The haematological kinetics of canine babesiosis in South Africa

by

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Table of contents

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List of Tables

List of Figures

List of Abbreviations

CBC	Complete blood count
CNP	Circulating neutrophil pool
EDTA	Ethylene diamene tetra-acetic acid
Epo	Erythropoietin
HCT	Haematocrit
HGB	Haemoglobin
IL	Interleukin
IMHA	Immune mediated haemolytic anaemia
ISA	In-saline agglutination
MCHC	Mean cell haemoglobin content
MCV	Mean cell volume
MHC	Major histocompatibility complex
MNP	Marginating neutrophil pool
MODS	Multiple organ dysfunction syndrome
OVAH	Onderstepoort Veterinary Academic Hospital
PCR	Polymerase chain reaction
PCV	Packed cell volume
PLT	Platelet
RBC	Red blood cell
RDW	Red cell distribution width
SIRS	Systemic inflammatory response syndrome
TNF	Tumour necrosis factor
TPP	Total plasma protein concentration
WBC	White blood cell

Summary

The haematological kinetics of canine babesiosis in South Africa

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The course of the haemopoietic response during canine babesiosis caused by *Babesia rossi* has not previously been studied. This prospective, descriptive longitudinal study on clinical cases describes the haematological kinetics during the first six days following treatment of natural babesiosis infection.

Ninety client-owned dogs diagnosed with *B rossi* infection, based on examination of a Cam's Quick-Stain-stained thin capillary blood smear and confirmed by polymerase chain reaction analysis, were included. At first consultation, 24 hours, three days and six days after first consultation, or until death, an EDTA sample was collected from the jugular or cephalic vein and submitted for a full automated blood count, using a CELL-DYN 3700 analyzer. Manual leukocyte differential counts were performed. Based on the treatment protocol, the dogs were divided into a blood transfusion group, and a non blood transfusion group.

A slightly to moderately regenerative normocytic normochromic anaemia occurred throughout the study period for both treatment groups. The anaemia was very severe at presentation in dogs that received a blood transfusion and moderate at presentation in dogs that did not receive a blood transfusion. Anaemia was still present by the end of the study period in both treatment groups. The regenerative response was moderate in severely anaemic dogs and mild in moderately anaemic dogs. A mild inflammatory leukocytic response was found in both treatment groups. The median segmented neutrophil count for both treatment groups was within the reference interval throughout the study period. A left shift occurred more commonly in dogs that received a blood transfusion, and was significantly influenced by the degree of anaemia at presentation. In dogs with a left shift, a degenerative left shift, not influenced by the degree of anaemia at presentation, was found more commonly. Severe thrombocytopaenia for both treatment groups, which resolved within a week in both groups, was found. Treatment with a blood transfusion reduced the anaemia, but had no significant effect on white blood cell or platelet responses. Blood cell responses were not significantly influenced by age, previous infection with babesiosis or duration of illness.

CHAPTER 1 INTRODUCTION

1.1 Background

Canine babesiosis is an economically important and potentially life-threatening disease. Clinically and pathologically it resembles human falciparum malaria. The clinical and haematological profile induced by canine babesiosis varies in different parts of the world depending on the species of babesia involved. The form of canine babesiosis that occurs in South Africa is particularly severe, and is characterized by anorexia, fever, splenomegaly and haemolytic anaemia. South African canine babesiosis is caused by *Babesia rossi*. Recently however, small pockets of *Babesia vogeli* have been described. The incidence of the latter parasite seems to be very low.

Historically a clinical classification system described babesiosis as uncomplicated if the clinical changes could be ascribed to haemolytic anaemia, and complicated if clinical changes could not be ascribed solely to haemolytic anaemia, but rather also to failure or dysfunction of other organ systems. More recently it has been proposed that this clinical classification system should be adapted to identify a single severe disease category.

1.2 Problem statement

Anaemia is central to the pathogenesis of babesiosis and to our understanding of the mechanisms of this disease. Correction of anaemia and resolution of the systemic inflammation is pivotal to recovery from this blood-borne infectious disease. To our knowledge, the course of the haemopoietic response during canine babesiosis has not previously been studied. As such the nature of the anaemia and inflammatory response in babesiosis in comparison to other diseases that also result in anaemia, such as blood loss and nutritional deficiencies, cannot be clearly distinguished. We believe the pathogenesis and response to treatment of babesia-induced anaemia differs from anaemia due to other causes. Presently, many clinicians in general veterinary practice make decisions on response to treatment and progression of disease based on assumptions that have not been tested.

1.3   Research questions

How do the results of complete blood counts of dogs naturally infected with *Babesia rossi* in the Onderstepoort area evolve over the first six days following diagnosis?

How does treatment with a blood transfusion, duration of illness before presentation, previous infection with babesiosis and patient age affect the results of complete blood counts of dogs naturally infected with *Babesia rossi*?

1.4   Objective

The objective of this study was to describe the haematological kinetics in dogs naturally infected with *Babesia rossi* presented to the Onderstepoort Veterinary Academic Hospital, Pretoria, South Africa.

As part of this objective, the following specific aims were achieved:

- A description of temporal changes in the results of complete blood counts of dogs naturally infected with babesiosis and requiring blood transfusion, over the first six days following diagnosis.

- A description of temporal changes in the results of complete blood counts of dogs naturally infected with babesiosis and not requiring blood transfusion, over the first six days following diagnosis.

- A description of the effects of duration of illness, previous infection with babesiosis and patient age on the results of complete blood counts of dogs natuarally infected with babesiosis.

1.5   Benefits

By measuring changes in haematologic results over time, the kinetics of blood cell production, demand or utilization, and destruction can be assessed following diagnosis and treatment of a natural babesiosis infection. This aspect of the natural history of this disease, despite its widespread incidence in South Africa, has to our knowledge, never been evaluated. Because the haematological kinetics of the canine haematopoietic response to infection is generally poorly documented, these results also may be applicable to other types of infections or disease processes in dogs. Evaluation of the effects of blood transfusion, duration of illness before presentation, previous infection with babesiosis and patient age on

the haematological results of dogs with babesiosis also have not previously been investigated in a prospective study and will be valuable for veterinarians evaluating patients before, during, and after treatment. This study will provide a database of haematologic results that can be used in the evaluation of different therapies against intraerythrocytic parasite-induced anaemia and the associated inflammatory response, and will allow comparisons to be made between anaemia induced by haemoprotozoal infection and anaemia caused by other aetiologies, as reported on by other authors. Finally, and importantly, the results of this study may be used to better compare haematologic changes in canine babesiosis and human malaria, and strengthen the basis for using canine babesiosis as a model for this devastating human disease.

CHAPTER 2 LITERATURE REVIEW

2.1   Introduction

As this study was at its core an investigation into blood cell kinetics, it was important that relevant information on bone marrow and blood cell kinetics be understood. This review initially focuses on mammalian bone marrow and blood cell kinetics, with reference to the dog. The laboratory evaluation of anaemia and causes of haemolytic anaemia in dogs are explained. Canine babesiosis is discussed in terms of aetiology, clinical manifestation and haematological aspects. Two more infectious diseases that cause haemolytic disease in dogs, namely African trypanosomiasis and haemotropic mycoplasmosis, are briefly discussed. Human falciparum malaria, an infectious disease that shares many similarities with canine babesiosis, is introduced. Finally some comparisons between canine babesiosis and human malaria are drawn.

