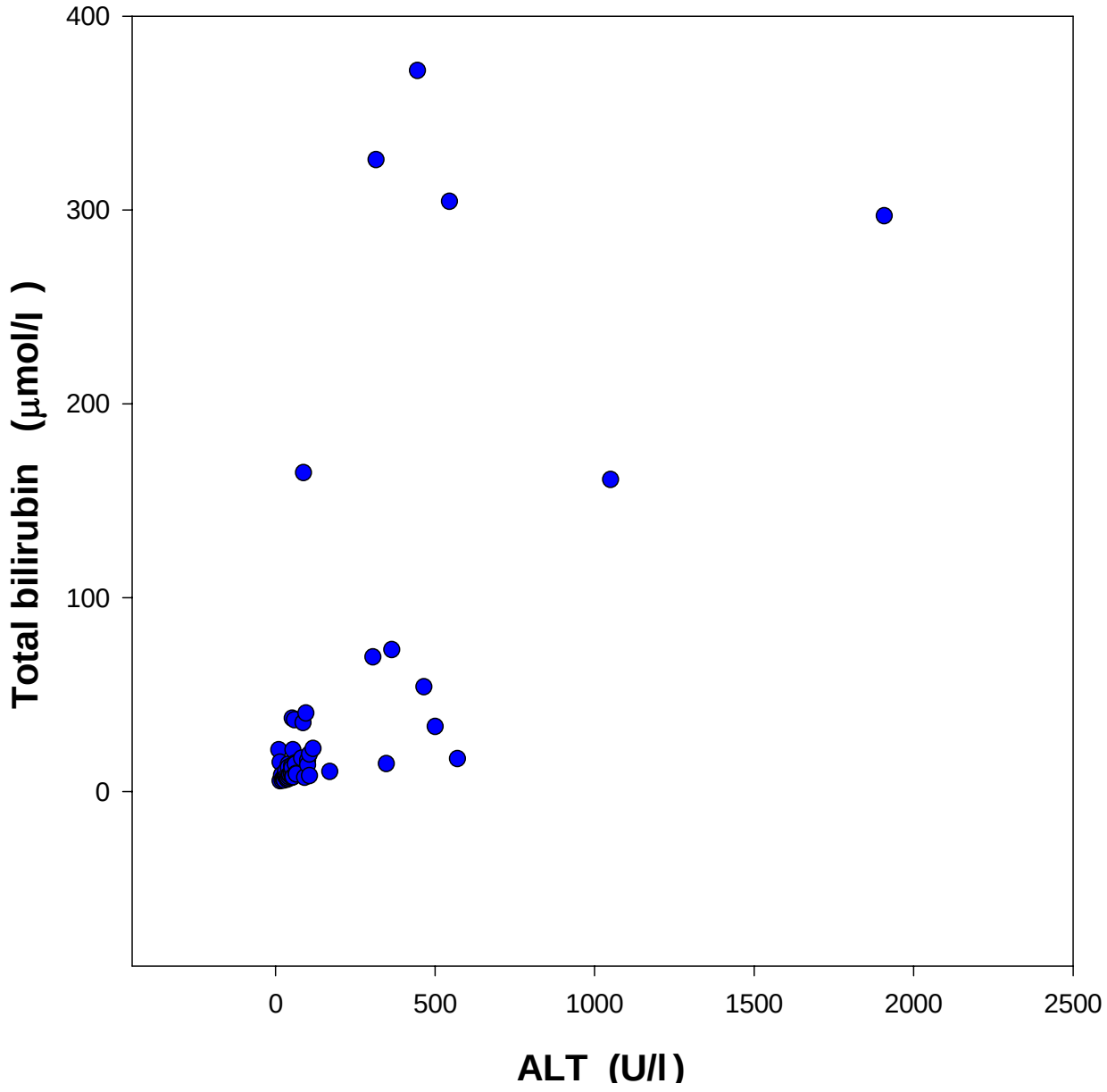


Figure 11: Correlation between total bilirubin and haematocrit in 56 cats with *B. felis* infection

A statistically significant negative correlation is seen, indicating that total bilirubin tends to increase while haematocrit decreases (r_s = –0.553; P<0.05).

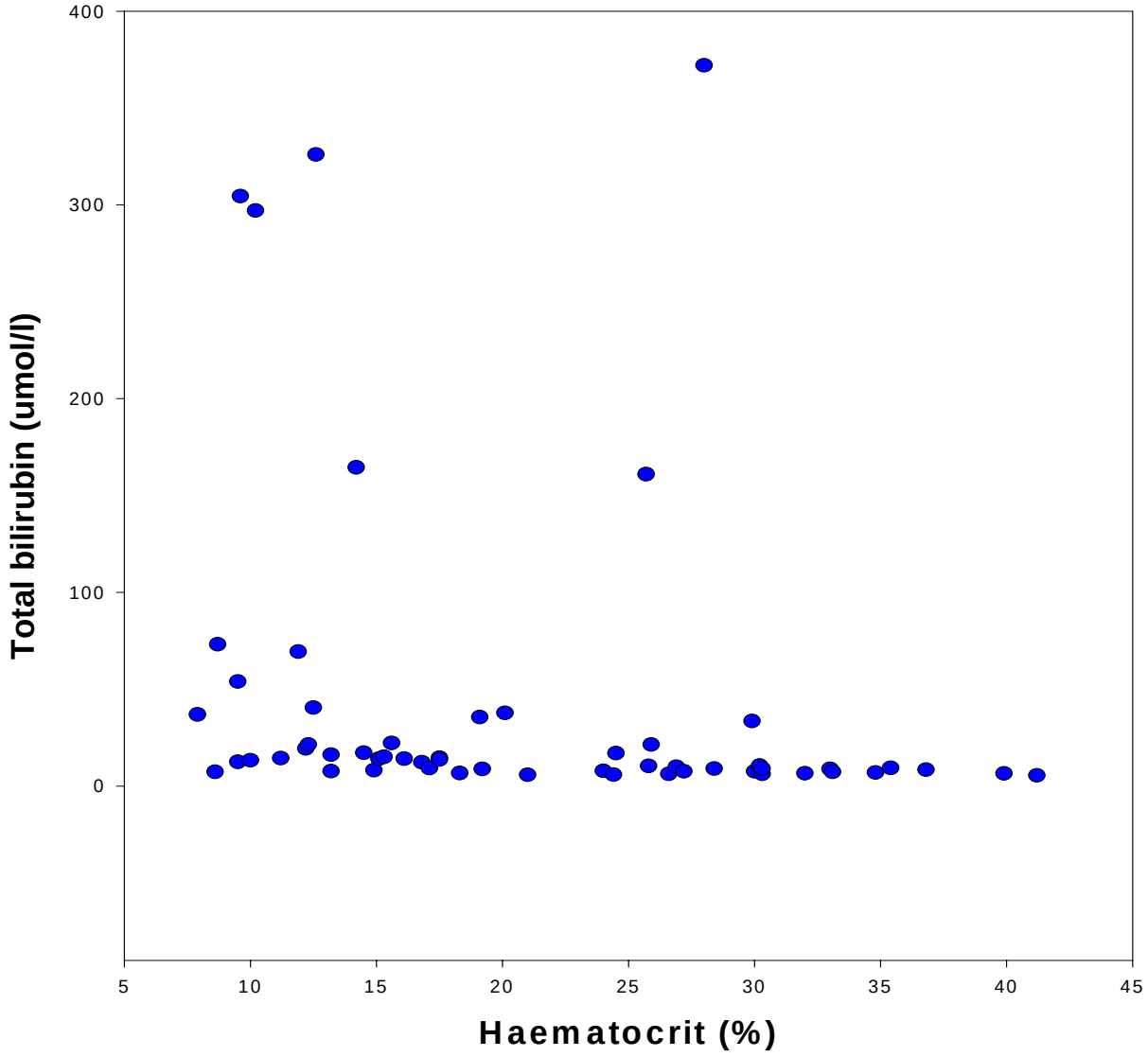


Figure 12: Correlation of urea and creatinine in 56 cats with *B. felis* infection

No significant relationship is seen between these values (*P*=0.117).

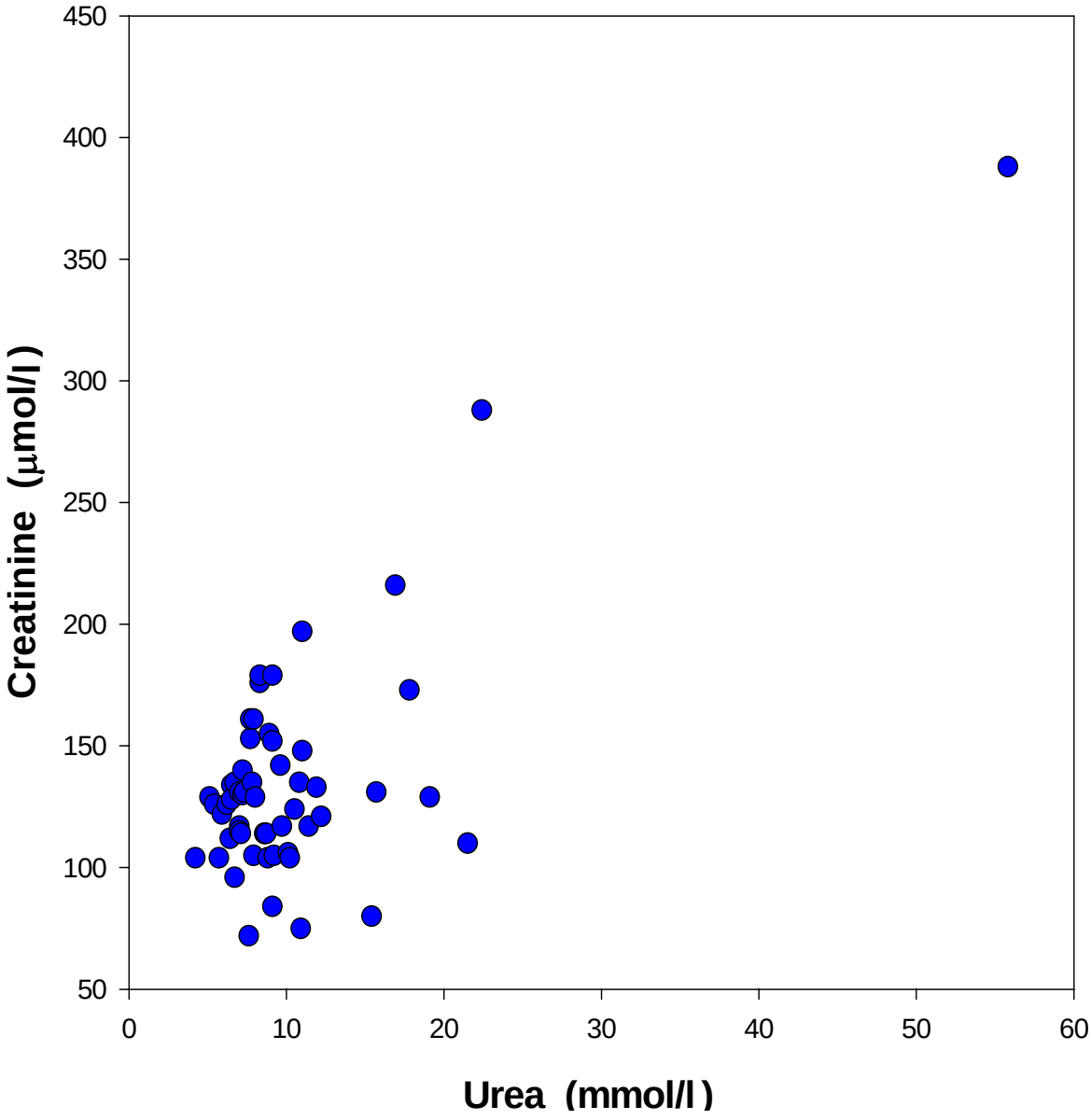


Figure 13: Correlation of central and peripheral parasitaemias in 56 cats with *B. felis* infection

A statistically significant strong positive correlation is seen, indicating that central and peripheral parasitaemias tend to increase together (r_s = 0.997; P< 0.05).

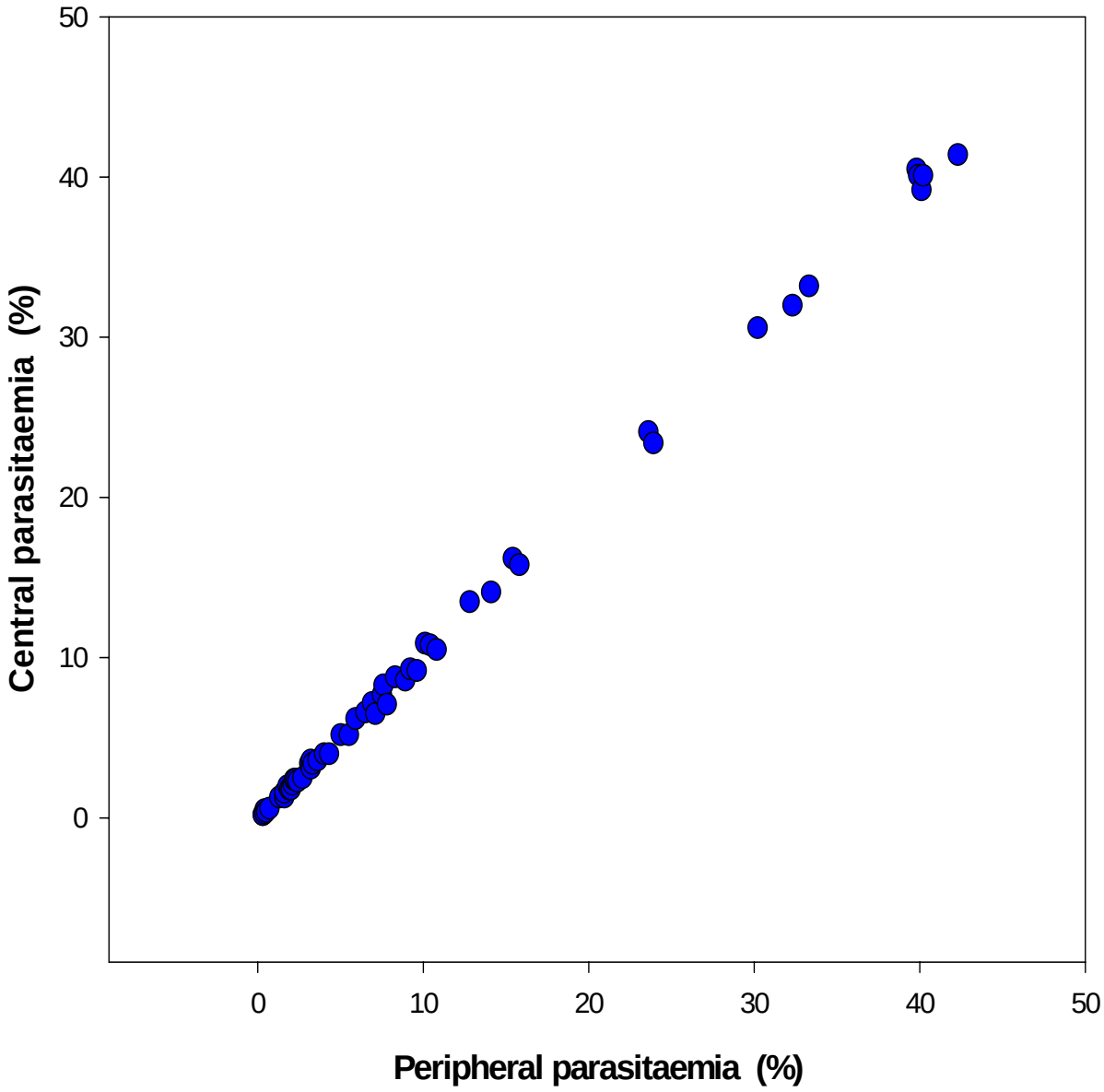


Figure 14: Correlation of haematocrit and central parasitaemia in 56 cats with *B. felis* infection

A negative correlation coefficient of –0.595 and a significant $P<0.05$ is seen, indicating that the haematocrit tends to decrease while the central parasitaemia increases.

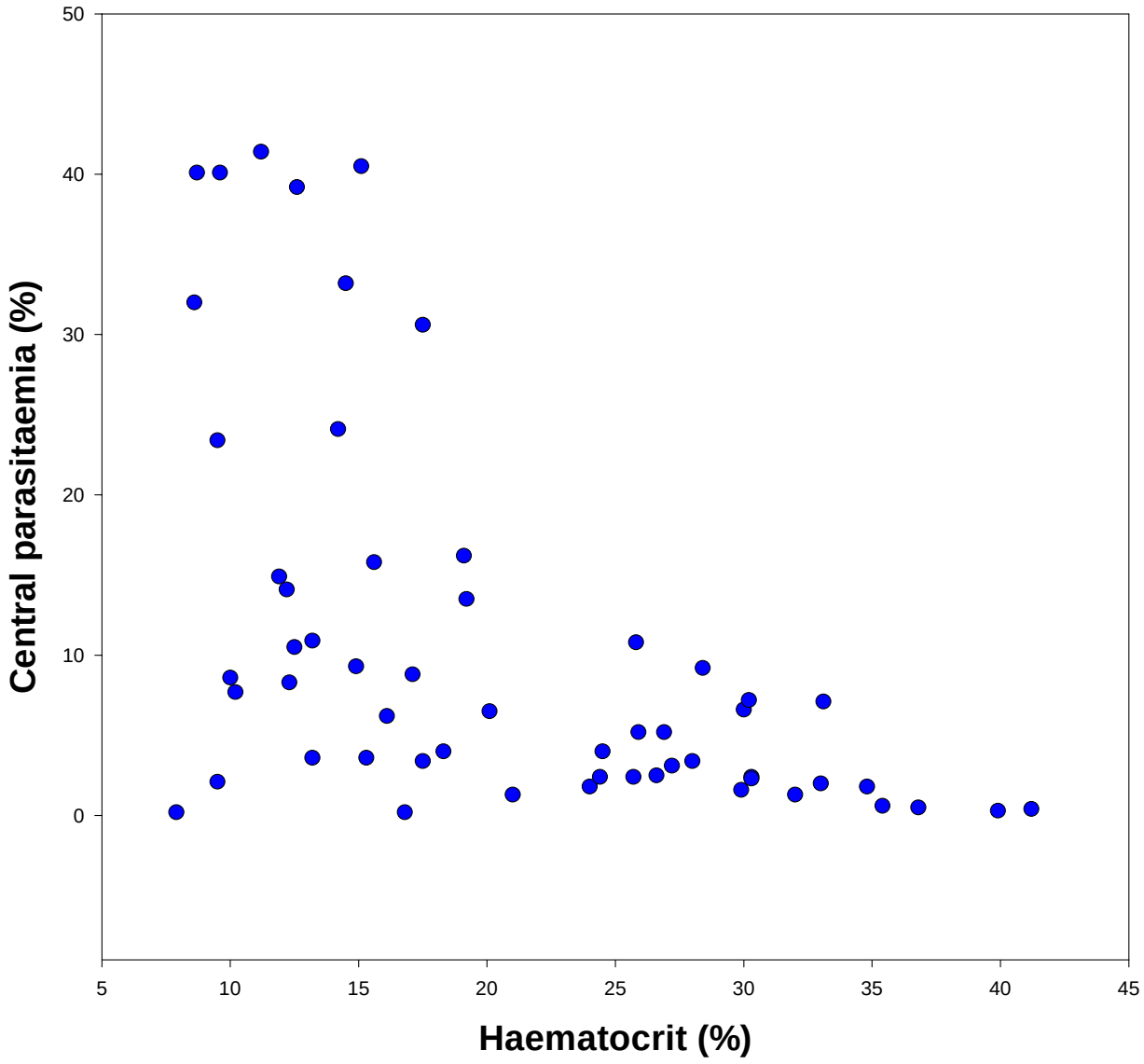


Figure 15: Effect of in-saline agglutination on haematocrit in 56 cats with *B. felis* infection

The ends of the boxes indicate the 25th and 75th percentiles, with a line at the median. T-bars indicate the 10th and 90th percentiles. Circles represent outliers. There is a statistically significant difference between the two groups (*P*=0.008).

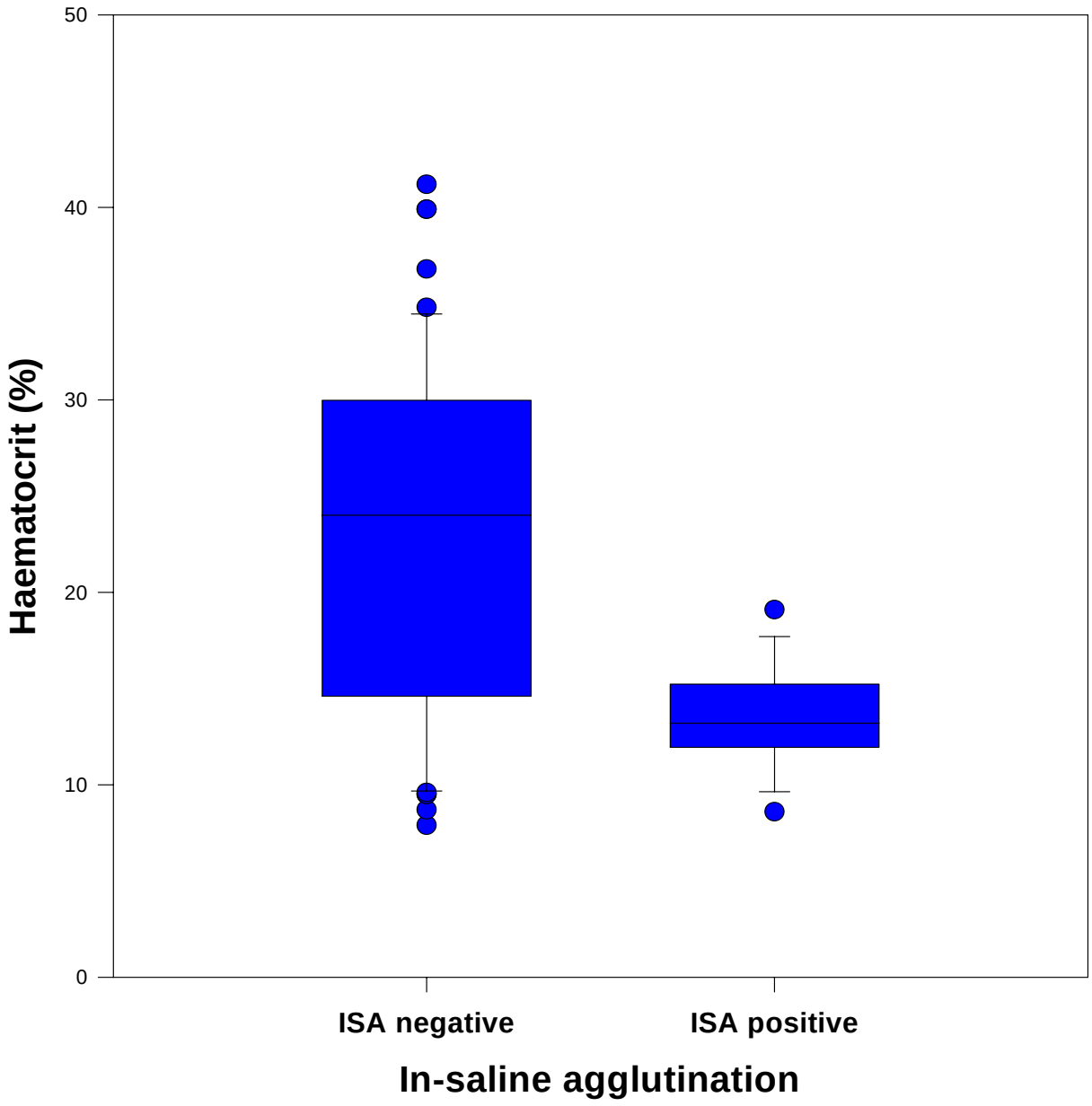


Figure 16: Effect of *H.felis* co-infection on haematocrit in 56 cats with *B. felis* infection

The ends of the boxes indicate the 25th and 75th percentiles, with a line at the median. T-bars indicate the 10th and 90th percentiles. Circles represent outliers. There is no statistically significant difference between the two groups (*P*=0.238).

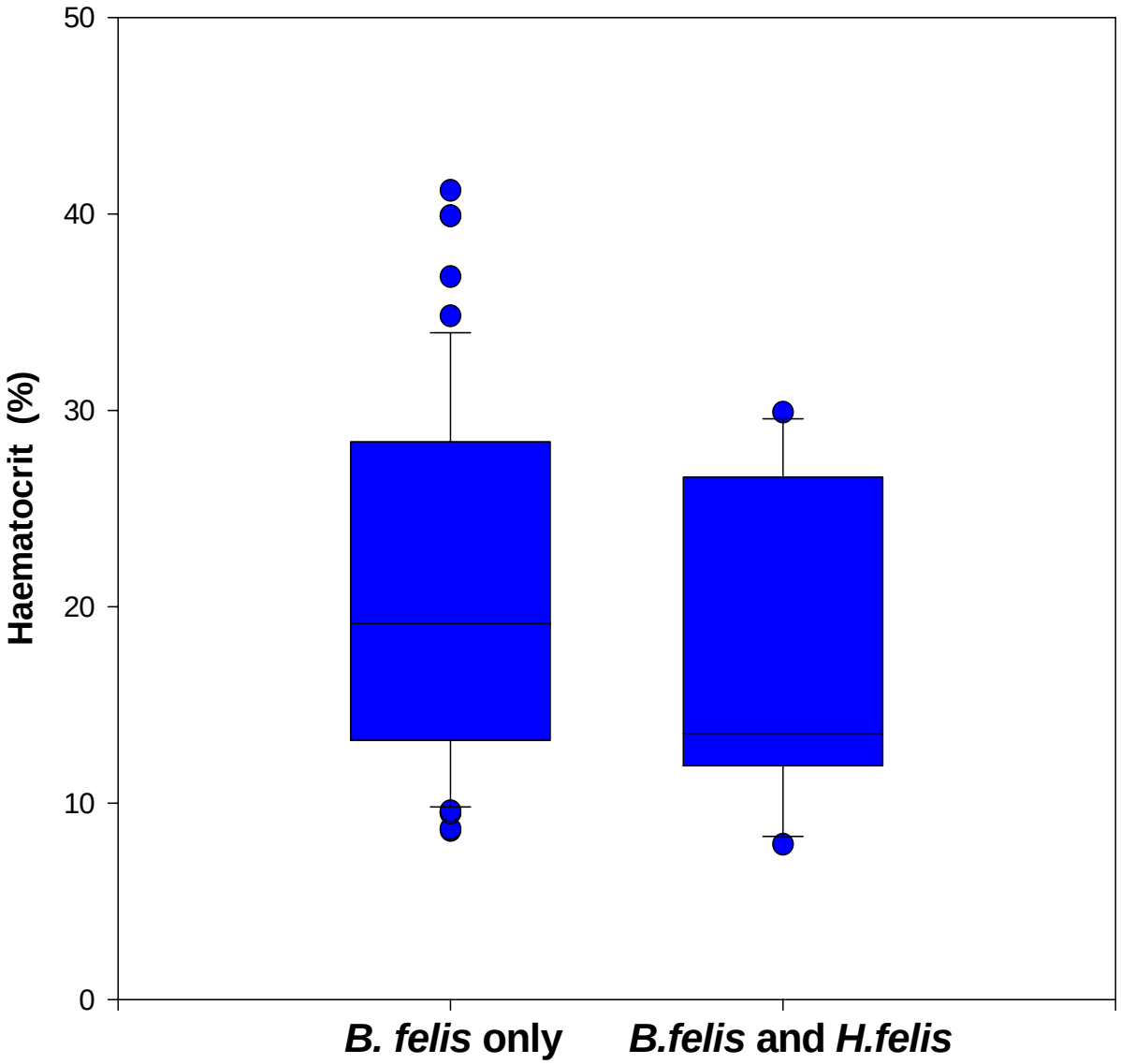
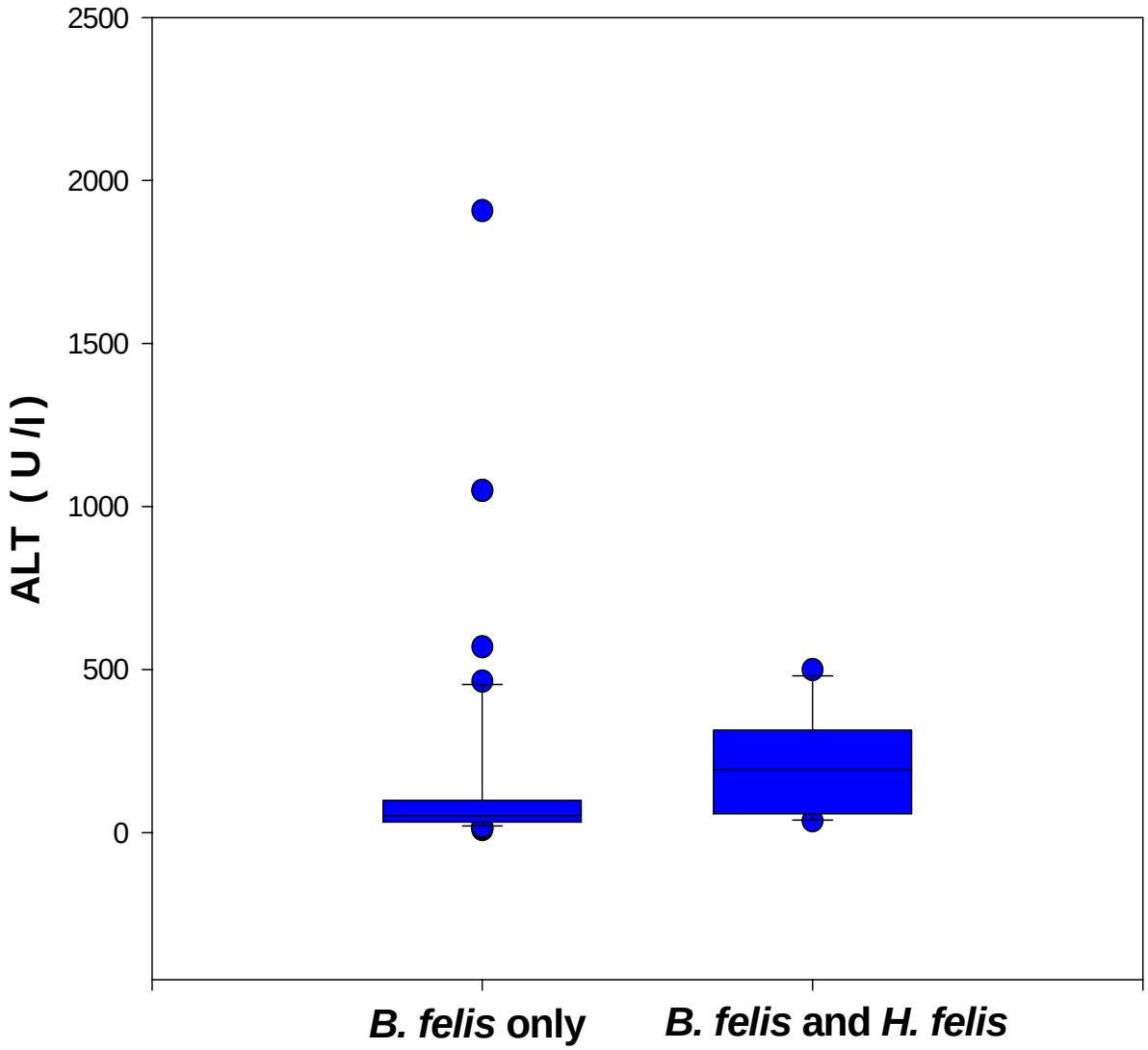


Figure 17: Effect of *H. felis* co-infection on ALT in 56 cats with *B. felis* infection

The ends of the boxes indicate the 25th and 75th percentiles, with a line at the median. T-bars indicate the 10th and 90th percentiles. Circles represent outliers. There is no statistically significant difference between the two groups (*P*=0.156).



CHAPTER 7

7. DISCUSSION

Babesia felis can potentially affect cats of any age[18], but in this study 80% of the affected cats were three years of age or younger. This is similar to what has been described previously for both feline babesiosis[18] and canine babesiosis[80] in South Africa. It is thought that dogs that recover from canine babesiosis may remain subclinical carriers of the parasite and enter a state of premunity where on reinfection, no clinical signs of babesiosis are generally seen[52,57,68,76]. The current recommended treatment for feline babesiosis, primaquine phosphate[1], is known not to sterilise the infection after treatment[61,65], which is crucial in the development of a premune state[60]. It is therefore possible that cats can also enter a state of premunity and become subclinical carriers of the parasite after initial infection. Cats in endemic areas would presumably contract the infection at initial exposure to the parasite early in life and then enter a state of premunity with no clinical signs of infection later on in life, which could explain the reduced prevalence of infection in cats over three years of age in this study. It can be speculated that relapses or infections of 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 dogs[45]. In this study, the majority (9/11) of affected cats older than three years had a concurrent illness or infection that could have influenced the cat's immune system. Of the eleven cats that were older than three years, two cats had concurrent FIV infections, two cats had concurrent FeLV infections, two cats had concurrent infections with both FeLV and FIV, one cat had concurrent infection with *Haemobartonella felis* and two cats had renal disease. One cat was a healthy asymptomatic carrier and therefore presumably in a state of premunity.