2.2   Mammalian bone marrow and blood cell kinetics in health and disease

Blood cell development is initiated in the embryonic yolk sac and continues during gestation in the foetal liver and bone marrow.[54] By late gestation, the bone marrow is the major site of blood cell production and produces granulocytes, monocytes, erythrocytes, megakaryocytes and a small portion of lymphocytes.[54] The major portion of lymphocytes are produced in extra-medullary tissues such as the spleen, lymph nodes, tonsils, gut-associated lymphoid tissue and bronchial-associated lymphoid tissue.[54] After birth, all blood cells are derived from pluripotential stem cells present in bone marrow and blood.[54] These pluripotential stem cells give rise to lymphoid and myeloid stem cells, with the former differentiating into B and T lymphocytes, and the latter forming progenitor cells that include erythroid burst-forming units (which differentiate into erythroid colony forming units), granulocyte/monocyte colony forming units, megakaryocytic colony forming units, eosinophil colony forming units and basophil colony-forming units.[24,54] In situations demanding a profound increase in blood cells, active haematopoiesis may resume in the extramedullary sites of the liver and spleen.[54]

2.2.1   Red blood cells

Erythropoiesis, the process by which committed haemopoietic progenitor cells develop into reticulocytes and erythrocytes, is regulated by stimulatory growth factors (e.g. erythropoietin

[Epo], Interleukin-6 [IL-6], IL-3, IL-11), inhibitory factors (e.g. tumour necrosis factor [TNF]-α, IL-2, glucocorticoids) and transcription factors.[12] Most of the growth factors are not capable of inducing substantial erythropoiesis *in vitro*, but synergize with Epo, the hormone primarily responsible for regulating the production of red blood cells (RBCs).[12] The kidney is the major site of Epo synthesis, while the liver may produce 10-15% of plasma Epo.[12] Hypoxaemia (i.e. low arterial $p0_2$) serves as the specific stimulus for Epo synthesis.[12] Epo is formed within minutes or hours, reaching maximum production within 24 hours.[12] As the approximate maturation time of prorubicytes into reticulocytes is 3-4 days,[12] and reticulocytes remain in the bone marrow for 2-3 days in most species including the dog,[24] RBCs will only appear in the circulating blood approximately five days later.[12] Epo inhibits apoptosis of newly formed progenitor cells, therefore allowing them to develop into mature erythrocytes.[12] In healthy dogs, maturation of reticulocytes occurs partially in peripheral blood, with the mature erythrocyte being organelle-free to facilitate optimal plasticity of shape, enabling passage through capillary beds.[12,24] The rubriblast is the earliest recognizable red cell precursor.[12,24] During maturation, cells become smaller and nuclei become smaller with aggregated chromatin.[12,24] The nucleus is extracted at the metarubricyte or normoblast stage.[24]

Pathological conditions affect the red cell mass by altering the balance between erythrocyte production and erythrocyte destruction, resulting in erythrocytosis or anaemia; the latter being defined as a reduction in the blood haemoglobin (HGB) concentration.[12] It is the kinetics of the progenitor cell compartment that normally determines the size of the red cell mass.[12] The average canine erythrocyte life span in health is 110 days.[24] Senescent erythrocytes are removed from circulation by phagocytosis by macrophages (mostly in the spleen but also in the liver and bone marrow) and less often, by intravascular lysis.[24]

Haemolysis is defined as a shortening of RBC life span (accelerated erythrocyte destruction), which causes kinetic changes in the mature (and immature) erythrocyte compartment.[12] Acute blood loss (haemorrhage) has a similar effect, as erythrocytes are lost from the peripheral blood. Reticulocytosis is the best single indicator of intensified erythropoiesis.[27] It is usually observed 2-4 days after an acute episode of blood loss or haemolysis, usually peaks at 4-7 days and then gradually declines in 2-3 weeks as the depleted red cell mass is replenished.[27] The regenerative response may take 2-5 days to become evident in circulation and therefore the anaemia may appear nonregenerative initially.[20] Normally a greater regenerative response occurs in haemolytic anaemias than in external blood loss anaemia, as the iron and proteins of destroyed RBCs are not lost from the body and are thus more readily available for erythropoiesis than stored iron.[20,24] Even in acute external blood loss anaemias, where iron stores are plentiful, the peak erythropoietic response is usually less than in haemolytic anaemias.[46]

2.2.2   White blood cells

Myeloid stem cells form progenitor cells, including granulocyte/monocyte colony-forming units, megakaryocytic colony-forming units, eosinophil colony-forming units and basophil colony-forming units.[24] These colony-forming units then differentiate into precursor cells and eventually into mature cells.[24] This means that individual white blood cell (WBC) lines develop independently, except for neutrophils and monocytes which originate from bipotential progenitor cells.[55,88]

Neutrophils are the most numerous blood leukocytes in health in dogs and changes in neutrophil numbers therefore most greatly affect the total WBC count.[55] The primary function of neutrophils is phagocytosis and killing of bacteria, but fungi, yeasts, algae, parasites and viruses can also be destroyed.[54,88] Neutrophils may induce antibody-dependent cellular cytotoxicity to destroy infected or damaged cells.[55,88] They are attracted to sites of inflammation by direct migration or chemotaxis.[88] During myeloid differentiation, several well-defined morphological stages of maturation can be identified within the bone marrow, namely the myeloblast, promyelocyte, myelocyte, metamyelocyte, band and segmenter stages.[24,88] Bone marrow neutrophils are divided into two compartments: a proliferation compartment (10-30%, including myeloblasts, promyelocytes, and myelocytes) and a maturation and storage compartment (65-90%, including metamyelocytes, bands, and segmented neutrophils).[88] Mature neutrophils are released in an age-ordered fashion (i.e. more mature cells are released first) into the blood, with the entire generative process from myeloblast to segmenter taking from 3.5-6 days in dogs.[55] Neutrophils in blood are distributed in two interchanging pools namely the marginating neutrophil pool (MNP) and the circulating neutrophil pool (CNP).[24,54,88] The MNP consists of slow moving neutrophils intermittently adhering to the vascular endothelium, facilitated by adhesion molecules on the neutrophils and endothelium.[54,88] The CNP consists of neutrophils moving with RBCs and plasma, and is sampled by venipuncture to yield the WBC count.[54,88] In the dog the size of the MNP approximates that of the CNP.[54,88] Neutrophils move in a unidirectional non age-ordered fashion from blood to tissues, with a circulating half-life of approximately 5.5-7.5 hours in dogs[54,88] and a tissue survival time of 1-2 days[55] or 1-4 days.[88] Neutrophils then undergo apoptosis, and are recognized and phagocytosed by macrophages without the release of potentially toxic intracellular components.[55,88] The circulating neutrophil count is determined by the rate of marrow release, distribution between the MNP and CNP and the rate of migration from blood to tissues.[88] If tissue demand for neutrophils increases, the bone marrow storage pool of mature segmenters becomes depleted, after which less mature forms appear in the circulation, constituting a left shift.[88,90] When immature neutrophils outnumber segmented (mature) neutrophils or when increased numbers of immature neutrophils are

present together with normal or low numbers of segmented (mature) neutrophils, the left shift is described as "degenerative".[90] A degenerative left shift is considered to be an inappropriate inflammatory response, as the bone marrow's ability to supply neutrophils to the blood is exceeded by the rate at which neutrophils move into inflamed tissue.[90] This occurs in severe disease and may warrant a guarded prognosis.[54] Neutrophilia may be caused by a physiological response to epinephrine, with fear, excitement or strenuous exercise, to cortisol release during stress, or by inflammation.[54,90] Physiological neutrophilia is uncommon in dogs.[24] Neutrophilia, together with lymphopaenia and monocytosis, occurs commonly with haemolytic anaemia.[24,31,88] The neutrophilia is caused by the release of inflammatory cytokines, including granulocyte-colony-stimulating factor associated with an immune response, necrosis and erythrophagocytosis by macrophages.[31] Neutropaenia occurs in a variety of disease conditions and may be due to defective bone marrow production, a shift from the CNP to the MNP or increased tissue demand in excess of bone marrow production.[55,88]

Lymphocytes are the second most numerous blood leukocyte in dogs.[54] Blood contains less than 5% of the total body lymphocyte population.[55] Lymphocytes are essential components of humoral and cell mediated immunity.[24,54] Committed lymphoid stem cells in the bone marrow form T cell precursors that then migrate to the thymus, the central lymphoid organ where T lymphocytes are educated and undergo positive and negative selection to ensure self-tolerance.[71] Lymphocytes surviving the selection process, mature and function as either CD4+ T-helper cells (that can recognize antigen only in the context of class II major histocompatibility complex [MHC] antigens) or CD8+ cytotoxic T cells (that can recognize antigen only in association with class I MHC antigens).[71] T lymphocytes form an important portion of the cell-mediated arm of the immune system[24,54] and modulate the activity of other cells.[55] B cell development takes place in the bone marrow, in this case then the major central lymphoid organ.[71] Stages in B cell development are not recognizable morphologically, with the exception of plasma cells.[71] B lymphocytes are involved in humoral immunity by producing antibodies.[55] B lymphocytes generally have a life span of days to weeks, while T lymphocytes have a life span from months to years.[54] Lymphocytes are the only leukocytes that recirculate[24] and that can undergo mitosis.[86] T lymphocytes are the predominant circulating lymphocyte, while B lymphocytes are transient members of the circulating population.[24] The blood transit time of a lymphocyte is approximately 30 minutes.[24] Lymphocyte recirculation is a non-random event, allowing lymphocytes an increased opportunity to perform immune surveillance and encounter antigens.[55] Lymphocytosis can be caused by epinephrine release with fear or excitement (in horses, cats, and pigs, but not dogs), hypoadrenocortism, chronic antigenic stimulation or inflammation[90] and lymphoid neoplasia.[55] Lymphopaenia can be caused by cortisol release

in stress or acute systemic infection, disruption of lymph node architecture, immunosuppressive drugs, radiation, loss of lymphocyte-rich lymph and acquired or congenital T lymphocyte deficiency.[55,90]