[1] Primaquine, Kyron, Johannesburg, South Africa

As the age distribution of the normal cat population in the study area was not known, it is also possible that the samples could have been taken from a predominantly young cat population, therefore not representing a specific age predisposition for the disease. In this study, only one kitten less than six months old was affected. This, again, could be a reflection of the normal age distribution of cats in the study area, but the protective role of passively acquired maternal antibodies should also be considered.

A possible breed predisposition for babesiosis has been described in dogs[52], but there is limited evidence for this. In this study, 77% of cats did not represent a specific cat breed, which is indicative of no breed predisposition in cats. Of the remaining study cat population, however, Siamese cats represented 12,5%. The significance of this proportion is difficult to determine, as the proportion of Siamese cats in the overall cat population in the study area was not known.

No sex predisposition for babesiosis is known to occur in the dog[52]. In this study no obvious sex predisposition for feline babesiosis was evident either. Infected cats were 57% females and 43% males – the significance of which should be determined by the sex distribution of cats in the overall normal cat population in the study area. Around 69% of the affected female and 67% of the affected male cats were neutered. These figures were probably representative of the average cat population and did not necessarily reflect a predisposition for disease in neutered animals.

Body condition of affected cats varied, but most cats were considered to be in normal body condition. Among the rest of the cats an equal distribution was seen between fat and thin cats. The habitus of affected cats also varied between alertness, moderate depression and severe depression at the time of presentation. The clinical appearance of affected cases seemed to vary according to the severity of the disease and the presence or absence of concurrent diseases or infections.

The most common owner complaints relating to the babesiosis were a combination of anorexia, listlessness, depression and anaemia in the majority of cases, which is in agreement with previous findings[18,32]. Other, less common, complaints included icterus, weight loss, dehydration, constipation and pica. Constipation has been described as a clinical sign of the disease[53], but it is more likely to be a secondary sign resulting from patient dehydration. Pica has also been described as an uncommon clinical sign of feline babesiosis[32]. It could be speculated that anorexia, as well as anaemia and hepatic involvement in feline babesiosis could lead to certain nutritional deficiencies that could, in turn, lead to pica.

Among the group of sick cats, four of them presented with owner complaints attributable to diseases other than babesiosis, but *B. felis* parasites were identified on examination of peripheral blood smears. One of these cats had moderate anaemia with a haematocrit of 17.5%, while the other three cats had haematocrit values towards the lower side of the normal range. In these cases the babesiosis could have been clinically significant, but it is also possible that these cats were, in fact, subclinical carriers of the disease.

Seven of the cats in this study had a history of previous illness with babesiosis and were on chronic extended primaquine phosphate therapy, at variable doses. These cats were not clinically ill at the time of presentation, but a check-up blood smear revealed the persistence of parasitaemia and chronic therapy was extended. The private veterinarians in charge were under the impression that these cats would relapse if chronic therapy were to be stopped. Only one of these cats had moderate anaemia with a haematocrit of 18.3%, while the other six cats had normal haematocrit values. It could be speculated that these cats were, in fact, chronically infected with the disease, but as primaquine phosphate is known not to sterilise the infection[61,65], residual parasites might be an expected finding on the blood smear after treatment. These cats could therefore also represent subclinical carriers of the parasite that had entered a state of premunity

after initial infection. Theoretically these cats could also represent cases of re-infection with the parasite, but the fact that none of these cats was clinically ill at presentation, as well as the fact that most of them had normal haematocrit values, would make that less likely. A comparison between this group of cats and the rest has been added as an addendum to the end of this dissertation.

Two of the cats were described as normal, healthy cats and had no signs of clinical disease, but *B. felis* parasites were seen on examination of blood smears. Both of these cats had normal haematocrit values and no remarkable clinico-pathological abnormalities were seen. These cats were likely to represent subclinical carriers in a premune state with protective immunity against the disease. The true prevalence of asymptomatic carriers is likely to be different to what is reflected here, as the sample population consisted mainly of sick cats that were examined by private veterinarians and did not reflect the average normal cat population in the sample area.

Diagnosis of the disease was based on identification of *B. felis* parasites within infected erythrocytes of both peripheral and central venous blood smears from all the cats. Staining with a Romanowsky-type stain (Cam'sQuick) proved to be satisfactory for identification of parasites. The most common morphologic parasite forms seen were round or signet ring forms. Both forms showed a marked variation in shape, number and distribution of chromatin. Maltese cross forms were less frequently seen than round forms, but became more prominent in cases where parasite counts were high.

A strong correlation between central and peripheral parasite counts was seen. This finding would imply that *B. felis* parasites do not cause sequestration of parasitised red blood cells in capillary beds, which is very different to what has been described in the dog[75,76].

Very high parasitaemias have been reported in domestic cats from South Africa and parasite counts of 50% and more have been previously described[18,30,69]. This was also seen in this study and the highest parasitaemia observed was 42.3%. This finding of a very high parasitaemia in babesiosis of domestic cats is in contrast to what has been reported for babesiosis in wild cats in Africa[13]. This raises the question again whether the *B. felis* parasite of domestic cats is, in fact, the same parasite that has been described in wild cats.

The high parasitaemias in domestic cats probably result from a combination of a 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 endotoxin[58]. Species that are refractory to endotoxin are also less susceptible to babesiosis and will only show symptoms of disease at a higher parasitaemia[10]. The high parasitaemias seen in cats with babesiosis differ substantially from those seen in the dog. In a study of 30 dogs with babesiosis, the average observed parasitaemia was 0.44%, with a standard deviation of 0.53% (L Jacobson et al., Department of Medicine, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, unpublished data). The development of illness at low parasitaemias in dogs may be related to the dog's high susceptibility to endotoxin[10].

A weak negative correlation was seen between the central parasite count and the magnitude of anaemia in this study, which indicates that the haematocrit drops as the parasitaemia increases. This effect seemed to be more pronounced when parasitaemias were higher than 20%, as corresponding haematocrits of 15% or less were commonly found in those cases.

The most significant haematological abnormality that occurred in a large number of cats in this study, was a macrocytic, hypochromic anaemia. The macrocytosis could be ascribed to reticulocytosis, as feline reticulocytes are large cells[16]. Absolute increases in reticulocytes indicate an actively responding bone marrow

and that the cause of the anaemia is likely to be extramarrow[16]; in this case implicating both intravascular and extravascular haemolysis (phagocytosis). Other possible explanations for the macrocytosis in a few of these cats could have been concurrent FeLV infection or concurrent agglutination of red blood cells[16], as macrocytic red blood cells were seen in all the cats that had these above-mentioned, concurrent disorders. Hypochromia, which was evidenced by low mean corpuscular haemoglobin concentrations, would also be expected with reticulocytosis, as normal reticulocytes do not have their full component of haemoglobin[16].

Macrocytic, hypochromic anaemia was thus suggestive of the presence of active red blood cell regeneration. Signs of bone marrow erythropoiesis were also evident on blood smear examinations and included the presence of reticulocytes, numerous nucleated red blood cells, marked anisocytosis, polychromasia, an increased amount of Howell-Jolly bodies (in excess of 1% of RBC) and basophilic stippling of some red blood cells. All these signs were indicative of a regenerative bone marrow response to anaemia and have also been reported previously for feline babesiosis[18,30,69].

The anaemia of feline babesiosis can thus be classified as a macrocytic, hypochromic regenerative anaemia. In dogs, the anaemia is initially normocytic, normochromic during the first few days after infection, but the anaemia also becomes macrocytic, hypochromic and regenerative as the disease progresses[76]. The anaemia of babesiosis can further be classified as a haemolytic anaemia that is caused by accelerated erythrocyte destruction[76]. This erythrocyte destruction probably occurs at both intravascular and extravascular sites in feline babesiosis.

Intravascular haemolysis occurs when erythrocytes are destroyed within the circulation, thereby releasing haemoglobin into the plasma where it is either removed by the liver or excreted by the kidneys[16]. With babesiosis, the primary

mechanism of intravascular red blood cell destruction is thought to involve disruption of the red blood cell membrane on exiting the red blood cell after intracellular multiplication[16,76], but induction of serum haemolytic factors and complement- and immunoglobulin-mediated destruction of both parasitised and unparasitised red blood cells are also important to the pathogenesis[31,75]. Clinical and laboratory characteristics of intravascular haemolytic anaemia include an acute onset of disease, regeneration, haemoglobinaemia (which can be detected by red discolouration of plasma and an increased MCHC), haemoglobinuria and hyperbilirubinaemia[16]. Intravascular haemolysis is thought to be the most prominent type of haemolysis found in canine babesiosis[75,76]. In this study, haemoglobinaemia was seen in some, but not all of the serum samples. Although urine samples were not collected for analysis and detection of haemoglobinuria, previous studies indicated that haemoglobinuria can occur in cats with babesiosis[20]. This provides evidence that intravascular haemolysis is also a feature of feline babesiosis.

Extravascular haemolysis occurs when erythrocytes are sequestered in the spleen or liver where they are phagocytosed or lysed[16]. Extravascular haemolysis has previously been described in canine babesiosis[75]. The primary mechanism of extravascular RBC destruction in babesiosis is thought to involve immune-mediated mechanisms or increased erythrophagocytic activity of macrophages[16,75]. Clinical and laboratory characteristics include a chronic onset of disease, regeneration of red blood cells associated with normal or increased plasma protein (TSP) concentrations, hyperbilirubinaemia and splenomegaly[16]. Most of these characteristics of extravascular haemolysis were evident in this study, as most cats had a slow onset of anaemia, marked regeneration was evident that was always associated with normal or increased TSP concentrations, and hyperbilirubinaemia was commonly seen. In addition to this, splenomegaly has been described as a possible clinical finding in cats with babesiosis[32]. Erythrophagocytosis of both infected and non-infected erythrocytes by mononuclear cells has also been reported previously for cats with

babesiosis[18]. It is therefore very likely that extravascular haemolysis is also a feature of feline babesiosis.

Nineteen cats (34%) in this study had severe anaemia with haematocrit values of 15% or less. All these cats were clinically either moderately or severely depressed at presentation, but seemed to have an ability to adapt to the severe anaemia. This ability has also been described in previous studies[18], as well as recognised in other forms of anaemia in cats[6,77]. Only one of the severely anaemic cats received a blood transfusion during therapy; the remainder were hospitalised and caged in a quiet area, with minimal handling. Most of these cats showed rapid improvement in clinical condition. One can postulate that the vast majority of dogs in a similar situation would have received blood transfusions as essential therapy for survival[41].

Normal haematocrit values were seen in twenty-four (43%) of the cats. It is possible that increased owner awareness of the disease in the area could have led to the fact that infections were noted earlier and cats were presented for treatment before the disease had progressed very far. This group also included those previously described cats that were likely to be subclinical carriers of the disease. As these cats were not suffering from clinical disease, normal haematocrit values were not at all surprising in these cases.

Auto-agglutination of red blood cells has not previously been described in feline babesiosis. The basis of autoantibody formation is not clear, but it is thought that immune-mediated haemolytic anaemia (IMHA) may result from a change in the antigenic structure of the body's own erythrocytes and from the formation of cross-reactive antibodies and/or from a change in the immune status of the patient[21]. Immune destruction of these erythrocytes is thus initiated by the binding of immunoglobulin G (IgG) or IgM antibodies and/or complement to the surface of the involved erythrocytes. In most cases this immune destruction is an extravascular process that depends on recognition of erythrocytes opsonised

with IgG and/or complement by specific receptors on reticuloendothelial cells with resultant erythrocyte destruction and haemolysis[21]. To definitively diagnose IMHA, one or more of the following three hallmarks must be present: (i) Marked spherocytosis, (ii) True autoagglutination, or (iii) Positive direct Coombs' test[21].

RBC autoagglutination occurred in 16% of the cats in this study group. The occurrence of irregular, small clumps of erythrocytes, often including polychromatic red cells, was considered indicative of the presence of an autoantibody. Agglutination of RBC was differentiated from rouleaux formation by its failure to disappear on dilution with saline[79]. Spherocytes were not assessed, as spherocytosis in cats is very difficult to identify because the normal feline erythrocyte is a small cell and naturally lacks an area of central pallor[21]. As the direct Coombs' test for cats is not available in South Africa, it was not performed.

In dogs, in-saline positive IMHA has been described as a possible severe complication of canine babesiosis[11,31,76]. Immune-mediated haemolytic anaemia in cats has rarely been documented, but it has been described secondary to *Haemobartonella felis* or FeLV infections and certain anti-thyroidal medications[8,26,64]. Of the nine cats in this study that were in-saline positive, none had concurrent infection with *H. felis* and none had received any anti-thyroidal medications. Six of these cats tested positive for FeLV infection, whereas none of the other three cats had evidence of any infections other than the babesiosis that could have been the cause of the IMHA. This autoagglutination of RBC seen thus provides evidence that IMHA can also occur secondary to babesiosis in the cat.

When haematocrit values for the group of in-saline positive cats were compared to those of the in-saline negative cats, a statistically significant difference was seen – the cats with concurrent IMHA had lower haematocrit values. The clinical importance or significance of this is, however, not known, as none of the in-saline positive cats was treated with immunosuppressive doses of corticosteroids, all of

them responded predictably to antibabesial therapy and none of them seemed to deteriorate without any immunosuppressive therapy.

Total white blood cell counts were done using an automated cell counter. When using these counters, various blood abnormalities may lead to counting errors. Normally all nucleated cells present, including nucleated RBC (nRBC), are counted by the automated counter. As most of these cats had high numbers of circulating nRBC, the total white blood cell counts thus had to be corrected and the resulting corrected white blood cell counts were remarkably different to the automated white blood cell counts in some cases. Additionally, normal feline platelets may be large and may also erroneously contribute to the automated white blood cell count[16].

A retrospective study in South Africa indicated that neutrophilia was a consistent feature of canine babesiosis[67] and a leukemoid response has even been described[40]. As only 11% of the cats in this study had elevated white blood cell counts, it is evident that these features are not consistent findings in feline babesiosis. In fact, the leukocyte abnormalities that occurred in cats in this study were more likely to be a result of concurrent problems or diseases than the babesiosis itself. This is similar to what has been reported previously[19].

In this study, absolute mature neutrophil counts were elevated in six cats. Mild mature neutrophilia without a left shift was seen in two cats. This could have resulted from physiological causes like fear or excitement at the time of blood collection, with mobilisation of marginated neutrophils and redistribution into the circulating neutrophil pool[16]. This physiologic response is most pronounced in cats, because the feline marginal neutrophil pool is approximately three times larger than the circulating neutrophil pool, which is different to what is seen in the dog[16]. Stress and endogenous corticosteroid release could also have caused the mature neutrophilia without an associated left shift. However, this response is observed less frequently in cats than in dogs[16]. Mature neutrophilia with an

associated left shift was seen in four cats, one of which had a marked neutrophilia of 39.3 x $10^9/\lambda$. This could have resulted from both inflammation and haemolysis[16].

Even though absolute mature neutropaenia occurred in 11 cats, these changes were mild and could have been the result of neutrophil margination[16]. An excess tissue demand for neutrophils during certain inflammatory conditions can also lead to neutropaenia when the rate of emigration into the tissues exceeds the rate of neutrophil release from the bone marrow[16]. This could have been the cause of the mature neutropaenia and associated left shift observed in one cat in this study, as this particular cat had a uterine infection and was aborting at the time of examination. Neutropaenia caused by ineffective granulopoiesis and reduced bone marrow production of neutrophils has also been described in cases with FeLV[16] and five of the neutropaenic cats in this study also had concurrent FeLV infections.

The absolute immature left shift neutrophilias which occurred in 17 cats in this study were generally regenerative and most likely of inflammatory origin, as inflammation causes a sudden demand for neutrophils which leads to depletion of the storage pool of mature neutrophils, with subsequent release of immature neutrophils into the blood stream[16]. Infectious agents could have caused this inflammation, but certain products of tissue injury could also have stimulated the release of inflammatory mediators. This could explain the immature neutrophilias in five of the cats where clinical icterus was seen and hepatocellular injury was evident. Left shifts can also occur in non-inflammatory diseases like IMHA[16] and four of the cats with left shifts in this study had concurrent IMHA. The metamyelocytes observed on examination of blood smears in certain cases were also indicative of a left shift neutrophilia[16].

Degenerative left shift neutrophilias were seen in only two cats; one had a uterine infection and was aborting and the other was positive for both FeLV and FIV infections and had concurrent IMHA.

Monocytosis occurred mostly in conjunction with neutrophilia and probably resulted from the same mechanisms[16]. Mild eosinophilia was present in one cat and was probably due to an external parasite load, as fleas were seen during clinical examination. No basophil abnormalities were recorded.

The lymphocytosis seen in eight cats could be ascribed to antigenic stimulation, but physiological causes such as fear and excitement should also be considered, as this commonly leads to lymphocytosis in the cat[16]. The lymphopaenia seen in 16 cats could have been the result of endogenous corticosteroid release during times of stress, but it could also have been due to acute systemic infections and, specifically, viral infections[16]. Nine of these 16 cats were, in fact, infected with concurrent FeLV and/or FIV.

Certain problems can occur when counting cat platelets by electronic counting methods. Blood samples taken from cats have a tendency to form platelet clumps easily and this will potentially generate grossly inaccurate (low) counts when counted electronically[16]. In this study, automatic platelet counts revealed thrombocytopaenia in 98% of cats. Once the automatic platelet counts were verified by examination of a stained blood smear, platelet scores revealed evidence of thrombocytopaenia in only 25% of the cats, while normal thrombocyte counts were seen in almost 71% and thrombocytosis was also seen occasionally. Platelet clots were noted on blood smears in the majority of cases. This study therefore also showed that the results of automatic platelet counts in cats can be unreliable and should be treated with caution. An additional manual platelet score should always be done.

Thrombocytopaenia is a consistent feature of canine babesiosis[28,76]. The mechanisms behind this thrombocytopaenia are thought to involve bone marrow suppression, sequestration of platelets in the liver or spleen, consumption of platelets, immune or non-immune destruction of platelets or a combination of these[5]. In contrast, it is clear from the above results that thrombocytopaenia is not a consistent finding in feline babesiosis.

Serum protein changes in dogs with babesiosis have not been well described and results have not been consistent. A recent study[42] has shown that the total serum proteins, albumin, albumin:globulin ratio and α globulin levels were significantly low in dogs with mild and severe babesiosis, whereas dogs with complicated babesiosis showed no typical serum protein changes. No statistical differences were seen between groups for total, β and γ globulin fractions. From the above, it is evident that marked differences exist between canine and feline babesiosis with regard to typical protein changes.

Changes in total serum proteins in this study resulted from cumulative changes in albumin and globulin concentrations. Hyperalbuminaemia can sometimes be seen with patient dehydration and is usually associated with concurrent hyperglobulinaemia and a normal albumin:globulin ratio[16]. This was seen in approximately 16% of these cases. Apart from one cat, hypoalbuminaemia was not seen in association with feline babesiosis, which is in contrast to what has been reported for canine babesiosis[42].

Hyperglobulinaemia was evident in 23% of cases and a polyclonal gammopathy was identified in all of these. Elevated α globulin levels are associated with an increase in acute-phase proteins. These acute-phase proteins are synthesised by the liver as part of the acute-phase response that occurs 2 to 5 days after acute tissue injury, infection or immunological disorders and usually leads to mild increases in protein levels[16]. An increase in α globulins was seen in 18% of cases. This could have been an indication of an acute phase response to the

babesiosis, but the effect of concurrent diseases on α globulin concentrations must also be considered. Feline haemobartonellosis has been associated with increases in α3, β2 and occasionally γ globulins and FeLV infection has been associated with increases in α3 and occasional increases in β and γ globulins[78]. The cases with elevated α globulins in this study did, however, not correspond to the cases that tested positive for co-infection with *H. felis* or FeLV.

Elevated β globulin levels were seen in conjunction with elevated γ globulin levels in 9% of the cats. This is usually an indication of increased production of immunoglobulins (chronic phase proteins)[16]. This increase in chronic phase proteins or immunoglobulins usually primarily causes elevation of γ globulins, but it can also extend into the β globulin region (especially during periods when an IgM response is prominent)[16], as was seen in this study. These immunoglobulins are secreted by B-lymphocytes and plasma cells in many tissues, particularly the lymphoid organs, and can lead to a marked increase in total globulin concentrations[16]. A primary elevation of γ globulins was seen in 29% of cases and could probably also be ascribed to the antibody response of the reticulo-endothelial system of the patient to *Babesia* antigens. This increase in γ globulin concentrations corresponds to what was reported in a previous study[20].

Hypogammaglobulinaemia was seen in two cases. This could be indicative of an immunodeficient state caused by FeLV infection in these cats, as both of them were positive for FeLV and concurrent lymphopaenia was also present[59].

Serum ALT is a soluble cytosolic enzyme that is specific to the liver in the cat[16]. Increases in serum ALT activity occur with alterations in hepatocyte cell membrane permeability, which leads to leakage of cytosolic ALT into the extracellular fluid. This leakage occurs with sublethal hepatocellular injury, inflammation and hepatic necrosis[16]. The magnitude of increase in serum ALT activity correlates with the number of cells or the amount of hepatic mass affected, but does not differentiate between reversible and irreversible hepatic

injury and does not correlate with the clinical manifestation of hepatic insufficiency[16]. Serum ALT enzyme activity rises within 12 hours and peaks in 1 to 2 days after a liver insult and gradually returns to normal over 2 to 3 weeks[16]. As marked increases in serum ALT levels were seen in the vast majority of cats in this study, feline babesiosis appears to be associated with significant hepatocellular involvement or damage. Repeat testing would have been necessary to determine whether this hepatic injury was reversible and whether ALT activity returned to normal over time. In a previous study, ALT levels did return to normal during the recovery stage in most cases of feline babesiosis, but a few cases showed an actual increase in ALT activity over time[20]. In dogs with babesiosis, liver enzyme activities may also be increased during severe disease[47,48]. In a recent retrospective study of canine babesiosis, raised serum ALT activity was seen in 33% of cases and raised ALP activity was seen in 22% of cases[54].

The hepatocellular damage most likely results from anaemia, but anaemia is probably not the only factor that plays a role. Anaemia can lead to hepatocellular hypoxia, which in turn causes progressive hepatic centrilobular necrosis and hepatocellular cytosol leakage with increased enzyme activity[16]. Centrilobular hepatic necrosis has been described as a consistent pathological finding in both feline[20] and canine babesiosis[22]. Even though a statistically significant negative correlation between ALT activity and haematocrit was found in this study, there was no consistent relationship between the two. The negative correlation seemed to be more pronounced when cats were moderately to severely anaemic (Ht 20% or less). There were, however, exceptions to this as a few cases with severely elevated ALT activity did not have severe anaemia – one cat, for example, had an ALT level of 1 050 U/λ, but a haematocrit of 25.7%. This is similar to what has been reported previously[20]. This indicates that hypoxia, resulting from anaemia, is probably not the only cause of the hepatic injury seen with feline babesiosis. Possible other causes of hepatic injury in these cats are not clear, but concurrent infections should always be considered. Of the five cats

with ALT levels in excess of 100 U/λ but haematocrit values still over 20%, two had concurrent infections (one had FIV, one had *H. felis),* which could have played a role in the development of hepatic injury[8,73]. However, the other three cats had no evidence of concurrent infections. The cause of the hepatic injury in canine babesiosis is not clear either. It is speculated that the hepatic insult could be due to hypoxic damage resulting from anaemia, or to inflammatory cytokines, or to a substance the parasite releases or a combination of these[54].

Increased serum ALP activity was seen in only 14 cats in our study. ALP is a membrane-bound hepatic enzyme with activity in both hepatocyte and canalicular membranes. Elevations of hepatic ALP levels in cats usually result from induction by cholestasis (bile acids)[16]. A bone ALP-isoenzyme also exists which can lead to increased serum activity in young, rapidly growing animals[16]. This bone isoenzyme is not likely to be the cause of the elevated ALP levels in this study, as all the cats less than a year of age in our study population had normal ALP levels. All the cats with elevated ALP levels in this study were over one year of age (range between 1 and 13 years). No correlation between ALT and ALP levels could be identified in this study, which again indicates that primary hepatocellular injury seems to predominate in these cases.

Hepatic GGT is mainly associated with microsomal membranes of the canalicular surfaces of hepatocytes and bile duct epithelium[16]. Like serum ALP, serum GGT activity increases with cholestasis and is more liver specific than ALP. In this study, increased GGT activity was seen in only two cats. Any primary hepatocellular injury can potentially be accompanied by cellular swelling that can compress bile canaliculi and cause some secondary cholestasis[16]. This is likely to be the reason for cholestasis and resultant increases in ALP and GGT activity in a few cases of feline babesiosis in this study.

A statistically significant negative correlation was seen between total bilirubin and haematocrit values, but the association was not a strong one. This negative

correlation seemed to be more pronounced when cats were moderately to severely anaemic - in cases where haematocrit values decreased to 20% or less, total bilirubin values tended to increase. There were, however, exceptions to this as severely elevated total bilirubin did not always correspond to severe anaemia – one cat, for example, had a total bilirubin concentration of 372 μmol/λ, but a haematocrit of 28%.

Even though hyperbilirubinaemia was seen in the majority of cats in this study, clinical icterus was observed in only a few cats. Only total bilirubin was measured and no differentiation was made between conjugated and unconjugated bilirubin. The hyperbilirubinaemia recorded in this study 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 to the hyperbilirubinaemia. A combination of unconjugated and conjugated bilirubin would thus be expected in these cases[16]. This is similar to what has been reported previously for feline babesiosis[20]. Hyperbilirubinaemia has also been reported during acute disease caused by *B. canis* in dogs[28]. As in cats, it seems to be a reflection of both erythrocyte destruction and intrahepatic cholestasis in dogs[49].

A positive correlation between total bilirubin and ALT levels was identified in this study. Most cases with severe hyperbilirubinaemia also had severe elevations in ALT activity, but these cases did not always correspond to the severely anaemic cases. This supports the concept that not only haemolysis, but also hepatocellular disease contributes to the total bilirubin concentrations found in cats with babesiosis. Similar observations have been made in dogs[34,81]. In dogs, bilirubin concentrations greater than 100 μmol/λ suggest co-existing hepatic dysfunction in haemolytic disease[81] and values exceeding 170 μmol/λ correlate positively with mortality[34].

When the group of clinically icteric cats in this study was compared to the group of non-icteric cats, a significant difference in ALT levels between the two groups was observed: the icteric cats had significantly higher hepatic ALT levels than the non-icteric cats. No significant difference was seen when the ALP levels of the two groups were compared. This supports the concept that clinical icterus only seems to occur where hepatocellular damage is present, and not where only haemolysis is present[71].

Icterus has also been described in advanced cases of canine babesiosis[31,46]. Similar to what has been described for dogs in general[71] and observed for cats with babesiosis in this study, it is also thought that haemolysis alone will not lead to clinical icterus in dogs with babesiosis[49]. 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[46,47].

Serum urea and creatinine levels were within normal limits in the majority of cats in this study and no correlation between these levels could be identified. Of the six cats with concurrently high urea and creatinine levels, only two had severely elevated levels. The cat with serum urea of 55.8 mmol/λ was 10 years old and had concurrently high creatinine levels of 388 μmol/λ and potassium levels of 5.25 mmol/λ. This cat developed signs of uraemic encephalopathy and subsequently died. Unfortunately, no post mortem was done to confirm the diagnosis of renal failure in this cat. The cat with urea of 22.4 mmol/λ and creatinine of 288 μmol/λ was three years old and also had concurrently elevated potassium levels of 5.82 mmol/λ. Even though babesiosis could have been the primary cause of renal failure in these cats, it is also likely that underlying renal disease was present in these cases and that acute decompensation with subsequent renal failure could have been precipitated by the babesiosis, which is similar to what has been described in dogs[50].

It is difficult to evaluate the elevated urea and creatinine concentrations without knowledge of a full urine analysis in any of the cases. It is therefore not possible to determine whether these elevations were of prerenal or renal origin. The fact that many of the cases with elevated urea and creatinine had concurrent elevations of albumin indicates that prerenal azotaemia would be more likely. In dogs with babesiosis, renal damage (which would not necessarily lead to renal failure) is evident by the presence of proteinuria, casts and renal tubular epithelial cells in the urine. This is a common finding in both complicated and uncomplicated babesiosis of dogs[50]. There was, however, little or no evidence of renal dysfunction in most dogs in a study of 30 cases of canine babesiosis (Lobetti et al., Department of Medicine, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, submitted). No urine analysis was performed in the current study, but a previous study on feline babesiosis showed bilirubinuria and haemoglobinuria to be the only significant findings on urine analysis, which were likely to be indicators of haemolysis rather than renal damage[20]. To definitively diagnose renal failure with babesiosis, one would need ongoing evaluations of urine volume, urine analysis and the degree of azotaemia[41]. Even if the above-mentioned two cats developed renal failure as a result of the babesiosis, it certainly does not appear to be a common complication of feline babesiosis. Similarly, in dogs, acute renal failure is an uncommon complication of babesiosis[31].

A disproportionate elevation in serum urea, when compared with serum creatinine, has been described in dogs with babesiosis[66]. This is likely of non-renal origin and could be related to catabolism of lysed erythrocytes with an increased protein load on the liver and subsequently an increased urea production[66]. It could also be related to more generalised protein catabolism resulting from the existing febrile, inflammatory disease[66]. An elevation of serum urea without a corresponding increase in serum creatinine levels was seen in nine cats, eight of which had normal creatinine values. This is also likely to be of non-renal origin and could be caused by the same factors as those described for

dogs. It seems less likely, though, that generalised protein catabolism resulting from fever and inflammation would be the cause of the elevated urea in cats, as feline babesiosis is not associated with fever[18,61]. As gastrointestinal haemorrhage was not ruled out in any of these cases, it should also be considered as a possible explanation for the disproportionate elevation of urea in these cases[16].

Elevated creatinine levels, without a concomitant rise in urea levels, were seen in eight cats. These elevations were mild and the highest creatinine level recorded in this group was 179 μmol/λ. As all these cats were anorexic, the most likely explanation would be that the true urea levels were falsely lowered by a low protein intake, while creatinine levels are not affected by dietary protein intake[16]. Low protein intake is also the most likely cause for the marginally low serum urea concentrations that were recorded in 11 cats in this study[16].

No remarkable changes in renal parameters could be observed in cats with babesiosis in this study, indicating that renal damage is not likely to be a consistent feature of feline babesiosis. These findings are similar to what has been reported previously[20].

Both hypernatraemia and hyponatraemia were seen in this study. The hypernatraemia observed in five cats was marginal with the highest sodium level being 159 mmol/λ. The most common cause of hypernatraemia in cats is dehydration, thus a relative increase in sodium concentration[72]. As all these cats were anorexic, decreased water intake was also likely and this could certainly have caused hypernatraemia. All the hypernatraemic cats also had elevated albumin levels and most of them had elevated creatinine levels, which further supports dehydration as a cause of the observed hypernatraemia. Even though none of the owner complaints included any gastrointestinal symptoms, it was not definitively ruled out and therefore gastrointestinal fluid losses could also be a possible explanation for the hypernatraemia seen[16].

Hyponatraemia was observed in 15 cats and the lowest sodium concentration recorded was 126 mmol/λ. Treatment to replace sodium is indicated when sodium levels drop below 125 mmol/λ[63]. This particular cat with severe hyponatraemia might, in fact, have benefited from sodium replacement therapy had its sodium levels been known, as this cat died despite therapy. When evaluating sodium concentrations, one should have knowledge of the patient's hydration status and/or serum osmolality[63]. In dehydrated patients, the most likely cause of true hyponatraemia is renal disease. This was the most likely explanation for the hyponatraemia seen in at least one of the cats in this study group, as this cat also had severe azotaemia and suspected renal failure. Hyperproteinaemia can artefactually decrease serum sodium concentrations and lead to pseudohyponatraemia[63]. This happens because sodium is contained in the aqueous fraction of serum and hyperproteinaemia will lead to a decrease in the proportion of the aqueous phase with a resultant decreased sodium concentration per litre of serum. Most of the cats with hyponatraemia in this study also had concurrent hyperproteinaemia. Another cause of hyponatraemia, which was not definitively ruled out in these cats, could have been an inappropriate secretion of antidiuretic hormone (ADH). This syndrome is characterised by excess ADH, which is unrelated to any osmotic or volume stimuli, but it is very rare in the cat[63]. Hyponatraemia can also occur in canine babesiosis – in a recent study of 30 dogs with babesiosis, hyponatraemia was present in 17% of cases (R Lobetti et al., Department of Medicine, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, submitted).