Monocytes (in peripheral blood) and macrophages (in tissues) comprise the mononuclear phagocytic system.[7] Macrophages phagocytose and digest cellular debris, micro-organisms and particulate matter, secrete cytokines and chemical mediators of inflammation, present antigen to lymphocytes and are cytotoxic against tumours and foreign cells.[7,54] Monocytes share a common parental stem cell with neutrophils, but unlike neutrophils they are released approximately six days after generation,[7] with no maturation or storage pool in the bone marrow.[54] While data on canine and feline monocytes are not available, it is known that human monocytes have a 1:3.5 distribution between the CNP and MNP, and have a short circulating half-life of 8.4 hours,[54] 24-36 hours[24] or 70 hours.[7] (The different values for circulating half life available in the literature are probably due to difference in experimental technique).[88] Monocytes randomly leave blood to enter tissues and do not return to the circulation.[24,54] Monocytes accumulate specifically at the site of inflammation, in lower numbers than neutrophils.[7] Most macrophages at inflammatory sites are recruited from blood monocytes.[7] The life span of tissue macrophages is unknown, but it is assumed that resident macrophages are long-lived, while macrophages or monocytes responding to inflammatory stimuli are short-lived.[7] While monocytopaenia is a rare occurrence in domestic animals,[7] monocytosis occurs commonly in acute and chronic diseases, including neoplasia, infections, immune-mediated diseases, haemorrhage, haemolysis, trauma and corticosteroid treatment.[54] Monocytosis due to cortisol is more prevalent in the dog than in the cat.[24]

Eosinophils reside in the loose connective tissue of organs that serve as entry points for foreign substances, predominantly the skin and the respiratory and gastrointestinal tracts.[101] They act as a defence against helminth parasites by releasing toxins and hydrolytic enzymes directly onto the target.[101] They also modulate the inflammatory process, as they remove immune complexes and granular debris released from mast cells and prevent degranulation of and inactivate mediators released from mast cells and basophils.[101] Eosinophils can phagocytose particulate matter, including bacteria, immune complexes, yeasts, protozoa and mast cell granules, though less effectively than neutrophils.[67] Eosinophils differentiate and mature in the bone marrow over a period of 2-6 days depending on the species.[101] Production and maturation parallels that of the neutrophil, with a maturation and storage pool.[24] IL-1 and IL-3 are the primary stimulating factors acting on primitive myeloid precursors and directing their development towards eosinophil progenitors.[67] IL-5 is the main cytokine controlling eosinophil production by stimulating committed eosinophil progenitors.[24,67] Blood transit time is 30 minutes in the dog.[24] There is an even distribution

between marginating and circulating pools.[24] Eosinophils emigrate from the blood randomly, and will enter the tissue within 12 hours of stimulation, accompanied by a variety of other leukocytes.[67] In the tissue, they function for several days.[24] As the eosinophil is predominantly a tissue cell, the number in peripheral blood is only a crude indicator of the dynamic state and eosinophilia is not consistently observed with eosinophilic inflammation.[67] The most common cause of eosinophilia in the dog is parasitism, by endo- and ectoparasites.[86] Eosinophilia is also observed in hypersensitivity disorders, mast cell neoplasia and hypoadrenocortism.[90] Stress-induced eosinopaenia is caused by cortisol, which is thought to slow the release of eosinophils from bone marrow.[67]

Basophils are the least numerous leukocyte in the blood of dogs.[54] Their functions include participation in immune-mediated inflammatory reactions and tumour cytotoxicity[54] and their activity is largely mediated by T lymphocytes.[54] They are a source of mediators of inflammation.[24] Kinetic data for production and circulation of basophils have not been determined in dogs.[54] Stages of proliferation and maturation parallel those of the neutrophil.[24] Some studies suggest that basophil development may be related to mast cell or eosinophil production, but there is no evidence that the basophil is a precursor of the tissue mast cell.[24,54] Bone marrow storage of basophils is minimal.[24] Basophilia usually occurs concomitant with eosinophilia, and heartworm infection is the most predictable cause of basophilia in dogs.[54] Unlike eosinophils, circulating basophils are not affected by cortisol.

2.2.3 Platelets

Platelets are small cytoplasmic fragments of megakaryocytic origin.[6] They primarily function in haemostasis, by adhering to the subendothelium, aggregating, recruiting additional platelets to the area and facilitating the localized formation of thrombin and fibrin to minimize blood loss.[32] They also play an essential role in inflammation and wound healing through direct cell-cell interactions and the release of soluble mediators from activated platelets.[32] Megakaryocytes in the bone marrow are derived from megakaryocyte progenitor cells, which are in turn derived from myeloid multipotential stem cells.[24] Platelets are produced by cytoplasmic demarcation of mature megakaryocytes, and are released directly into the blood.[24] Platelet circulating life span is approximately ten days[24] or 5-9 days.[83] They are removed from circulation by age-dependant attrition.[83] Thrombocytopaenia may be caused by decreased production, or increased destruction, sequestration or loss.[6] Thrombocytosis may be secondary (reactive) to acute or chronic inflammation, iron deficiency and hyperadrenocortism.[6]

2.3 Anaemia

2.3.1 Laboratory evaluation of anaemia

Anaemia is defined by an absolute decrease in RBC count, HGB concentration and haematocrit (HCT).[6,24,46] These three measurements of red cell mass are interrelated and tend to parallel each other.[68] HCT is most easily measured by the general practitioner, and therefore most frequently used.[68] The RBC indices mean cell volume (MCV) and mean cell haemoglobin concentration (MCHC) are beneficial in the assessment of an anaemic patient.[6] It is common practice to initially classify an anaemia morphologically on the basis of the MCV and MCHC,[24,46] as macrocytic, normocytic or microcytic and hypochromic or normochromic, respectively.[6,24] After classifying the anaemia morphologically, the degree of reticulocytosis is then examined to evaluate the bone marrow response, and the anaemia is thus further classified into responsive (regenerative) or non responsive (non regenerative).[46] The absolute reticulocyte count is the best indicator of effective erythropoiesis.[20,46] The RBC indices are insensitive indicators of regeneration. Markedly regenerative anaemias may occasionally present as macrocytic and hypochromic.[20] If the regenerative response is less pronounced, the MCV will not increase, as a large change in the number of macrocytes is required to increase the MCV.[74] If there is significant microcytosis or macrocytosis (as with reticulocytosis), red cell distribution width will increase.[24] The two broad pathogenic mechanisms associated with regenerative anaemia are acute blood loss and haemolysis,[6,46] with haemolysis usually causing a greater regenerative response than external blood loss,[46] as explained before.

2.3.2 Causes of haemolytic anaemia in dogs

Haemolytic anaemia may ensue when an immune response targets erythrocytes.[33] In primary immune mediated haemolytic anaemia (IMHA), no underlying cause of the immune response can be identified, while in contrast secondary IMHA is associated with an underlying condition.[5,66] Anti-RBC antibodies, including IgG, IgM and IgA, attach directly or indirectly to various components of the RBC membrane.[5] Causes of secondary IMHA include infection (such as babesiosis, dirofilariasis, pyometra, pyelonephritis), neoplasia (such as lymphoma, hemangiosarcoma) and drugs (such as penicillins, cephalosporins).[5] Immune-mediated haemolysis may occur alone, or in conjunction with other causes of haemolysis, such as direct parasite destruction of erythrocytes in babesiosis.[5]

Haemolytic anaemia may also be caused by physical damage to RBCs leading to fragmentation and intra- and extravascular haemolysis, as occurs in microangiopathic haemolytic anaemia.[33] This may be caused by near drowning in fresh water as result of

hypotonicity, heat stroke, heart valve disease, hemangiosarcoma and vasculitis.[33] Haemolytic anaemia can also result from oxidative damage induced by various agents, drugs and food additives and food components. Denatured and precipitated haemoglobin, known as Heinz bodies, causes erythrocytes to become rigid with reduced deformability, which can then lead to lysis or removal by macrophages.[33] Heinz bodies may be caused by onions, garlic, methylene blue and acetaminophen. Zinc, copper and naphthalene may cause direct oxidative and other types of RBC damage leading to intravascular haemolysis. Hypophosphataemia may also induce haemolysis due to depletion of adenosine triphosphate (ATP).[33] Inherited erythrocyte defects, such as phosphofructokinase deficiency in English Springer Spaniels and pyruvate kinase deficiency in Basenjis, West Highland White Terriers, Pugs and Dachshunds, may also cause haemolytic anaemia.[33] Infectious diseases that cause haemolytic anaemia in dogs include African trypanosomiasis, haemotropic mycoplasmosis and canine babesiosis.