Both hyperkalaemia (11 cats) and hypokalaemia (nine cats) were observed in this study. Anuric renal disease is an important cause of hyperkalaemia in the cat[16,72]. This could be the explanation for the hyperkalaemia seen in only two of these cats, as they also had concurrent severe azotaemia. None of the other hyperkalaemic cats were azotaemic, which makes renal disease a less likely cause for hyperkalaemia in these cats.

Normal intracellular potassium concentrations are higher than extracellular concentrations and pseudohyperkalaemia can develop if there is leakage of potassium from the intravascular fluid into the extravascular fluid[16]. Even though intraerythrocytic potassium concentrations are relatively low in cats when compared to other species[16], it has been shown that haemolysed blood samples can be a cause of fictitious hyperkalaemia[72]. This is a possible explanation for the hyperkalaemia seen in a few cats in this study, as haemolysed serum was noted in most of these cases. Five of the 11 cats with hyperkalaemia also had evidence of IMHA. Reticulocytes have higher intracellular potassium concentrations than mature erythrocytes[16]. The IMHA in these cats also involved agglutination of reticulocytes, as was evidenced on examination of blood smears. Haemolysis of these reticulocytes could thus be another explanation for the hyperkalaemia seen in some cats. Thrombocytes also contain high amounts of intracellular potassium and severe thrombocytosis may also produce pseudohyperkalaemia[16,72]. Thrombocytosis was seen in two cats, one of which had severe hyperkalaemia. The thrombocytosis might have been a contributing factor to the hyperkalaemia seen in this cat.

Another cause of hyperkalaemia is acidosis. This occurs when there is an exchange of intracellular potassium for extracellular hydrogen ions in an attempt by the body to correct acidosis[16,72]. As acid-base determinations were not done in this study, it is impossible to say if acidosis played a role in the development of hyperkalaemia in these cats. Previous studies on feline babesiosis have shown that the blood pH remained within normal limits during the course of the disease and no evidence of acidaemia was found[20]. It is possible, however, that underlying acid-base changes were present, but that these were not reflected in the blood pH due to adequate compensation or mixed acid-base disturbances. Acidaemia has been reported in severe canine babesiosis[7]. Hyperkalaemia was also noted in severely affected dogs with babesiosis in one study[28].

Hypokalaemia was seen less frequently than hyperkalaemia in this study. As all of these cats were anorexic, the most likely explanation for the hypokalaemia is a decreased oral intake of potassium[63]. Other possible explanations for the hypokalaemia seen in these cats could include increased urinary and gastrointestinal losses and intracellular shifts of potassium[16,63]. Hypokalaemia can also occur in severe canine babesiosis, most likely due to decreased oral intake of potassium[76].

Even though 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 for any cat affected with babesiosis, as some individual cases might need to be treated accordingly.

Various studies have shown the prevalence of FeLV and FIV infections in different sick cat populations in various countries[4,29,51]. These include:

	FeLV Positive	FIV positive	Both positive
France	19.4%	15.4%	4.3%
Italy	12.2%	23.4%	4.2%
United Kingdom	18.0%	12.8%	0.7%
Germany	13.4%	8.4%	2.1%
Austria	14.2%	2.7%	1.0%
Switzerland	11.3%	1.8%	Not available
Japan	10%	43.9%	12.4%
Australia	1.4%	20.8%	Not available
United States	13.3%	7.4%	1.5%

In this study, 14% of the cats tested positive for FIV, 32% tested positive for FeLV and concurrent infections with both FIV and FeLV were seen in 9% of the cats. This would indicate a high prevalence of these viruses in the study area.

However, no survey has ever been done to determine the prevalence of FeLV or FIV infections in cats in South Africa. Data obtained from a local veterinary laboratory indicated that a total of 3 414 cats had been tested for FeLV/FIV infections over a five-year period. Among these, 18% tested positive for FIV and 2.9% tested positive for FeLV infection (Johan Müller, Golden Vet Lab, Johannesburg, South Africa, personal communication). Unfortunately, no data on combined infections with these viruses could be obtained.

Interpretation of serological test results can be difficult without detailed knowledge of the epidemiology of the disease in question. The reliability or predictive value of a positive test result may be quite poor in a population of cats with very low prevalence of infection, even when the sensitivity and specificity of the tests is high. The positive predictive value of a test increases as the prevalence of the infection increases and the negative predictive value of the test decreases at the same time. In contrast to positive test results, negative test results are useful in predicting that the cat is indeed not infected when the prevalence of disease is low. Therefore, because the overall prevalence of FeLV infection in cats tends to be low, the FeLV ELISA test kits are extremely useful in predicting the disease-free status of a cat when a negative result is obtained. A positive test result, however, is of limited use in predicting that the cat is truly infected[1,44,70].

Feline immunodeficiency virus antibodies were identified in 14% of our study population. The overwhelming majority of cats that seroconvert to FIV following infection remain infected with FIV. This results in a high correlation between antibodies to FIV and persistent infection, thus a positive FIV antibody test is considered to be indicative of a persistent FIV infection[1,59]. The major exceptions are (1) tests performed on kittens with passively acquired maternal antibodies and (2) false-positive tests because of failure of the tests[1]. As none of the cats positive for FIV was younger than one year of age, false positives because of

maternal antibody transfer are not likely and these cats are therefore more likely to be truly infected with FIV.

A negative FIV result indicates that FIV-specific antibodies are not present and that the cat is not infected. The major exceptions include (1) tests performed on cats that are infected but have not yet seroconverted, (2) false-negative tests because of failure of the tests, and (3) FIV-infected cats that have become antibody-negative late in the disease process because of debilitation of the immune system[1]. It is therefore theoretically possible that false-negative test results could have occurred in this study.

Male cats are two to three times more likely to be infected with FIV than female cats[1,4,51,73]. This was not observed in this study, as three FIV-positive cats were male and five were female. The mean age of FIV-infected cats has been shown to be 5 to 6 years, with very few kittens and adolescent cats being infected[1,51]. This was also seen in this study, as the mean age of FIV-infected cats in our study population was 5.2 years. The fact that most of the cats in this study population were very young may have artificially reduced the observed prevalence of FIV, as young cats are less likely to acquire this infection. Outdoor cats have also been shown to be at greater risk of infection than indoor cats[1,4]. This was also seen in this study, as all eight cats were outdoor cats.

There is a direct relationship between a sample's FeLV antigen concentration and the degree of ELISA activity or colour change in the test well, therefore equivocal FeLV test results probably indicate the detection of low antigen concentration[44]. The interpretation of an equivocal test result is difficult and current recommendations are that the cat should be retested after a month[44]. As equivocal test results were obtained for six cats in this study, proper interpretation of those results was not possible unless the cats were retested[51].

A clear-cut positive ELISA test for FeLV indicates that soluble p27 viral core protein has been detected in the sample and the cat is most probably infected with FeLV[1,59]. False positive results can, however, occur because of improper use or failure of the test. As previously discussed, the overall low prevalence of FeLV infection in cats also leads to a high percentage of false-positive results, even though the sensitivity and specificity of the test kit is high[1,44,70].

Because a positive ELISA test detects the presence of soluble FeLV antigen as early as the primary viraemic stage, it can neither indicate nor predict whether the cat is transiently or persistently infected. 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[39,59]. To therefore determine if 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 six to eight weeks[59]. A second positive test result most likely indicates continued antigenaemia or viraemia, thus persistent infection. Development of a negative second test result indicates that the cat is no longer viraemic or antigenaemic, but unfortunately does not exclude the minor possibility of viral latency or the development of FeLV-associated disease at a later stage[1,59]. In this study, 10 of the 12 cats that were retested after 12 weeks were negative for FeLV, representing 42% of all the cats that had tested positive for FeLV initially. These cats were most likely to have seroconverted to a negative FeLV state, but it is also possible that the initial positive FeLV results could have included some false positives.

Nine of the original 24 cats that tested positive or equivocal for FeLV had died after a period of only 12 weeks. Even though all of these cats were positive for babesiosis, this is unlikely to be the primary cause of death as none of the other cats that were affected by babesiosis without any concurrent diseases died within that time period. One might argue that these cases were likely to be truly positive for FeLV, as it is known that cats persistently infected with FeLV have a

shortened life span[82]. One study reported that 49 of 100 cats with FeLV-associated anaemia required euthanasia within two weeks of diagnosis and that the remaining cats had a median survival time of about four months only[82].

A negative test for FeLV indicates that viral core p27 antigen is not present in the sample and that the cat is not likely to be affected with FeLV. False-negative results could, however, also have occurred in this study because of improper use or failure of the tests or if testing was done during very early infection before primary viraemia[1,59].

Young cats (mean age of three years) are more likely to be infected with FeLV than older cats[1]. This was also seen in this study, as 20 of the 24 cats were less than three years of age and the mean age of all the cats that tested positive or equivocal for FeLV was 3.1 years. This study could, however, have had an artificially high prevalence of FeLV positives, because 80% of the study population was less than three years old at presentation and thus also represented the high-risk age group for FeLV infection.

It has also been shown that male cats are more likely to be infected with FeLV than female cats[1]. This was not observed in this study, as only nine cats that tested positive or equivocal for FeLV were males and the other 15 were females.

The differentiation between feline babesiosis and haemobartonellosis can be difficult, as both diseases cause clinical signs of anorexia, depression, anaemia, weakness, weight loss and occasional icterus during the acute phase of the disease[8,26]. A chronic carrier phase that could relapse into clinical illness has also been described in cats with haemobartonellosis. Also, in both diseases the anaemia tends to be regenerative, the erythrocytes are usually macrocytic and hypochromic and the total and differential white blood cell counts are quite variable and of limited diagnostic value. Autoagglutination of erythrocytes has

also been described in cats with haemobartonellosis[8,26]. This, again, is similar to what was seen in cats with babesiosis in this study.

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. The differentiation of *H. felis* parasites from *B. felis* parasites, Howell-Jolly bodies and basophilic stippling on a blood smear can be difficult, as all of these can have a very similar appearance[26]. Additionally, *H. felis* parasites only appear in the blood in a cyclical manner within discrete parasitaemic episodes and disappear from the blood again afterwards[26]. 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[8,26].

Because the two diseases are difficult to differentiate clinically and the diagnosis of haemobartonellosis can be very difficult when examining stained blood smears only, it is possible that more than six cats in this study could have had concurrent babesiosis and haemobartonellosis. A PCR test has been developed as an aid to the diagnosis of feline haemobartonellosis[2], but it is not available in South Africa and was therefore not used in this study.

A positive correlation between *H. felis* and FeLV infection has been well described and it is thought that about half of the cats with clinical haemobartonellosis are also positive for FeLV[26]. However, no cause and effect have been established. It is possible that FeLV may activate latent *H. felis* infections to induce clinical disease, but it is also feasible that cats already infected with *H. felis* are more susceptible to infection by FeLV[8,26]. This association was also seen in our study, as three of the six cats with *H. felis* also tested positive for FeLV. One of these three cats died of severe disease, one was lost to follow-up and the third tested negative for FeLV after 12 weeks.

When cats with concurrent *H. felis* and *B. felis* infections were compared to cats with only *B. felis*, no significant differences in haematocrit or ALT values were found. Even though co-infection with *H. felis* does not seem to cause significant worsening of clinical parameters in cats with babesiosis, it is still important to be aware of this co-infection, as feline haemobartonellosis should not be left untreated and a different treatment regimen is used in these cats[26].

This study confirmed that concurrent infections with *H. felis* and *B. felis* certainly exist in cats. As the clinical and clinico-pathological differentiation between the two diseases can be difficult and the definitive diagnosis of haemobartonellosis can also be difficult, it may therefore be advisable to treat for both diseases simultaneously whenever co-infection is suspected in a case of feline babesiosis.

CHAPTER 8

8. CONCLUSIONS

8.1 The results of the study have addressed all the research questions:

i. *Signalment - Is there a specific predilection for infection with B. felis in cats with regard to age, breed and sex?*

An age predilection appeared to be present and young adult cats less than three years of age seemed to be predisposed. No breed or sex predilection for disease could be identified in this study; however, Siamese cats seemed to be over-represented amongst purebred cats.

ii. *What are the haematological changes seen in cats infected with B. felis, as reflected by changes in the erythrocyte, leukocyte and thrombocyte parameters?*

The most significant finding regarding the erythrocyte parameters was a macrocytic, hypochromic, regenerative anaemia – this was present in 57% of the cats. No characteristic changes were seen in total or differential leukocyte counts and the leukocyte abnormalities that occurred were more likely to be a result of concurrent problems or diseases than the babesiosis itself. Thrombocyte counts were variable and thrombocytopaenia was an inconsistent finding.

iii. *What are the clinico-pathological changes seen in cats infected with B. felis, as reflected by changes in serum proteins, hepatic parameters, renal parameters and serum electrolytes?*

- Serum protein values were mostly normal, but increased values were seen in some cases. Polyclonal gammopathies were observed in all cats with increased total globulin levels and were ascribed to a combination of acute- and chronic-phase proteins that were produced by the body in response to the *Babesia* antigens.
- Hepatic cytosol enzyme activity was elevated in the vast majority of cases, which was indicative of primary hepatocellular injury as a consistent feature of the disease. Total bilirubin concentrations were also elevated in the vast majority of cases and was likely to be a result of both intra- and extravascular haemolysis, but hepatocellular disease and secondary intrahepatic cholestasis were also likely to contribute.
- No remarkable changes in renal parameters were observed and values tended to be within normal limits, indicating that renal damage is not likely to be a consistent feature of the disease.
- No consistent changes in serum electrolyte concentrations were observed, but a variety of electrolyte abnormalities did occur in a number of cases.

iv. What is the prevalence of concurrent infection with feline immunodeficiency virus among cats that are infected with B. felis?

The prevalence of concurrent FIV infection was 14%.

v. What is the prevalence of concurrent infection with feline leukemia virus among cats that are infected with B. felis?

The initial prevalence of concurrent FeLV infection was 32%, but on retesting the prevalence was substantially lower.

vi. *What is the parasitaemia found on peripheral blood smears and how does it compare to the parasitaemia found on central venous smears?*

The peripheral parasitaemias were variable and ranged from 0.3% to 42.3%. A very close correlation was seen between peripheral and central parasitaemias, strongly suggesting that *B. felis* does not sequester.

vii. *Do cats infected with B. felis show positive in-saline agglutination of red blood cells?*

In-saline agglutination tests showed positive agglutination of red blood cells in 16% of cases.

viii. *Are any other haematological parasites present on peripheral and/or central venous blood smears?*

Concurrent infection with *Haemobartonella felis* was noted on both peripheral and central venous blood smears of 11% of all the *B. felis* cats tested. No other haematological parasites were seen on any blood smears.

8.2 In conclusion, this study has addressed all the research questions, and the findings have identified possible areas for further research in this fascinating disease.

ADDENDUM

The original data was subsequently compared between the treated cats (7) and the untreated cats (49), to establish if treatment had influenced the observed abnormalities of the disease.

RESULTS

When the haematocrit values of the treated cats were compared to the untreated cats, no statistically significant difference was seen (*T*=263.5; *P*=0.116). The median haematocrit of the treated cats was 26.6% (range 18.3%-41.2%), while that of the untreated cats was 17.5% (range 7.9%-39.9%). Graphical representation of this comparison is shown in Figure A1.

When central parasitaemias of the treated cats were compared to the untreated cats, no statistically significant difference was seen (*T*=156.5; *P*=0.292). The median central parasitaemia of the treated cats was 4.0% (range 0.4%-8.8%), while that of the untreated cats was 7.1% (range 0.2%-41.4%). Graphical representation of this comparison is shown in Figure A2.

When ALT values of the treated cats were compared to the untreated cats, a statistically significant difference was seen (*T*=111.5; *P*=0.03). The median ALT value of the treated cats was 37 U/λ (range 14-64 U/λ), while that of the untreated cats was 61 U/λ (range 10-1908 U/λ). Graphical representation of this comparison is shown in Figure A3.

When total bilirubin concentrations of the treated cats were compared the untreated cats, a statistically significant difference was seen (*T*=84.0; *P*=0.004). The median total bilirubin concentration of the treated cats was 6.7 µmol/λ (range 5.5-14.1 µmol/λ), while that of the untreated cats was 14.1 µmol/λ (range 5.8-372 µmol/λ). Graphical representation of this comparison is shown in Figure A4.

Figure A1: Comparison of the haematocrit values between 7 treated and 49 untreated cats with *B. felis* infection

The ends of the boxes indicate the 25th and 75th percentiles, with a line at the median. T-bars indicate the 10th and 90th percentiles. Circles represent outliers. There is no statistically significant difference between the two groups (*P*=0.116).

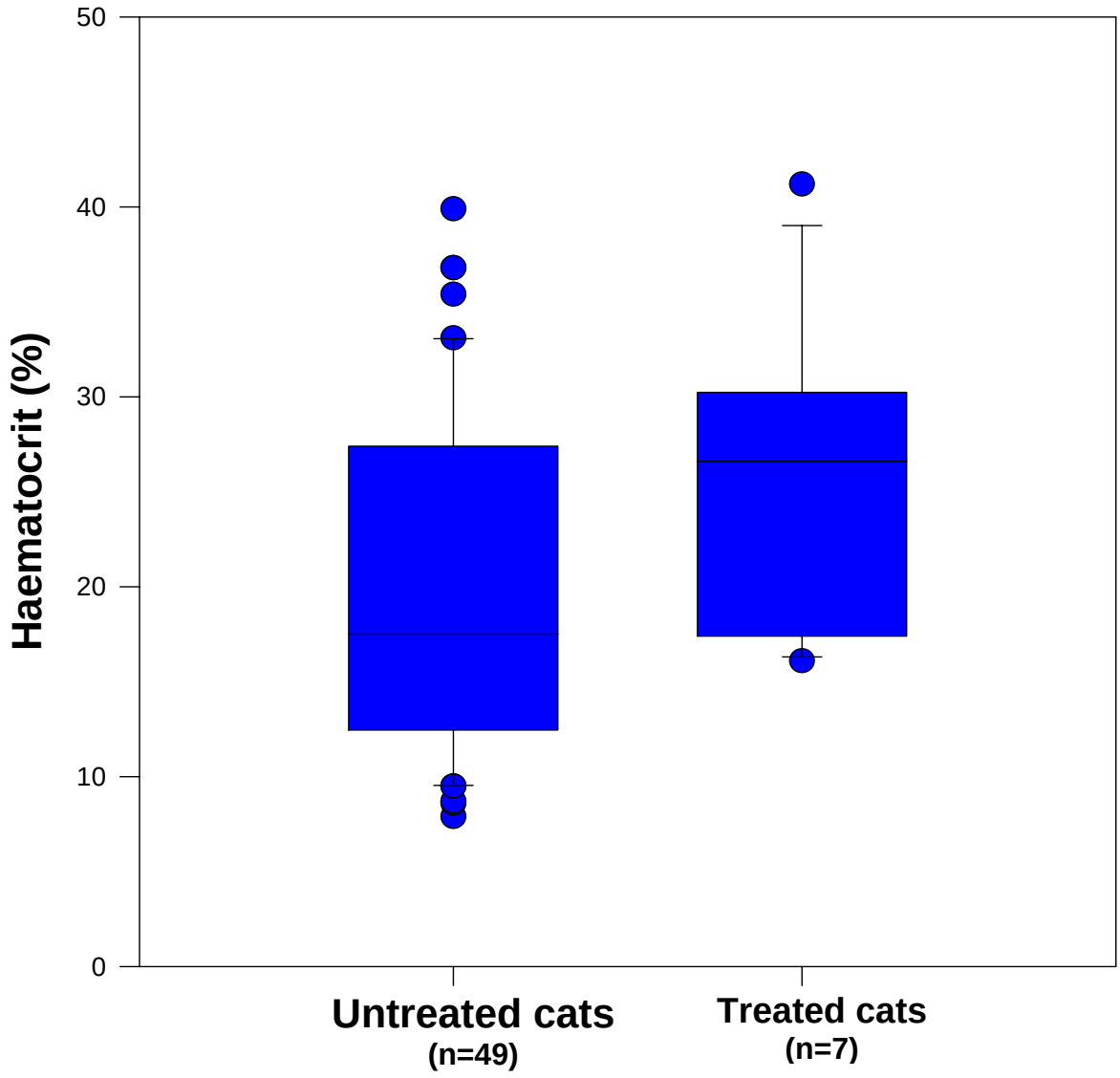


Figure A2: Comparison of central parasitaemias between 7 treated and 49 untreated cats with *B. felis* infection

The ends of the boxes indicate the 25th and 75th percentiles, with a line at the median. T-bars indicate the 10th and 90th percentiles. Circles represent outliers. There is no statistically significant difference between the two groups (*P*=0.292).

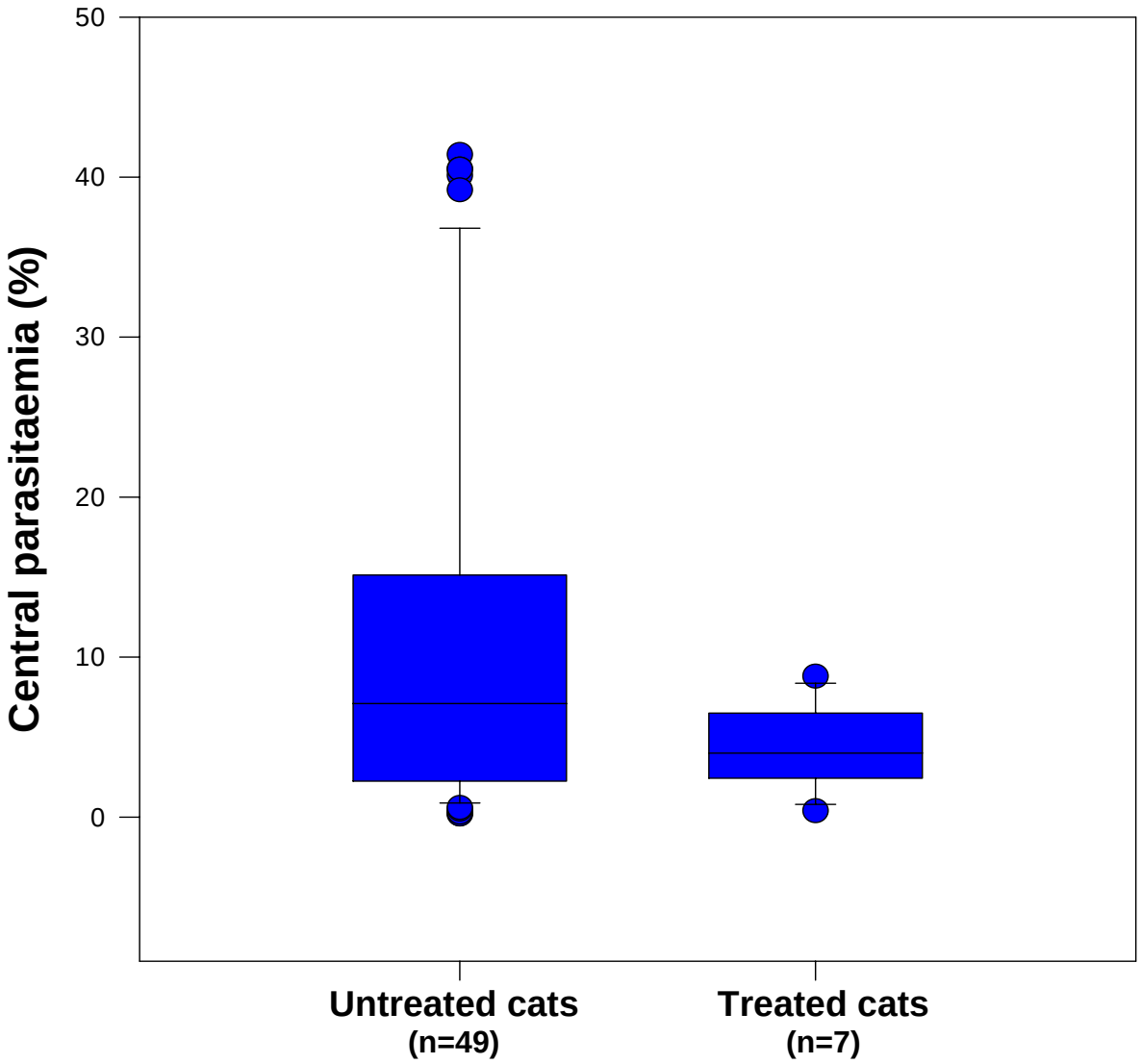


Figure A3: Comparison of ALT values between 7 treated and 49 untreated cats with *B. felis* infection

The ends of the boxes indicate the 25th and 75th percentiles, with a line at the median. T-bars indicate the 10th and 90th percentiles. Circles represent outliers. There is a statistically significant difference between the two groups (*P*=0.030).

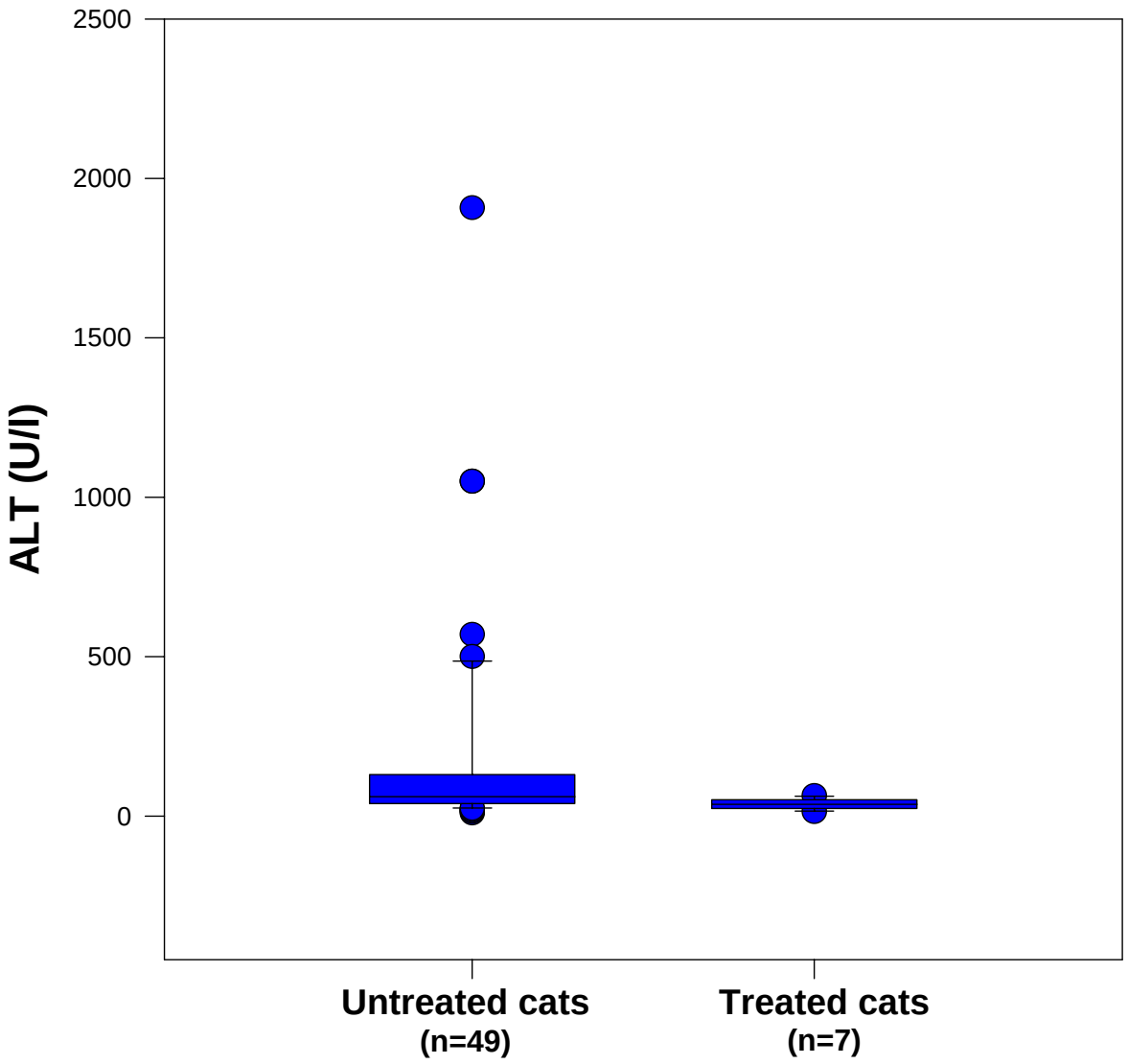
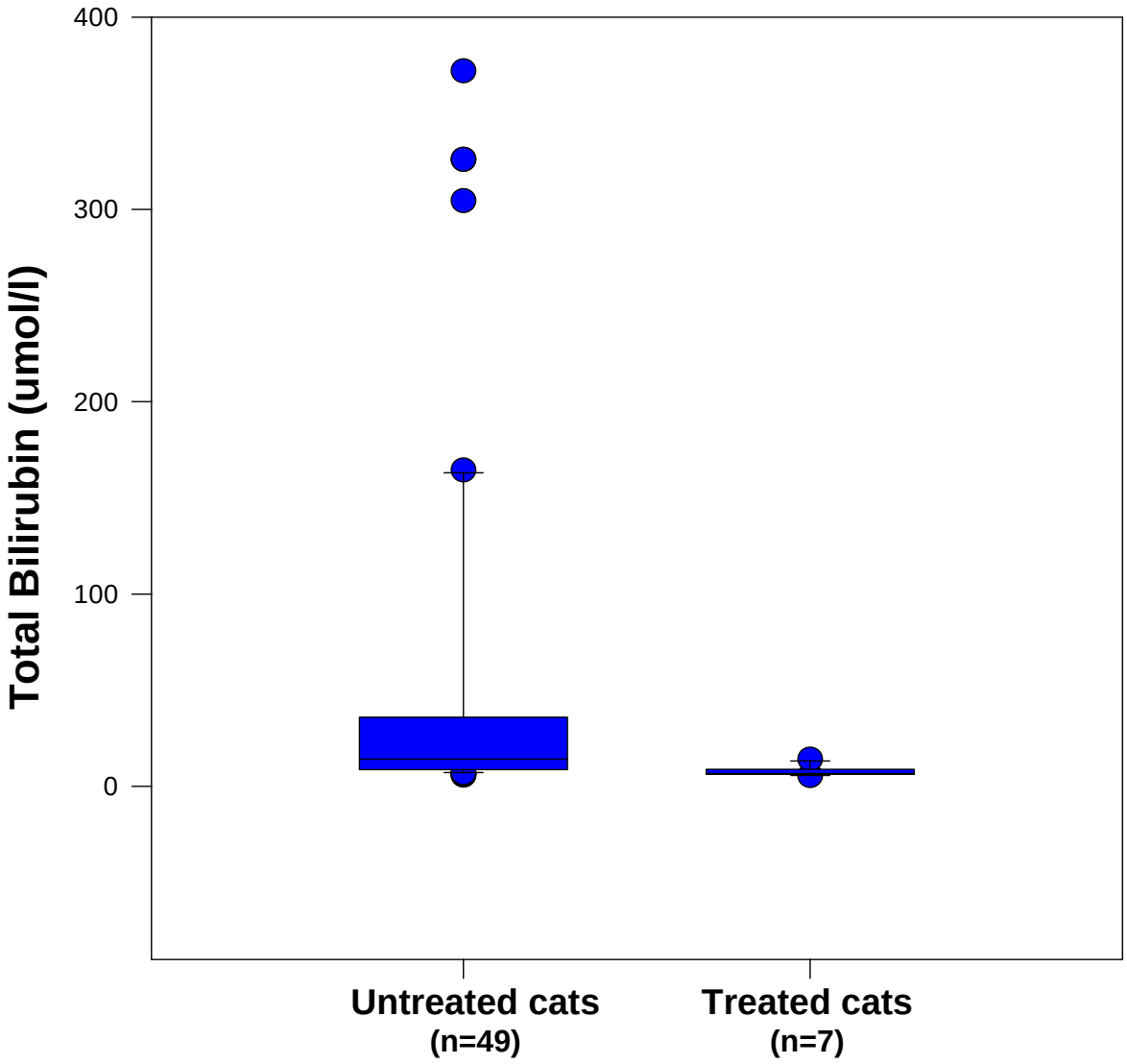


Figure A4: Comparison of total bilirubin concentrations between 7 treated and 49 untreated cats with *B. felis* infection

The ends of the boxes indicate the 25th and 75th percentiles, with a line at the median. T-bars indicate the 10th and 90th percentiles. Circles represent outliers. There is a statistically significant difference between the two groups (*P*=0.004).



DISCUSSION

Even though the difference was not statistically significant, the untreated cats had a lower median haematocrit value, which indicates that treatment with primaquine phosphate appears to result in an an improvement or correction of anaemia when cats are suffering from feline babesiosis. Almost all of the treated cats had normal haematocrit values and only one cat had moderate anaemia with a haematocrit of 18.3%, despite the fact that parasites were still identified on blood smears. One could speculate that these treated cats were either chronically infected with the disease or that they represented cases of re-infection with the parasite. The most likely explanation, however, is that these cats were subclinical carriers of the parasite that had entered a state of premunity after treatment with primaquine phosphate, which has been reported not to sterilise the infection[61,65].