2.4   Canine babesiosis

2.4.1   Aetiology of canine babesiosis

Canine babesiosis, a potentially life-threatening tick borne disease with haemolytic anaemia as its hallmark, is caused by the haemoprotozoan parasites *Babesia canis* and *B gibsoni*.[29,91] *B canis* is characteristically larger (2.5-5.0 μm in diameter) than *B gibsoni* (1.2-1.9 μm). The taxonomy of both these organisms has been revisited recently. Based on phylogenetic studies of the 18S ss-rRNA gene, *B conradae*[51] and a *B microti*-like[11,102] canine isolate named *Theileria annae*[9,11] (or *B annae*)[10] have been described as two small canine piroplasms genotypically distinct to *B gibsoni*.[50] The taxonomy of *B canis* has also been elucidated further. Based on cross-immunity and serological testing, Uilenberg *et al.*[95] proposed three subspecies of *B canis*: *B canis canis* with a European distribution transmitted by *Dermacentor spp*; *B canis vogeli* with a tropical and subtropical distribution in all continents transmitted by *Rhipicephalus sanguineus*, and the African subspecies *B canis rossi*, transmitted by *Haemaphysalis leachi (H leachi* has recently been redescribed as *H elliptica*[4]). A later study also suggested that the large *Babesia* species be classified into three subspecies.[38] With the use of in vitro polymerase chain reaction (PCR) techniques, genotypes determined by sequencing, segregated *B canis* isolates into three groups near the species level.[103] More recently it was proposed that these three subspecies be considered true species, based on sequence analysis of DNA fragments using restriction fragment length polymorphism and phylogenetic analyses.[14] This author found that the three (sub) species belong to the same clade (even though they cluster differently in this clade) therefore corresponding to *Babesia* species *senso stricto*. Recently a new large *Babesia* species

capable of infecting domestic dogs has been described, albeit in an immunocompromised dog.[8]

For many years, the only known *Babesia* species in dogs in South Africa was *B (canis) rossi*.[42] Since 2004 it has been reported (in a study based on PCR analysis) that although *B rossi* is the most prevalent piroplasm in South Africa, *B vogeli* also occurs in this region.[64] *B vogeli* was found in blood collected from clinically healthy dogs from a shelter in Bloemfontein, South Africa, and also in one dog from the Onderstepoort area near Pretoria, South Africa.[64] This dog was presented to the Veterinary Academic Hospital, Onderstepoort showing clinical signs suspicious for canine ehrlichiosis.[64] *B rossi* is the most virulent species of all the canine babesial parasites.[9,85,91,95,103]

2.4.2   Clinical presentation of canine babesiosis

Babesia spp cause anaemia purportedly by both antibody mediated erythrocyte destruction and direct parasite damage, resulting in both extravascular and intravascular haemolysis.[62,91] While haemolytic anaemia is a hallmark of this disease, disease phenotype may vary.[91] This variable clinical presentation has resulted in the clinical classification of canine babesiosis into uncomplicated and complicated forms.[43] Uncomplicated canine babesiosis typically presents with signs attributed to mild or moderate acute haemolysis only (with no additional organ failure or clinically important dysfunction), such as fever, anorexia, depression, pale mucous membranes, splenomegaly and a water hammer pulse. In a study of 662 hospitalized cases, 50% had severe anaemia, 32% moderate anaemia and 18% were non-anaemic.[81] Complicated canine babesiosis involves clinical manifestations in addition to the haemolytic disease and includes acute renal failure, cerebral babesiosis, coagulopathy, icterus and hepatopathy, in-saline agglutination (ISA) positive immune-mediated haemolytic anaemia (although all babesia infected dogs are regarded as having a haemolytic anaemia, a sub-set of infected dogs will develop haemolytic anaemia that is ISA positive), peracute babesiosis, pulmonary oedema and acute respiratory distress syndrome, haemoconcentration (so called "red biliary") and shock.[43] Very severe anaemia is also regarded as a complicated form of the disease by some clinicians. It seems that the manifestations of complicated babesiosis occur as a result of the host inflammatory response.[97] In a retrospective study of 91 cases of complicated babesiosis, 87% were regarded as positive for the systemic inflammatory response syndrome (SIRS).[97] It was concluded that SIRS and subsequent organ dysfunction resulting from SIRS known as multiple organ dysfunction syndrome (MODS), occur frequently in complicated canine babesiosis.[97]

In a 2006 review of the South African form of severe and complicated babesiosis it was proposed that an important overlap existed between "severe uncomplicated" (i.e. severe haemolytic anaemia) cases and "complicated" cases, that all these cases should simply be called "severe babesiosis" and that the focus should be on identifying those dogs in need of intensive treatment.[42] Since the 1994 review of babesiosis,[43] various authors have added to our knowledge of the pathogenesis and clinical manifestations of canine babesiosis caused by *B rossi*. Pancreatitis was described as an additional but rare complication of canine babesiosis.[70] It has been recognized that hypotension,[45] hypoglycaemia,[48] hyperlactaemia[75] and acid base disturbances[56] occur regularly in hospitalised cases of canine babesiosis. Renal failure, as previously described, is now also considered rare,[60] but may occur in association with rhabdomyolysis, which is another very unusual complication of babesiosis.[44] The cardiovascular system has also recently been investigated, with Dvir *et al.*[25] finding electrocardiographic changes in babesiosis consistent with myocarditis and myocardial ischemia. Lobetti[58] measured cardiac troponin I and T, and found that high cardiac troponin I correlated with disease severity, also supporting myocardial complications. This author suggested that cardiac and renal involvement could be linked.[57] It is clear, as proposed previously,[19] and in light of these clinical advances described since 1994, that severe babesiosis may share many similarities with other pro-inflammatory states, such as bacterial sepsis, endotoxaemia and human malaria.

2.4.3 Haematological features of canine babesiosis

The most important haematological findings in canine babesiosis are anaemia[42,81] and thrombocytopaenia.[49,81] The haematological and clinical profile induced by canine babesiosis varies markedly in different parts of the world. This variation probably is due to differences in pathogenicity and virulence among the three vector-specific (sub) species.[85,95]

Although reports from all over the world have been published on the haematology of canine babesiosis, case numbers are usually small or they provide little data. The following reports are on case series of less than 40 dogs. One case of canine babesiosis was reported in a Basset Hound from California with anaemia and lymphocytosis; the *Babesia* species was not mentioned, but from photomicrographs, it appeared to be a large *Babesia* species.[3] Haematologic findings in a litter of puppies from a kennel in north Florida infested with *R sanguineus* (the vector *of B vogeli*[95]) included regenerative anaemia, leukocytosis with neutrophilia and lymphocytosis, and thrombocytopaenia.[37] Haematologic values from 32 dogs with babesiosis in Thailand indicated neutropaenia and eosinopaenia; however, concurrent infections with other organisms such as *Ehrlichia sp* and *Mycoplasma haemocanis* were not ruled out.[84] Similarly, a Pit Bull terrier with babesiosis from Korea had

lymphocytosis, but otherwise had neutropaenia, anaemia, and thrombocytopaenia.[87] The *Babesia* species from Thailand and Korea were not speciated but *B canis* and *B vogeli* have recently been identified by PCR analysis to be prevalent in Thailand.[21] Two studies reported on the haematological findings of dogs with babesiosis in Spain. Domina[22] described promyelocytes in circulation in 40 dogs with canine babesiosis. Furlanello *et al.*[30] reported on 23 dogs (of which 17 travelled to Bosnia, Herzegovina, Croatia or Hungary before becoming ill) with mildly regenerative normocytic normochromic anaemia and variable leukocyte abnormalities. The babesial species involved in these two studies were not determined by PCR analysis; both *B canis* or *B vogeli* are prevalent in these and surrounding areas.[21,23,28] In a study of 32 dogs infected with a large *Babesia* species in northern Australia, a wide range of haematological results (ranging from severely anaemic to nonanemic and neutrophilic to neutropaenic) was found.[41] The *Babesia* species again was not determined, but *B canis* and *B vogeli* are prevalent in northern Australia.[47] The following four reports provided limited data on canine babesiosis occurring on the African continent. Omamegbe[76] reported on 20 dogs in Nigeria with canine babesiosis, and described anaemia, eosinophilia and neutrophilia. Matthewman[65] reported on nine dogs in Harare, Zimbabwe with babesiosis that had regenerative or non-regenerative anaemia and thrombocytopaenia. However, it was unclear whether or how accurately concurrent ehrlichiosis was ruled out. Moore reported on 12 cases of canine babesiosis from South Africa and described a wide variation in both total WBC counts and packed cell volumes.[72] A leukaemoid response (i.e. a total WBC count of more than $50 \times 10^9/l$[31]) was described by Lobetti in two dogs with canine babesiosis.[59] The *Babesia* species involved in these four studies from the African continent were not determined by PCR analysis.

Reports on more substantial case numbers (40 cases or more) were found from all over the world. Reports from the European continent on canine babesiosis include 133 cases from France,[78] 45 cases from Spain[82] and 248 cases from Poland.[104] Reports published from the African continent include 70 cases from Nigeria[1] and 662 cases from South Africa.[81] There is also a report on 291 cases from the Philippines.[13] All of these reports documented haematological data from a single blood sample usually taken soon after diagnosis based on examination of a peripheral blood smear, but it was never stipulated exactly when the sample was collected. The *Babesia* species was not determined in any of these studies, but *B canis* has been reported to occur in France,[9] *B canis* and *B vogeli* have been reported to occur in Spain,[21] Nigeria[95] and Poland,[95] and *B rossi* and *B vogeli* have been reported to occur in South Africa.[42,64,95]

The haematological profiles of dogs from France, Spain, Poland and the Philippines were similar, apart from higher lymphocyte counts in dogs from the Philippines.[13] Compared to these above-mentioned four studies, dogs with the South African disease had a more severe anaemia, neutrophilia and monocytosis. Some of the haematological profiles in dogs from Nigeria were similar to those of dogs in South Africa. This concurs with the fact that the most prevalent African species (*B rossi*) causes more severe haematological changes than the less virulent *B canis* found in Europe. The acute presentation of canine babesiosis, characterized by life-threatening haemolytic anaemia, is most commonly encountered in South Africa.[42] As *H leachi* was found on 77% of dogs with babesiosis from the Onderstepoort area near Pretoria,[39] it can be concluded that most cases of canine babesiosis studied in this area of South Africa were caused by *B rossi*.[42] In one molecular study in the Onderstepoort area, *B rossi* was found in 18 of 56 (32.1%) canine blood samples and *B vogeli* was found in 1 of 56 (1.8%) samples, based on PCR analysis.[64]

The retrospective South African study of 662 cases hospitalised at the Onderstepoort Veterinary Academic Hospital was conducted to establish whether there were significant differences or changes in haematological or other laboratory values in dogs grouped according to degree of anaemia and mortality.[81] The findings in this study lent credence to the hypothesis that a proportion of dogs develop a complicated form of babesiosis similar to the complicated form of falciparum malaria in humans, with the same age predisposition and the tendency to develop a severe inflammatory response. The study comprised the largest number of cases of canine babesiosis caused by *B (canis) rossi* that has been published to date. Fifty percent of dogs had severe anaemia (HCT less than 0.15 l/l), and 32% had moderate anaemia (HCT 0.15-0.29 l/l). Dogs in both these groups had leukocytosis, neutrophilia with a left shift, monocytosis and thrombocytopaenia. Lymphocyte counts were within the reference interval. Eighteen percent of the dogs were not anaemic (HCT greater than 0.29 l/l), and had total WBC, segmented neutrophil and monocyte counts within reference intervals, but marginal lymphopaenia and thrombocytopaenia. These authors also found that differential leukocyte counts for segmented neutrophils, band neutrophils and lymphocytes were inversely related to HCTs, with severely anaemic dogs having moderate leukocytosis and non-anaemic dogs having marked lymphopaenia (46% were lymphopaenic). The mean age of the non-anaemic group was three times higher than that of the severely anaemic group. A high HCT, low lymphocyte count and high number of metamyelocytes were indicators of poor prognosis. In summary, the most important findings of this study were the lack of inflammatory response and older age distribution in the non-anaemic group versus the presence of an inflammatory response and younger age distribution of the anaemic group. It was speculated that older dogs mount a massive inappropriate inflammatory response, with or without being primed by a first infection causing

haemolysis. As this was a retrospective study, there was insufficient data on previous exposure. It was also speculated that the lack of inflammatory response was due to the disease process being rapid and overwhelming in the non-anaemic group, with no time for an inflammatory reaction to become evident. This hypothesis could not be tested, as no follow-up blood samples were analysed. The study was not designed to evaluate haematological kinetics.

2.4.4   Studies of haematological kinetics of canine babesiosis

No substantive reports involving serial haematological sampling or describing the blood cell kinetics of a natural infection of canine babesiosis could be found in the literature. Maegraith[62] reported reticulocyte percentages over a period of eight to 20 days following the experimental infection of 60 dogs with *Babesia sp* and total WBC counts in ten puppies from infection until death. A rising reticulocyte percentage, proportional to the severity of anaemia, was found in all cases except in peracute deaths (within one or two days after first appearance of parasites in the blood). Leukocytosis occurred in eight of the ten puppies, while two became leukopaenic. To investigate the haematologic abnormalities caused by local Nigerian and Mexican strains of *Babesia sp,* four splenectomised dogs were sampled for two weeks after experimental infection with both strains.[2] The case numbers were too low and the dogs not spleen-intact, making valid conclusions about the natural disease impossible. In a case report from Germany, HCT and platelet (PLT) counts were reported in samples collected over a ten day period from one canine patient with babesiosis.[40] Again no valid conclusions can be drawn for the canine population from this study of one dog. In a study on the prophylactic activity of imidocarb in seven dogs experimentally infected with a *Babesia sp* strain from Gibraltar, HCT and PLT counts were reported over a period of three weeks, with anaemia and thrombocytopaenia still present after a week.[96] A French study of 48 dogs naturally infected with *Babesia sp*, reported serial changes in lymphocyte and monocyte counts within hours after taking an initial blood sample.[36] It was found that thrombocytopaenia and eosinopaenia persisted throughout the 15 day observation period, but the initial lymphopaenia found in the first few hours, normalised. In another study of 31 cases of canine babesiosis in India, haematological sampling was done on consecutive days over a seven day period, but the results were not reported.[73] In a Croatian study on the acute phase response in 50 dogs with *Babesia sp* infection, HCTs, WBC counts and PLT counts were reported over a period of seven days after antibabesial treatment.[63] The median HCT values showed an upward trend and increased to within the reference interval four days after antibabesial treatment. Median WBC counts were significantly higher after treatment. Median PLT counts showed an upward trend, but were still below the reference interval four days after treatment and did not increase to within the reference interval until seven days

after treatment. The *Babesia* species was not determined in any of these studies, but was in most cases not *B rossi*, as this parasite has never been described in any of these areas, except for possibly Nigeria and Gibraltar, where *B (canis) rossi* has been described as the African (sub) species.[95]

2.5 Infectious diseases other than babesiosis causing haemolytic anaemia in dogs

2.5.1 African trypanosomiasis

In Africa, dogs are susceptible to infection with *Trypanosoma brucei, T congolense* and *T evansi*.[35] Trypanosomiasis is transmitted by tsetse flies of the genus *Glossina*.[35] Clinical signs in acutely infected dogs include anorexia, weakness, fever, oedema of the face and the genitalia, purulent ocular and nasal discharge, pale mucous membranes, lymphadenomegaly, splenomegaly, petechial haemorrhage and mucosal bleeding.[26,35] Chronically infected animals will show weight loss. Neurological abnormalities include mental dullness (sleepiness, as occurs in people) and are progressive, resulting in behaviour changes similar to those observed in rabies.[35] Anaemia is a consistent feature of trypanosomosis and is probably due to the increased destruction of RBCs by the trypanosome organism and increased erythrophagocytosis.[26] Haematological features therefore include macrocytic anaemia with marked reticulocytosis, neutropaenia, lymphopaenia, eosinopaenia and thrombocytopaenia.[26,35] Some dogs may have leukocytosis with neutrophilia.[35,77]

2.5.2 Haemotropic mycoplasmosis

Haemotropic mycoplasmosis in dogs is caused by *Mycoplasma haemocanis* (formerly known as *Haemobartonella canis*).[34,69] While the natural mode of transmission in the dog remains unknown, experimental transmission by *R sanguineus* has been demonstrated.[34,69] The disease may vary from acute life-threatening haemolytic anaemia to more subtle chronic anaemia, ill-thrift and infertility.[69] Organisms attach to and grow on the RBC surface.[16] Clinically important haemotrophic mycoplasmosis usually occurs only in splenectomised dogs, but cases have been described in dogs with concurrent babesiosis, ehrlichiosis, and bacterial and viral infections and dogs on immunosuppressive drug therapy.[34] Clinical signs include anorexia, lethargy and fever.[69] Haematological features include severe anaemia, reticulocytosis and thrombocytopaenia, with mild anaemia and leukopaenia in chronically infected dogs.[69]