When cats are treated for feline babesiosis, parasitaemias are expected to decrease as parasites are cleared away from the circulation. This was demonstrated in these cats, as the median central parasitaemia of the treated group was lower than that of the untreated group, even though this difference was not statistically significant. Therefore, even though primaquine phosphate therapy does not sterilise the infection[61,65], it does seem to effectively reduce the parasite burden.

Increased ALT values and total bilirubin concentrations are seen in the majority of cats with babesiosis. When these parameters were compared between treated and untreated cats, the treated cats had statistically significant lower values. A possible explanation for this is that primaquine phosphate therapy reduces the amount of hepatocellular damage and haemolysis normally caused by the infection. This normalisation of ALT and total bilirubin concentrations during the recovery phase of the disease has also been recorded previously for feline babesiosis[20]. Elevated ALT levels have been previously described as a toxic effect of primaquine phosphate therapy[20]. Even though these cats were on

chronic primaquine phosphate therapy, this toxic effect was not seen, as the treated cats had much lower ALT values than the untreated cats. This could be explained by the fact that the cats in this study were receiving lower doses of primaquine phosphate than the cats in the previous study[18].

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APPENDICES

Appendix 1: Signalment of 56 cats with naturally occurring babesiosis

No	Age (yrs)	Breed	Condition	Habitus	Clinical icterus
1	5.0	Domestic Shorthair (DSH)	Fat	Alert	No
2	1.0	Siamese	Normal	Moderately depressed	No
3	3.0	Siamese	Normal	Moderately depressed	No
4	1.0	DSH	Fat	Moderately depressed	No
5	2.0	DSH	Fat	Alert	No
6	2.0	Persian	Normal	Moderately depressed	No
7	13.0	DSH	Normal	Alert	No
8	2.0	DSH	Normal	Alert	No
9	2.0	DSH	Fat	Alert	No
10	2.0	Domestic Longhair (DLH)	Normal	Moderately depressed	No
11	3.0	Siamese	Normal	Moderately depressed	No
12	3.0	DLH	Normal	Alert	No
13	1.0	DSH	Fat	Alert	Yes
14	3.0	DLH	Normal	Alert	No
15	3.0	DSH	Normal	Alert	No
16	2.0	Oriental	Thin	Moderately depressed	No
17	1.0	DSH	Thin	Moderately depressed	Yes
18	7.0	DSH	Normal	Moderately depressed	Yes
19	1.0	DSH	Normal	Alert	No
20	13.0	DSH	Normal	Moderately depressed	No
21	8.0	DSH	Fat	Moderately depressed	No
22	1.0	DLH	Thin	Moderately depressed	Yes
23	1.0	Russian Blue	Normal	Moderately depressed	No
24	1.0	DSH	Normal	Moderately depressed	No
25	13.0	Siamese	Thin	Severely depressed	No
26	1.0	DLH	Thin	Severely depressed	No
27	2.0	DSH	Fat	Moderately depressed	Yes
28	10.0	Siamese	Thin	Severely depressed	No
29	1.0	Chinchilla	Normal	Moderately depressed	No
30	8.0	DSH	Fat	Alert	No
31	3.0	DSH	Fat	Severely depressed	No
32	1.0	DSH	Thin	Alert	No
33	0.8	DSH	Normal	Moderately depressed	Yes
34	0.8	DLH	Normal	Alert	No
35	1.0	DLH	Normal	Severely depressed	No
36	2.0	Siamese	Thin	Severely depressed	No
37	10.0	DLH	Fat	Moderately depressed	No
38	3.0	DSH	Thin	Severely depressed	No
39	3.0	Burmese	Fat	Severely depressed	No
40	1.0	DSH	Normal	Alert	No
41	1.0	DSH	Normal	Alert	No
42	2.0	DSH	Thin	Moderately depressed	Yes
43	4.0	DSH	Fat	Alert	No
44	1.0	DSH	Fat	Alert	No
45	0.5	Siamese	Normal	Moderately depressed	No
46	1.0	DSH	Normal	Moderately depressed	Yes
47	1.0	DLH	Thin	Moderately depressed	Yes
48	0.8	DLH	Thin	Alert	Yes
49	1.0	DSH	Normal	Severely depressed	Yes
50	5.0	DSH	Fat	Alert	No
51	1.0	DSH	Normal	Alert	No
52	2.0	DLH	Normal	Alert	No
53	2.0	DSH	Thin	Severely depressed	No
54	1.5	Russian Blue	Thin	Moderately depressed	No
55	1.0	DSH	Normal	Severely depressed	Yes
56	1.5	DSH	Normal	Alert	No
Median	2.0				
25%	1.0				
75%	3.0				

Appendix 2: Red blood cell parameters of 56 cats with babesiosis

No	Ht (%)	RBC count (x10^{12}/λ)	Hgb (g/λ)	MCV (fλ)	MCH (pg)	MCHC (g/λ)	Normoblasts (cells/100 WBC)	In-saline agglutination
Norm. range	24-45	5-10	8-15	39-55	12.5-17.5	30-36	0	Negative
1	19.1	2.71	5.9	70.3	21.7	30.8	77	Positive
2	36.8	7.79	12.2	47.3	15.6	33.0	0	Negative
3	32.0	5.45	10.5	58.8	19.3	32.8	5	Negative
4	21.0	3.17	6.9	66.4	21.7	32.8	12	Negative
5	33.1	5.94	10.8	55.7	18.1	32.6	0	Negative
6	20.1	3.41	6.5	59.0	19.0	32.2	141	Negative
7	39.9	8.10	13.6	49.2	16.8	34.1	0	Negative
8	34.8	6.66	11.3	52.2	16.9	32.4	0	Negative
9	30.0	5.02	9.8	59.8	19.6	32.7	0	Negative
10	35.4	8.01	12.2	44.2	15.2	34.4	0	Negative
11	17.5	1.96	5.1	88.9	25.9	29.1	0	Negative
12	18.3	3.71	6.0	49.4	16.1	32.5	14	Negative
13	29.9	5.73	9.9	52.2	17.2	32.9	0	Negative
14	26.6	5.23	9.1	50.9	17.5	34.4	0	Negative
15	30.3	5.55	10.2	54.7	18.3	33.5	0	Negative
16	24.0	4.18	7.9	57.3	18.8	32.9	4	Negative
17	24.5	5.87	8.1	41.8	13.9	33.2	2	Negative
18	25.7	5.36	8.6	47.9	16.0	33.5	0	Negative
19	24.4	4.84	9.0	50.3	18.5	36.8	0	Negative
20	13.2	1.59	3.8	82.8	23.9	28.9	437	Positive
21	25.9	5.20	8.9	49.8	17.1	34.4	23	Negative
22	28.0	6.15	10.1	45.5	16.3	35.9	0	Negative
23	26.9	5.48	9.1	49.2	16.6	33.8	0	Negative
24	13.2	1.72	3.8	76.9	22.0	28.6	17	Positive
25	15.1	1.86	4.4	81.4	23.5	28.8	38	Positive
26	11.2	1.03	2.8	109.3	27.2	24.9	59	Positive
27	8.7	0.80	2.2	108.6	27.8	25.6	87	Negative
28	12.2	1.27	2.8	95.4	22.3	23.3	35	Positive
29	33.0	6.95	10.9	47.5	15.7	33.1	0	Negative

No	Ht (%)	RBC count (x10^{12}/λ)	Hgb (g/λ)	MCV (fλ)	MCH (pg)	MCHC (g/λ)	Normoblasts (cells/100 WBC)	In-saline agglutination
30	41.2	7.87	13.4	52.3	17.0	32.6	0	Negative
31	14.9	1.48	4.1	100.6	27.5	27.3	297	Negative
32	15.6	2.27	4.6	68.7	20.4	29.7	66	Positive
33	12.6	1.31	3.9	96.5	29.7	30.7	329	Negative
34	17.1	3.59	5.8	47.6	16.1	33.8	63	Negative
35	12.3	1.87	3.5	65.5	18.8	28.7	125	Negative
36	17.5	1.90	4.8	91.9	25.1	27.3	65	Negative
37	14.5	2.49	4.8	58.1	19.2	33.1	11	Negative
38	10.0	1.61	3.4	62.0	21.0	33.9	17	Negative
39	16.8	1.80	4.9	93.1	27.1	29.1	2	Negative
40	16.1	1.73	5.0	93.0	28.8	31.0	6	Negative
41	25.8	4.64	9.3	55.6	20.1	36.1	3	Negative
42	9.6	1.23	3.2	77.9	26.0	33.3	53	Negative
43	19.2	2.74	5.8	70.3	21.4	30.4	37	Negative
44	28.4	5.50	9.4	51.7	17.0	33.0	7	Negative
45	12.5	1.67	3.6	74.9	21.7	29.0	36	Positive
46	9.5	1.03	2.8	92.3	27.1	29.3	25	Negative
47	8.6	1.00	2.9	85.2	28.6	33.6	72	Positive
48	10.2	1.07	3.2	95.9	30.2	31.5	9	Negative
49	11.9	1.54	3.4	77.2	21.8	28.3	814	Negative
50	15.3	2.57	5.0	59.4	19.5	32.8	0	Negative
51	30.3	5.59	10.9	54.3	19.5	35.9	10	Negative
52	27.2	3.88	8.4	70.1	21.6	30.8	1	Negative
53	7.9	1.18	2.6	66.7	21.9	32.9	53	Negative
54	9.5	0.89	2.5	106.6	28.3	26.5	87	Negative
55	14.2	2.06	4.8	69.0	23.2	33.7	147	Negative
56	30.2	5.92	10.8	51.0	18.2	35.7	7	Negative
Median	18.7	3.0	5.9	60.9	19.9	32.8	10.5	
25%	12.9	1.6	3.8	51.4	17.2	29.2	0.0	
75%	28.2	5.5	9.6	82.1	23.7	33.6	61.0	

Ht – haematocrit; RBC count – red blood cell count; Hgb – Haemoglobin concentration; MCV – mean corpuscular volume; MCH – mean corpuscular haemoglobin; MCHC – mean corpuscular haemoglobin concentration

Appendix 3: White blood cell parameters of 56 cats with babesiosis

No	White blood cells ($x10^9/\lambda$)	Corrected white blood cells ($x10^9/\lambda$)	Absolute Neutrophils (mature) ($x10^9/\lambda$)	Absolute Neutrophils (immature) ($x10^9/\lambda$)	Absolute Lymphocytes ($x10^9/\lambda$)	Absolute Monocytes ($x10^9/\lambda$)	Absolute Eosinophils ($x10^9/\lambda$)	Absolute Basophils ($x10^9/\lambda$)
Norm. Range	5.5-19.5	5.5-19.5	2.5-12.5	0.0-0.3	1.5-7.0	0.0-0.85	0.0-1.5	0.0-0.2
1	37.6	21.2	6.2	0.2	14.9	-	-	-
2	2.8	2.8	1.5	0.2	0.8	0.0	0.1	-
3	3.1	3.0	1.4	0.1	1.1	0.3	-	-
4	9.0	8.0	2.3	0.1	5.2	0.4	-	-
5	12.3	12.3	4.8	-	6.5	0.1	0.9	-
6	21.4	8.9	4.4	0.3	2.8	0.8	0.4	0.1
7	7.7	7.7	4.1	-	2.7	0.2	0.8	-
8	7.2	7.2	1.7	-	5.2	-	0.3	-
9	9.4	9.4	7.1	-	2.3	-	0.1	-
10	6.0	6.0	5.2	-	0.7	0.1	-	-
11	12.1	12.1	4.1	0.2	7.3	0.2	0.2	-
12	17.4	15.3	9.8	0.2	3.7	-	1.7	-
13	18.3	18.3	13.4	0.2	4.0	0.4	0.4	-
14	12.3	12.3	8.1	0.1	3.6	-	0.5	-
15	9.4	9.4	5.5	-	3.2	-	0.8	-
16	24.8	23.8	17.2	-	5.7	-	0.7	-
17	13.0	12.7	9.4	0.1	2.3	0.5	0.4	-
18	13.2	13.2	11.4	-	0.8	0.9	0.1	-
19	9.6	9.6	6.9	0.2	2.0	0.3	0.2	-
20	14.2	2.6	1.0	0.0	1.3	0.4	-	-
21	6.3	5.1	3.9	0.5	0.6	0.2	-	-
22	41.8	41.8	39.3	1.3	0.4	0.8	-	-
23	10.0	10.0	4.5	-	4.5	0.2	0.8	-
24	14.6	12.5	7.6	0.7	2.1	1.2	0.7	-
25	15.4	11.2	9.4	0.2	0.6	1.0	-	-
26	22.1	13.9	6.8	0.7	5.7	0.6	0.1	-
27	18.3	9.8	3.7	0.2	4.7	0.8	0.4	-
28	21.2	15.7	5.2	0.3	7.7	2.5	-	-
29	4.3	4.3	1.8	-	2.4	0.1	-	-
30	3.3	3.3	2.0	-	0.5	0.5	0.3	-
31	52.4	13.2	10.6	0.7	0.7	1.2	0.1	-
32	21.7	13.1	6.0	0.8	4.8	1.2	0.3	-
33	42.4	9.9	3.6	0.4	3.5	1.3	1.2	-
34	18.8	11.5	3.1	0.2	7.0	0.5	0.7	-
35	29.8	13.2	5.8	0.7	6.2	0.3	0.3	-
36	25.4	15.4	4.3	0.3	9.9	0.8	0.2	-
37	32.8	29.5	21.3	1.5	1.2	5.3	0.3	-
38	6.6	5.6	3.9	0.2	0.8	0.6	0.1	-
39	9.4	9.2	7.7	-	0.8	0.5	0.2	-
40	9.4	8.9	6.7	0.4	1.3	0.1	0.4	-
41	10.5	10.2	7.8	-	1.8	0.2	0.3	-
42	54.2	35.4	20.2	5.0	7.1	3.2	-	-
43	7.5	5.5	2.3	-	2.8	-	0.3	-
44	11.6	10.8	3.3	-	7.2	0.2	0.2	-
45	-	-	-	-	-	-	-	-
46	5.3	4.2	1.6	0.1	2.3	0.2	-	-
47	5.9	3.4	2.3	0.4	0.7	0.1	0.0	-
48	18.7	17.2	7.4	0.5	9.1	-	0.2	-
49	58.9	6.4	2.6	0.3	2.8	0.5	0.3	-
50	19.7	19.7	13.8	2.0	2.6	1.4	-	-
51	-	-	-	-	-	-	-	-
52	9.6	9.5	5.4	0.3	3.3	0.2	0.3	-
53	22.6	14.8	6.2	1.0	3.5	3.2	0.6	0.1
54	30.7	16.4	6.2	1.0	7.1	1.3	0.8	-
55	18.7	7.6	4.2	0.2	3.0	0.3	-	-
56	3.5	3.3	1.2	1.1	0.9	0.1	-	-
Median	13.1	10.1	5.3	0.2	2.8	0.3	0.2	0.0
25%	9.0	7.2	3.3	0.0	1.2	0.1	0.0	0.0
75%	21.7	13.9	7.7	0.5	5.2	0.8	0.4	0.0

Appendix 4: Thrombocyte parameters of 56 cats with babesiosis

No	Thrombocyte count ($x10^9/\lambda$)	Thrombocyte score (smear)
Normal range	300-800	-
1	4	Clot,normal
2	3	Low
3	2	Low
4	5	Clot,low
5	218	Clot,normal
6	64	Clot,normal
7	294	Clot,normal
8	114	Clot,normal
9	6	Clot,normal
10	6	Clot,normal
11	6	Low
12	320	Normal
13	200	Clot,normal
14	173	Clot,normal
15	240	Normal
16	106	Normal
17	20	Clot,normal
18	36	Clot,normal
19	84	Clot,low
20	6	Clot,normal
21	4	Normal
22	154	Clot,normal
23	23	Clot,normal
24	4	Clot,normal
25	73	Clot,normal
26	5	Clot,normal
27	5	Clot,normal
28	31	Low
29	10	Normal
30	148	Clot,normal
31	18	Low
32	No count	Normal
33	37	Low
34	14	Normal
35	95	Clot,normal
36	41	Normal
37	7	Clot,normal
38	6	Low
39	8	Clot,low
40	38	Clot,normal
41	4	Clot, low
42	14	Clot,low
43	94	Normal
44	30	Normal
45	8	Clot,high
46	2	Clot,high
47	4	Low
48	52	Clot,normal
49	68	Clot,normal
50	281	Normal
51	8	Clot,normal
52	291	Normal
53	37	Clot,normal
54	46	Normal
55	3	Low
56	17	Clot,normal
Median	23.0	
25%	6.0	
75%	91.5	