2.6   Human malaria

2.6.1   Aetiology and epidemiology of human malaria

In humans, malaria is caused by *Plasmodium falciparum, P vivax, P malariae* and *P ovale,* but usually only *P falciparium* produces fulminant disease.[15,98]   In areas of intense and continuous transmission of *P falciparum* (holoendemic malaria) mortality is highest in children between one and four years of age, but is followed by acquisition of solid immunity so that by the time adulthood is reached, the infection is largely asymptomatic.   Many infections present as an uncomplicated febrile illness.[15]   In areas where transmission is intense but seasonal (hyperendemic malaria), immunity within the population is lower and all age groups suffer from symptomatic malaria.[100]   Severe anaemia in young children is the principal manifestation under these circumstances.[98]   In areas where transmission is weak and epidemics may occur, (hypoendemic malaria) the disease is described as unstable.[98]   Symptomatic disease is seen at all ages, and the principal manifestation of severe malaria under these circumstances is cerebral malaria.[98]   Passive transfer of maternal immunity protects newborns from severe anaemia in endemic areas, but severe anaemia affects children aged 1-3 years in areas of high transmission and cerebral malaria affects older children in areas of lower transmission.[15]

2.6.2   Clinical presentation of human falciparum malaria

Acute malaria may be complicated by cerebral malaria, severe anaemia or other severe manifestations such as respiratory distress and hypoglycaemia.[53,100]   Chronic malaria, which results from repeated or chronic parasitism in semi-immune individuals, results in an afebrile manifestation of severe anaemia with a low parasitaemia.[100]

2.6.3   Haematological features of human falciparum malaria

The anaemia of human malaria is normocytic and normochromic, although microcytosis and hypochromasia due to iron deficiency and/or thalassaemia, may be present.[15]   Most patients with cerebral malaria have variable and sometimes severe anaemia at presentation that worsens in two thirds of cases, reaching a nadir 1-17 days after initiating treatment.[100]   Reticulocytosis does not occur for many days.[98]   There is in fact a notable absence of reticulocytes.[15]   The anaemia of malaria results from a combination of parasitized erythrocyte destruction at merogony, accelerated removal of unparasitized red cells and ineffective erythropoiesis.   There is modest leukocytosis, but both leukemoid reactions and leukopaenia have been described.[15]   The neutrophil count may be increased during the first two days of fever; thereafter it may be decreased.[100]   Neutrophil counts may rise in severe disease

(which may also suggest concurrent bacterial infection[15]) and neutrophilia indicates a poor prognosis.[98] Patients with acute falciparum malaria have eosinopaenia, with the eosinophil count then increasing in convalescence over 5-10 weeks.[100] Monocytosis and lymphocytosis are seen with acute infections.[15] There is mild to moderate thrombocytopaenia without hemorrhagic manifestations, which correlates with the degree of parasitaemia[100] but not with disease severity.[15] Bone marrow megakaryocytes are normal in number in malaria.[98] There is no evidence for platelet-specific alloantibodies, and no defect in thrombopoiesis has been demonstrated.[15]

2.7   Comparisons between human malaria and canine babesiosis

The protozoan parasites that cause babesiosis and those that cause malaria, are both transmitted to mammals by arthropod vectors, both invade the erythrocyte, both look somewhat similar on Giemsa-stained blood smears and both produce remarkably similar diseases.[19] Canine malarial organisms have not been identified, but mice and humans are susceptible to both babesiosis and malaria.[19] Studies of murine babesiosis and malaria have led to the proposal that TNF and similar mediators are common to their pathogenesis.[17] Studies in mice, dogs and humans using recombinant TNF have shown that it is possible to reproduce the illness and pathology seen with babesiosis and malaria, giving credence to the hypothesis that this cytokine is central to the disease process.[18,19]

The clinical similarities between human malaria and canine babesiosis have been summarized.[43] The following clinical signs or syndromes occur in both diseases: pyrexia, severe anaemia often disproportionate to the parasitaemia, severe haemoglobinuria, acute renal failure, cerebral involvement, coagulopathy, hypotensive shock, icterus and pulmonary oedema. Hypoglycaemia[48,79,99] and hyperlactaemia[52,75] have also been described in both disease conditions. Immune mediated haemolysis has not been demonstrated in human malaria. Results of a retrospective study of 662 hospitalised cases of canine babesiosis, in which older dogs developed an acute overwhelming systemic inflammatory response, lend support to the hypothesis that a small but identifiable proportion of dogs develop a complicated form of babesiosis similar to the complicated form of falciparum malaria.[81]

2.8   Conclusions drawn from literature review

It is clear that available data on the haematological features of canine babesiosis caused by *B rossi,* are limited to one (or possibly two) report(s),[1,81] even though this South African form of canine babesiosis causes life-threatening disease, is comparable to human falciparum malaria and shares similarities with other canine infectious diseases causing haemolysis.

There are no studies reporting on haematological kinetics after infection with *B rossi*. It is therefore not possible to compare the haematological response to this parasite-induced haemolytic anaemia with other diseases causing anaemia. The major purpose of this study was to address this paucity of knowledge on the haematological kinetics of canine babesiosis in South Africa.

CHAPTER 3 MATERIALS AND METHODS

3.1　Experimental design

This was a prospective, descriptive longitudinal study of clinical cases of canine babesiosis.

Ninety dogs presented to the Outpatients Clinic of the Onderstepoort Veterinary Academic Hospital (OVAH) that were diagnosed with babesiosis were included in the study.

Inclusion criteria:

- Dogs diagnosed with *Babesia sp* infection, based on microscopic examination of an air-dried Cam's Quick-Stain-stained thin capillary blood smear, obtained from the anterior edge or hairless ventral surface of the ear;

- Dogs diagnosed by polymerase chain reaction (PCR) analysis to be positive for *Babesia rossi* infection;

- Dogs of any breed, age and either sex;

- Dogs weighing more than 3 kg;

- Dogs without a positive identification of *Ehrlichia canis* morulae on a peripheral blood smear.

Exclusion criteria:

- Dogs suspected of having concurrent *E canis* infection, based on having two or more of the following findings: chronic weight loss, peripheral lymphadenopathy, leukopaenia (based on examination of peripheral blood smear), total plasma protein concentration > 80 g/l (as determined by refractometer, while being clinically hydrated), and petechiae and/or ecchymosis and epistaxis;

- Dogs diagnosed by PCR analysis to be positive for *E canis* or *B vogeli* infection;

- Dogs that had been vaccinated or had unrelated metabolic illness or babesiosis within the previous month;

- Dogs that were in-saline auto agglutination positive, requiring cortisone treatment;

- Dogs that were receiving cortisone treatment for any other reason;

- Dogs that had been included in the study, but then were euthanised or were denied treatment based on financial grounds during the course of the study (i.e. within the first six days of entry into the study);

- Dogs that developed any concurrent disease except complications of babesiosis (i.e. acute renal failure, cerebral babesiosis, coagulopathy, icterus and hepatopathy, in-saline agglutination (ISA) positive immune-mediated haemolytic anaemia, pulmonary oedema and acute respiratory distress syndrome, haemoconcentration ("red biliary"), shock and pancreatitis) during the course of the study;

- Any case with incomplete data or that was lost to follow up.

All owners of sick dogs meeting the criteria were fully informed of the nature of the study **(Appendix A)** and were then required to sign a consent form **(Appendix B)** to include their dog in the study.

3.2   Experimental procedures

All dogs admitted to the study were managed according to the standard treatment protocol for babesiosis followed in the OVAH, which involves anti-babesial treatment with diminazene aceturate at a dosage of 3.5 mg/kg, and a blood transfusion if required **(Appendix C)**. Supportive treatment (i.e. fluid, electrolyte and glucose supplementation, anti-emetic therapy and enteral feeding) was given as deemed necessary. The dogs were divided into two groups, based on treatment protocol:

- Blood transfusion group, decided at first consultation (usually dogs with packed cell volume ≤ 15% or a clinical evaluation that in the experience of the clinician warranted blood transfusion)

- Non-blood transfusion group, decided at first consultation (usually packed cell volume > 15% or a clinical evaluation that in the experience of the clinician did not warrant blood transfusion)

At first consultation the history was taken. This included enquiries into previous infection with babesiosis and duration of illness. Duration of illness was taken as the first day that the dog was lethargic (in the owner's opinion), and was not based on anorexia.

At first consultation an air-dried thin blood smear was made from capillary blood obtained from the anterior edge or hairless ventral surface of the ear, stained with Cam's Quick-Stain (Kyro-quick Solution®, Kyron Laboratories (Pty) Ltd, Benrose, South Africa) and examined microscopically for *Babesia* organisms. Packed cell volume (PCV), total plasma protein

concentration (TPP) and ISA were determined using heparin- or EDTA-anticoagulated whole blood. The sample was centrifuged for five minutes using a microhaematocrit centrifuge. The PCV was determined using a Hawksley haematocrit reader. PCV, TPP and ISA were done in order to determine treatment and possible complications such as immune-mediated haemolytic anaemia (requiring cortisone treatment) or concurrent *E canis* infection, which would have lead to exclusion.

At first consultation, and 24 hours, three days and six days after first consultation, or until discharge or death, an ethylene diamene tetra-acetic acid (EDTA) whole blood sample (3 ml Vacutainer tube or 0.5 ml paediatric tube for dogs weighing 3-5 kg) was collected from the jugular vein of the dog. The EDTA samples were submitted to the Clinical Pathology Laboratory, and a complete blood count (CBC) **(Appendix D)** was performed, using a CELL-DYN® 3700 analyser, validated for use in the dog.[80] Manual leukocyte differential counts were performed by an experienced veterinary haematolgy technologist (not the principal investigator) by counting 100 cells on a Cam's Quick-stained peripheral bloodsmear. When counting immature neutrophils, only band neutrophils were included in the count. Nucleated RBCs were not enumerated. All EDTA samples collected at first consultation were also submitted for PCR analysis **(Appendix E)**.

At first consultation, each dog underwent a clinical examination, the results of which were listed on a data capture sheet **(Appendix F)**. The clinical examination was repeated at 24 hours, three days and six days after first consultation.

At first consultation a faecal analysis was done. The worm species were identified from the eggs and the egg count was scored from 1 + to 4 + (where 1 + means < 1 egg/10 × field, 2 + means 1 egg/10 × field, 3 + means 2-5 eggs/10 × field and 4 + means > 5 eggs/10 × field) This data was collected to evaluate any possible role of verminosis in the event of microcytic hypochromic anaemia. All dogs were dewormed with Drontal® at one medium tab/10 kg, and treated for ectoparasites with Frontline® at first consultation.

Data regarding outcome, i.e. occurrence of complications specifically related to babesiosis, recovery, discharge from hospital or death (natural or euthanasia requested by the owner or duty clinician) were also recorded **(Appendix G)**.

The principal investigator was responsible for collecting all data.

3.3   Observations

- Signalment, general history and clinical data **(Appendix F)**

- History of previous babesiosis infection **(Appendix F)**

- Duration of illness in days **(Appendix F)**

- Treatment group **(Appendix F)**

- Peripheral blood smear **(Appendix F)**

- PCV, TPP, ISA **(Appendix F)**

- Serial CBC (**Appendix D**)

- Blood smear made from central circulating blood **(Appendix D)**

3.4   Statistical analysis

Data were captured into an Excel spreadsheet (Microsoft Excel 2003, Microsoft Corp, Redmond, WA, USA).

The medians and range of each haematologic variable for each of the treatment groups (blood transfusion and non-blood transfusion) at each time point were tabulated. Medians and range were used rather than mean and standard deviation as the vast majority of data were not normally distributed.

The values of each haematological variable for each treatment group at each time point were screened for normality, log-transformed as required and used for statistical analysis. The means and standard deviations of the log-transformed values for each group were plotted over time. These geometric means were compared between groups at each time point by regressing the log-transformed value on treatment group (blood transfusion vs. non-blood transfusion), and including age as a covariate.

Multiple regression models were also fitted in order to investigate the effects of age, treatment group, duration of illness before presentation and previous illness due to babesiosis, on each of the haematological variables. When models were fitted using all four predictors, it was found that the duration of illness before presentation and previous illness due to babesiosis had no significant effect on any of the haematological variables throughout the study period. Models were then fitted with the predictors treatment group and age only. This increased the power of the study, as fewer observations had to be discarded due to

missing values. A significance level (α) of 0.05 was used throughout. Data analysis was performed using NCSS 2004 (NCSS, Kaysville, Utah, USA).

Haematology reference intervals established by the Clinical Pathology Laboratory, Faculty of Veterinary Science, Onderstepoort, as shown in Table 3.1, were used for interpretation of data. Severity of anaemia was classified as described by Tvedten,[93] as shown in Table 3.2. Reticulocyte responses were classified as described by Cowgill[20] and Tvedten,[93] as shown in Table 3.3. A degenerative left shift was defined as segmented neutrophils $\leq 11.5 \times 10^9$/l and band neutrophils $> 0.5 \times 10^9$/l.

Table 3.1   Haematology reference intervals for dogs (Clinical Pathology Laboratory, OVAH[a]).

Variable	Units	Reference interval
Haemoglobin (HGB)	g/l	120-180
Red Blood Cell (RBC) Count	$\times 10^{12}$/l	5.5-8.5
Haematocrit (HCT)	l/l	0.37-0.55
Mean Cell Volume (MCV)	fl	60-77
Mean Cell Haemoglobin Concentration (MCHC)	g/dl rbc	32-36
Red Cell Distribution Width (RDW)	%	15.5-19.5
Reticulocytes	$\times 10^9$/l	60-80
White Blood Cell (WBC) Count	$\times 10^9$/l	6.0-15.0
Segmented neutrophils	$\times 10^9$/l	3.0-11.5
Band neutrophils	$\times 10^9$/l	0.0-0.5
Lymphocytes	$\times 10^9$/l	1.0-4.8
Monocytes	$\times 10^9$/l	0.15-1.35
Eosinophils	$\times 10^9$/l	0.10-1.25
Basophils	$\times 10^9$/l	0.0-0.1
Platelet (PLT) Count	$\times 10^9$/l	200-500

Table 3.2   Classification of severity of anaemia for dogs.[93]

HCT (l/l)	Severity of anaemia
0.30-0.37	Mild
0.20-0.29	Moderate
0.13-0.19	Severe
< 0.13	Very severe

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[a] Based on reference intervals for the dog published by Boehringer Mannheim for the Deutsche gesellschaft für Klinische Chemie, 1983:89

Table 3.3   Classification of regenerative response for dogs.[20,93]

Reticulocyte count ($\times 10^9$/l)	Regenerative response
< 60	Normal/none
60-150	Mild
150-300	Moderate
> 500	Severe/strong

3.5   Ethical considerations

- All clinical cases were naturally occurring canine babesiosis infections and no experimental infections were induced.

- All clients were informed about the study and required to give their informed consent for the inclusion of their dog in the study.

- The research was non-invasive with the most invasive procedure performed being the collection of blood samples, which is part of standard procedure in most cases of canine babesiosis presented to the OVAH.

- If a dog was in any way suffering due to the frequent collection of blood (i.e. unexpected drop in PCV, or PCV below 6%, difficulty in finding veins for venipuncture) this would be been deemed unethical and that animal would be excluded from the study. However, it was never required during the course of this study to exclude any dog based on these grounds.

- The study was approved by the Animal Use and Care Committee of the University of Pretoria.

CHAPTER 4 RESULTS

4.1   Study population

Of 96 dogs with babesiosis (based on examination of a peripheral blood smear) that were initially considered for this study, 90 dogs met the inclusion criteria. Six dogs were excluded because of concurrent disease, including one dog with an intestinal linear foreign body confirmed on post mortem, one dog with acute hind limb paralysis of unknown aetiology that was euthanised due to financial constraints, one dog with post mortem changes consistent with parvovirus infection, two dogs with *Ehrlichia canis* infection confirmed by polymerase chain reaction (PCR) analysis and one dog with both *E canis* and *Babesia vogeli* infection confirmed by PCR analysis.   Three more dogs were excluded during the study due to receiving prednisolone therapy, but their complete blood counts (CBCs) prior to receiving prednisolone therapy (i.e. CBCs at admission and 24 hours later for two dogs and a CBC at admission for one dog) were included in the data analysis. The 90 dogs included in this study were confirmed by PCR analysis to be infected with *B rossi.*

4.2   Signalment

Of the 90 dogs included in this study, 56 were male and 34 were female. The median age of the dogs was 16 months (range 3 months-15 years 2 months).   Forty-eight dogs were younger than one year, while 42 dogs were one year or older. The median age for the dogs that received a blood transfusion was 9.5 months (range 3 months-6 years).   The median age for the dogs that did not receive a blood transfusion was 1.5 years (range 5 months-15 years 2 months). Breeds included Crossbreed (14), Boerboel (12), Jack Russell Terrier (11), Rottweiller (8), German Shepherd Dog (6), Labrador Retriever (5), Husky (4), Maltese Poodle (4), Dachshund (4), Fox Terrier (4), Sharpei (3), Pittbull Terrier (2), Boxer (2), English Bulldog (1), Toy Pomeranian (1), Bull Mastiff (1), Great Dane (1), Miniature Pincher (1), Bouvier (1), Dalmation (1), Bullterrier (1), Miniature Schnauzer (1), Basset (1) and Bernese Mountain Dog (1). The weight distribution of the dogs was as expected for age and breed. The median weight was 14.8 kg and the range was 3 kg-49 kg. No dog weighed less than 3 kg.