Appendix 5: Clinical pathology of 56 cats with babesiosis

No	TSP (g/l)	ALB (g/l)	GLOB (g/l)	ALT (U/l)	ALP (U/l)	GGT (U/l)	UREA (mmol/l)	CREAT (µmol/l)	BILI (µmol/l)	Na (mmol/l)	K (mmol/l)
Norm. range	60-80	25-35	22-48	<23	<20	<10	7.1-10.7	<141	<6.8	141-156	4.0-5.1
1	93.5	38.7	54.8	86	2	1	5.4	126	35.6	154.0	5.92
2	76.1	34.9	41.2	18	2	5	7.9	161	8.5	151.0	3.61
3	86.1	32.2	53.9	24	6	0	7.8	135	6.6	150.0	3.19
4	57.6	27.3	30.3	20	9	0	7.2	130	5.8	140.0	3.94
5	78.4	38.5	39.9	52	26	0	10.8	135	7.3	154.0	4.75
6	75.8	32.9	42.9	52	0	0	6.4	112	37.8	149.0	5.28
7	74.2	34.0	40.2	35	31	0	8.3	176	6.5	158.0	4.99
8	74.3	36.5	37.8	38	9	2	7.7	153	7.0	159.0	4.39
9	78.4	32.4	46.0	55	0	1	6.2	126	7.6	154.0	5.10
10	73.8	26.0	47.8	31	0	7	6.5	134	9.4	132.0	3.58
11	74.5	37.3	37.2	61	0	2	9.6	142	14.7	152.0	5.35
12	81.6	29.6	52.0	23	20	0	9.7	117	6.7	155.0	4.27
13	83.5	35.0	48.5	500	14	11	7.2	140	33.6	153.0	5.17
14	72.8	34.7	38.1	37	12	1	6.7	135	6.3	155.0	4.33
15	79.0	36.6	42.4	27	15	0	9.1	179	6.3	157.0	3.84
16	63.7	34.4	29.3	30	18	0	7.0	117	7.9	151.0	5.13
17	76.2	36.5	39.7	570	62	1	6.5	128	17.0	152.0	4.43
18	82.7	36.8	45.9	1050	75	8	5.7	104	161.0	139.0	4.05
19	79.4	39.9	39.5	27	42	1	7.0	131	5.9	134.0	4.51
20	91.7	25.2	66.5	100	14	1	8.3	179	16.2	138.0	4.28
21	74.1	23.5	50.6	10	5	0	8.0	129	21.5	146.0	4.10
22	109.1	38.4	70.7	445	71	10	9.1	84	372.0	152.0	5.26
23	80.0	42.1	37.9	49	30	0	9.2	105	10.0	157.0	4.73
24	86.4	43.6	42.8	42	15	0	11.9	133	7.7	154.0	5.60
25	82.4	31.4	51.0	62	1	0	10.5	124	14.1	142.0	5.96
26	65.7	29.0	36.7	347	2	0	10.1	106	14.4	127.0	4.12
27	62.3	31.2	31.1	364	6	3	21.5	110	73.2	142.0	4.43
28	96.8	49.2	47.6	106	1	1	55.8	388	19.4	143.0	5.25
29	80.9	39.8	41.1	44	32	4	5.9	122	8.8	141.0	4.43
30	85.3	33.6	51.7	14	16	0	11.0	197	5.5	142.0	4.19
31	85.2	34.2	51.0	106	3	2	22.4	288	8.2	137.0	5.82
32	72.6	35.5	37.1	117	0	0	11.4	117	22.2	133.0	4.40
33	80.1	35.7	44.4	315	0	3	10.2	104	326.0	133.0	4.05
34	79.6	43.0	36.6	64	17	1	8.6	114	9.3	134.0	5.17
35	71.9	34.1	37.8	54	13	0	19.1	129	21.5	129.0	3.86
36	94.1	39.4	54.7	100	0	0	9.1	152	13.7	128.0	6.22
37	74.2	33.4	40.8	81	15	0	7.0	115	17.2	150.0	5.18
38	62.6	30.6	32.0	50	9	0	11.0	148	13.3	152.0	3.33
39	96.9	36.4	60.5	51	0	5	5.1	129	12.3	141.0	4.05
40	73.9	34.8	39.1	40	18	0	8.6	114	14.1	151.0	4.69
41	108.9	47.3	61.6	170	35	1	7.3	131	10.4	145.0	4.35
42	79.6	31.4	48.2	545	0	0	10.9	75	304.5	139.0	4.78
43	79.1	44.9	34.2	43	16	0	8.9	155	8.8	144.0	5.23
44	69.7	37.6	32.1	65	25	1	7.1	114	9.0	141.0	4.83
45	79.8	40.8	39.0	95	20	0	6.7	96	40.5	141.0	6.94
46	82.3	37.7	44.6	465	30	0	7.9	105	54.0	140.0	4.03
47	72.7	29.6	43.1	91	6	3	17.8	173	7.3	148.0	3.82
48	75.2	39.9	35.3	1908	29	0	7.6	72	297.1	139.0	4.72
49	65.0	29.5	35.5	305	10	4	15.4	80	69.4	126.0	4.58
50	60.8	24.9	35.9	14	6	0	16.9	216	15.1	130.0	3.95
51	71.5	33.8	37.7	50	0	2	4.2	104	9.0	153.0	4.70
52	78.3	33.2	45.1	33	36	0	7.7	161	7.6	156.0	4.58
53	68.9	39.3	29.6	59	6	1	15.7	131	37.0	158.0	4.09
54	74.5	33.1	41.4	40	24	0	12.2	121	12.5	155.0	5.03
55	71.8	34.0	37.8	87	11	0	8.8	104	164.5	154.0	4.69
56	75.9	34.9	41.0	31	8	1	8.7	114	10.6	149.0	4.72
Median	77.3	34.9	41.1	54.5	11.5	0.0	8.6	128.5	12.9	147.0	4.6
25%	72.8	32.3	37.5	36.0	2.0	0.0	7.1	113.0	7.8	139.0	4.1
75%	82.4	38.5	48.0	106.0	22.0	2.0	10.9	145.0	27.9	153.5	5.2

Appendix 6: Globulin fractions of 56 cats with babesiosis

No	α globulin (g/λ)	β globulin (g/λ)	γ globulin (g/λ)
Normal range	8-16	6-14	12-22
1	10.9	11.7	32.9
2	21.8	10.7	12.4
3	19.1	12.2	23.6
4	10.1	8.9	12
5	10.3	11.5	14.6
6	10.6	12.6	18.4
7	8.4	12	18.8
8	9.3	11.4	18.7
9	8.1	11.9	25.7
10	20.8	10.3	16.5
11	10	12.3	15
12	8.1	9.8	34.2
13	17.3	10.9	21.7
14	12.7	10.1	16.8
15	9.9	10.5	23.5
16	11.2	11.5	11
17	10.6	10.4	19.8
18	14.1	11.4	22.4
19	9.5	12	19.3
20	14.8	18.5	34.2
21	16.1	12.6	24
22	20	13	42
23	15.4	12.7	16.1
24	13.8	14.2	18.3
25	13.2	15.6	24.5
26	7.8	8.9	20.4
27	11.4	10.3	11.5
28	12.9	18	17.1
29	6	8.2	24.4
30	14.4	12.8	27.3
31	12	13	27
32	15.2	13.4	12.6
33	15.7	11.7	19.7
34	13.1	13.5	15.4
35	13.6	12.7	15.9
36	13.6	15.6	29
37	9.8	12.3	22.1
38	16.5	11.8	5.8
39	11.5	16	34.1
40	10.9	9.8	18.7
41	11	20	33
42	13	13	21.6
43	9.5	10.2	15.9
44	9.7	12	13.4
45	15.1	12.7	14
46	13.8	14.4	18.5
47	11.7	12.6	19.6
48	13.3	10.4	12.9
49	11.1	10.5	15.8
50	13.5	9	15.2
51	20.7	7.6	12.6
52	16.1	7	25.6
53	17.3	10.1	7
54	16.9	8.7	17.5
55	13.9	10.1	15.4
56	24.4	7.9	12.2
Median	13.1	11.8	18.6
25%	10.5	10.3	15.1
75%	15.3	12.8	23.8

Appendix 7: Concurrent infections in 56 cats with babesiosis

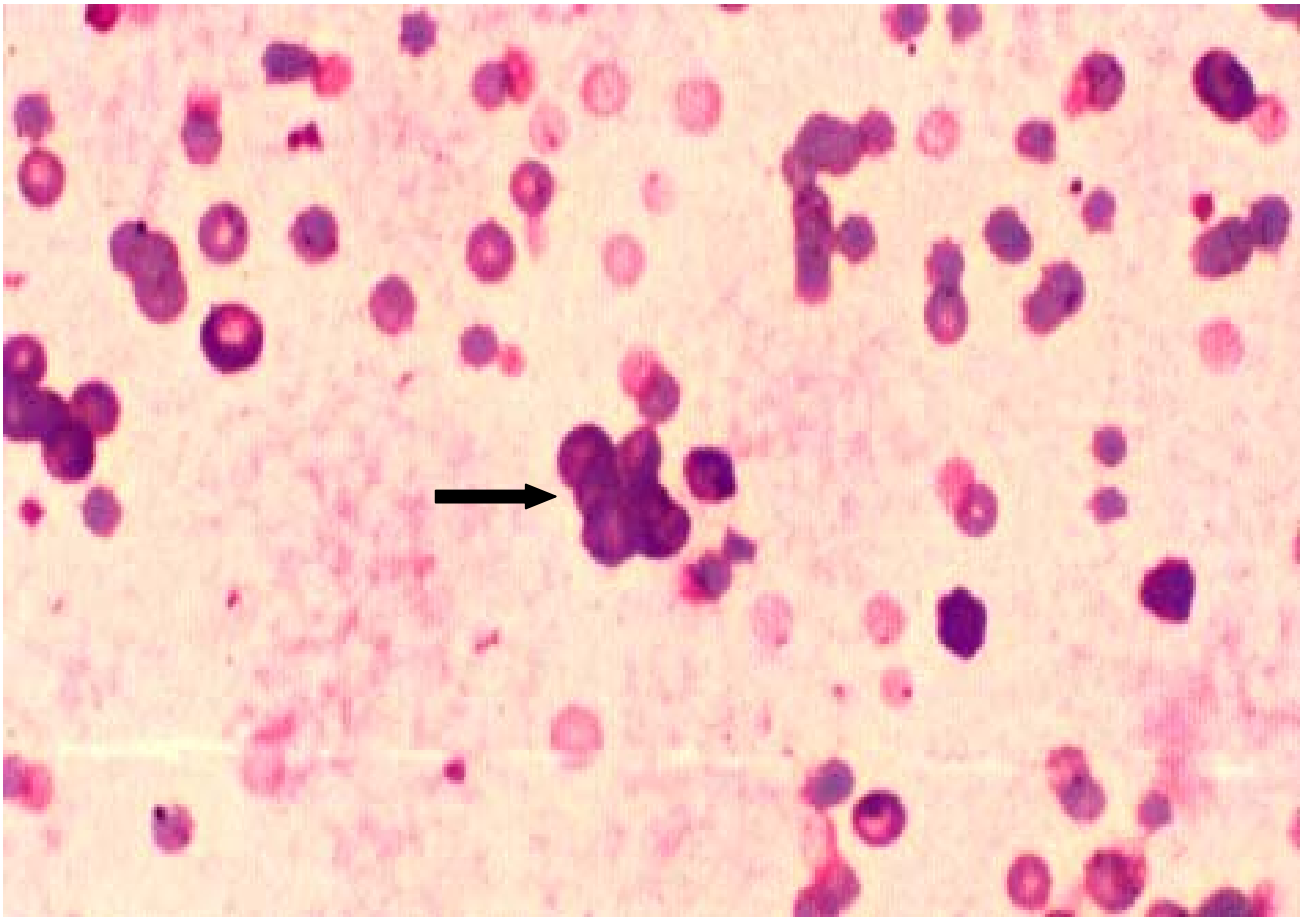
No	*Haemobartonella felis*	FeLV	FIV	Repeat FeLV
1	Negative	Negative	Negative	
2	Negative	Negative	Negative	
3	Negative	Negative	Negative	
4	Negative	Positive	Positive	Not possible: died
5	Negative	Negative	Negative	
6	Negative	Negative	Negative	
7	Negative	Negative	Negative	
8	Negative	Negative	Negative	
9	Negative	Negative	Negative	
10	Negative	Negative	Positive	
11	Negative	Positive	Negative	Negative
12	Negative	Negative	Negative	
13	Positive	Negative	Negative	
14	Positive	Negative	Negative	
15	Negative	Negative	Negative	
16	Negative	Negative	Negative	
17	Negative	Negative	Negative	
18	Negative	Negative	Positive	
19	Negative	Negative	Negative	
20	Negative	Positive	Positive	Not possible: euthanased
21	Negative	Positive	Positive	Positive
22	Negative	Negative	Negative	
23	Negative	Negative	Negative	
24	Negative	Positive	Negative	Positive
25	Negative	Positive	Negative	Not possible: euthanased
26	Negative	Positive	Negative	Not possible: lost to follow-up
27	Negative	Positive	Negative	Negative
28	Negative	Positive	Negative	Not possible: euthanased
29	Negative	Negative	Negative	
30	Negative	Negative	Positive	
31	Negative	Positive	Negative	Not possible: died
32	Negative	Positive	Negative	Negative
33	Positive	Positive	Negative	Negative
34	Negative	Positive	Negative	Negative
35	Negative	Positive	Negative	Negative
36	Negative	Positive	Negative	Negative
37	Positive	Negative	Negative	
38	Negative	Positive	Negative	Not possible: euthanased
39	Negative	Positive	Negative	Negative
40	Negative	Negative	Negative	
41	Negative	Negative	Negative	
42	Negative	Positive	Negative	Negative
43	Negative	Negative	Negative	
44	Negative	Negative	Negative	
45	Negative	Negative	Negative	
46	Negative	Positive	Negative	Negative
47	Negative	Positive	Positive	Not possible: died
48	Negative	Positive	Negative	Not possible: lost to follow-up
49	Positive	Positive	Negative	Not possible: died
50	Negative	Negative	Negative	
51	Negative	Negative	Negative	
52	Negative	Negative	Negative	
53	Positive	Positive	Negative	Not possible: lost to follow-up
54	Negative	Positive	Positive	Not possible: died
55	Negative	Negative	Negative	
56	Negative	Negative	Negative	

Appendix 8: Parasitaemias of 56 cats with babesiosis

No	Peripheral parasitaemia (%)	Central parasitaemia (%)
1	15.4	16.2
2	0.4	0.5
3	1.6	1.3
4	1.3	1.3
5	7.8	7.1
6	7.1	6.5
7	0.4	0.3
8	2.0	1.8
9	6.5	6.6
10	0.7	0.6
11	3.3	3.4
12	4.3	4.0
13	1.6	1.6
14	2.7	2.5
15	2.2	2.4
16	1.9	1.8
17	4.0	4.0
18	2.2	2.4
19	2.3	2.4
20	10.1	10.9
21	5.0	5.2
22	3.1	3.4
23	5.5	5.2
24	3.2	3.6
25	39.8	40.5
26	42.3	41.4
27	39.9	40.1
28	14.1	14.1
29	1.8	2.0
30	0.5	0.4
31	9.2	9.3
32	15.8	15.8
33	40.1	39.2
34	8.3	8.8
35	7.6	8.3
36	30.2	30.6
37	33.3	33.2
38	8.9	8.6
39	0.3	0.2
40	5.9	6.2
41	10.4	10.8
42	40.2	40.1
43	12.8	13.5
44	9.6	9.2
45	10.8	10.5
46	23.9	23.4
47	32.3	32.0
48	7.5	7.7
49	No count possible	14.9
50	3.6	3.6
51	2.4	2.3
52	3.2	3.1
53	0.3	0.2
54	2.1	2.1
55	23.6	24.1
56	6.9	7.2
Median	5.9	6.4
25%	2.2	2.4
75%	12.3	13.8

Appendix 9: In-saline agglutination on a stained bloodsmear of a cat with *B. felis* infection (100x oil immersion)

Note that the agglutinating cells are not only mature, but also immature red blood cells (reticulocytes) and are not parasitised (black arrow).



Appendix 10: Concurrent *H. felis* infection on a stained bloodsmear of a cat with *B. felis* infection (100x oil immersion)

H. felis parasites are visible as small rods on the periphery of the red blood cell (thick black arrow), and as ring-shaped organisms on the surface of the red blood cell. A red blood cell containing a Maltese-cross form of *B. felis* can also be seen (thin black arrow).