4.3   Treatment protocol

Of the 90 dogs included in this study, 54 (60%) were admitted for treatment to the Onderstepoort Veterinary Academic Hospital, of which 32 (59.3%) received a blood

transfusion, and 22 (40.7%) received fluid therapy. The remaining 36 (40%) dogs were discharged at first consultation with only antibabesial treatment. Out of the total study population of 90 dogs, 32 (35.6%) received a blood transfusion while 58 (64.4%) did not.

4.4   Previous infection with babesiosis

The diagnosis of babesiosis was a first infection in 60 (66.7%) dogs, a repeat infection in 12 (13.3%) dogs and unknown to owners in 18 (20%) dogs. Of the 60 dogs that were infected for the first time, 19 (31.7%) received a blood transfusion and 41 (68.3%) did not receive a blood transfusion. Of the 12 dogs that had a repeat babesiosis infection, four (33.3%) received a blood transfusion and eight (66.7%) did not receive a blood transfusion.

4.5   Duration of illness

The duration of illness before presentation was known for 75 (83.3%) dogs and unknown for 15 (16.7%) dogs. The mean was 2.20 days and the median was two days for the dogs that received a blood transfusion. The mean was 1.83 days and the median was two days for the dogs that did not receive a blood transfusion. The mean was 1.99 days and the median was two days for the 76 dogs for which duration of illness was known.

4.6   Death and survival

Of the 90 dogs included in the study, 83 dogs recovered, six dogs died of babesiosis during the course of the study, and one dog with cerebral babesiosis was euthanised on Day 3 due to financial constraints and a poor prognosis. Three dogs developed in-saline agglutination (ISA) positive immune-mediated haemolytic anaemia and were treated with prednisolone, and were therefore excluded from the study. Of the six dogs that died of babesiosis during the course of the study, one died because of complications of the haemo-concentrating form of the disease (so called "red biliary"), three died of suspected acute respiratory distress syndrome and two died without the exact babesia-related cause being known.

4.7   Datasets analysed

The number of dogs in the two treatment groups for which data were analysed per day during the study period, are given in Table 4.1. The decline in numbers over the study period was due to the factors described in 4.6 above, and in most cases, due to lack of client cooperation in returning dogs for follow-up blood collection or because of returning on the incorrect day, for which data was then excluded.

Table 4.1   Number of dogs in treatment groups per day for the six days analysed.

Day	Blood	Non-blood	Total (n)
0	31	57	88*
1	29	52	81
3	26	44	70
6	18	28	46

* Day 0 samples from two of 90 dogs were analyzed incorrectly with the Cell Dyn automated cell counter (set on the incorrect species) and thus were excluded. "Blood" indicates that a blood transfusion was given on Day 0; "Non-blood" indicates no blood transfusions were given.

A complete data set is provided in **Appendix H**.

4.8 Red blood cell responses

4.8.1 Anaemia

Tables 4.2, 4.4, 4.6, 4.8 and 4.10, indicate the medians and ranges for each erythrocyte variable for each treatment group at each time point. Compared with reference intervals, the anaemia was normocytic and normochromic throughout the study period for all dogs. The anaemia (see Table 3.2) at presentation was very severe in dogs that received a blood transfusion and moderate in dogs that did not receive a blood transfusion. The median haemoglobin (HGB) concentration, red blood cell (RBC) count and HCT were still below the reference interval for both treatment groups on Day 6.

Figures 4.1-4.5 indicate the mean log-transformed values for each erythrocyte variable for each treatment group at each time point.

Tables 4.3, 4.5, 4.7, 4.9 and 4.11 indicate the predictors (treatment group and age) that had a significant effect on erythrocyte variables, based on multiple regression models. The duration of illness and history of previous illness due to babesiosis had no significant effect on these erythrocyte variables throughout the study period. There was a significant effect of treatment on Day 0 (p<0.001), Day 1 (p=0.002) and Day 3 (p=0.004) for HGB concentration, with dogs that received a blood transfusion having a lower mean HGB concentration than those that were not transfused. There was a significant effect of treatment on Day 0 (p<0.001) Day 1 (p=0.004), Day 3 (p=0.001) and Day 6 (p=0.011) for RBC count, with dogs that received a blood transfusion having a lower mean lower RBC count than those that were not transfused. There was a significant effect of treatment on Day 0 (p<0.001), Day 1 (p=0.002), Day 3 (p=0.003) and Day 6 (p=0.022) for HCT, with dogs that received a blood transfusion having a lower mean HCT than those that were not transfused. There was a significant effect of treatment on Day 0 (p=0.016) and Day 6 (p=0.039) for mean cell volume (MCV), with dogs that received a blood transfusion having a higher mean MCV than those that were not transfused. There was a significant effect of age on Day 0 (p=0.049) for MCV, with older dogs having a higher mean MCV than younger dogs.

Table 4.2   Median, minimum, maximum and range values (g/l) for HGB concentration (reference interval 120-180 g/l) over time for the two treatment groups.

Note: the number of dogs evaluated decreased by day as indicated in Table 4.1

Treatment group	Blood transfusion				Non-blood transfusion			
Days	0	1	3	6	0	1	3	6
Median	34	58	81	97	92	75	94	119
Minimum	23	33	47	64	30	31	52	70
Maximum	77	102	124	152	205	154	159	163
Range	54	69	77	88	175	123	107	93
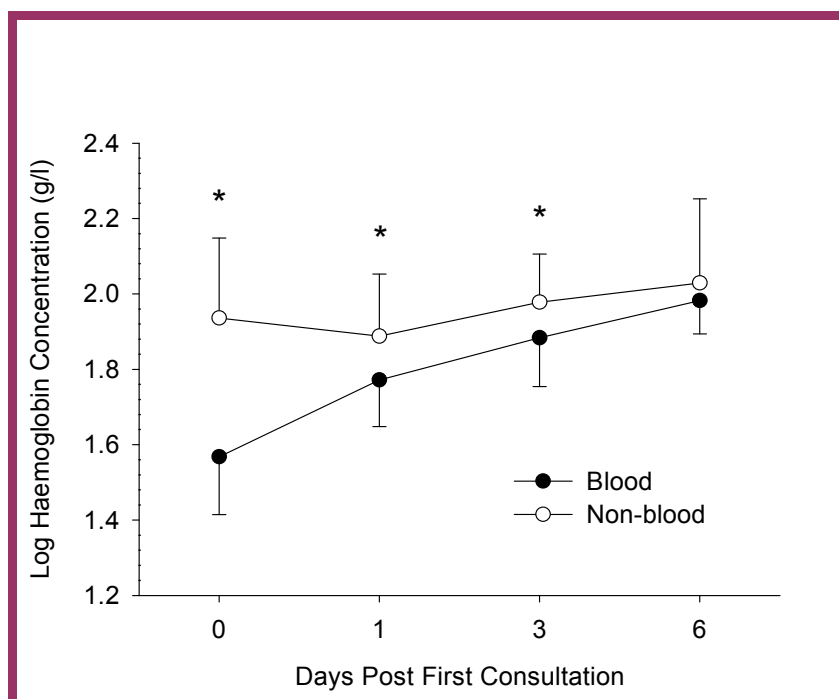
Figure 4.1   Mean log-transformed values for HGB concentration over time for the two treatment groups.

Asterisks indicate a significant difference between the two groups adjusted for age, using multiple regression models. Error bars represent standard deviation. "Blood" indicates that a blood transfusion was given on Day 0; "Non-blood" indicates no blood transfusions were given. Note: the number of dogs evaluated decreased by day as indicated in Table 4.1

Table 4.3 Effect of treatment (blood transfusion vs. non-blood transfusion) and age on log-transformed HGB concentration: output of multiple regression models for Day 0, 1, 3 and 6.

The shaded values indicate significance (p<0.05)

Day post first consultation	n	Predictor	ß[a]	SE (ß)[b]	P[c]
0	88	Treatment group	-0.3576	0.0437	< 0.001
		Age	0.0010	0.007	0.160
1	81	Treatment group	-0.1167	0.0358	0.002
		Age	-0.0001	0.0007	0.895
3	68	Treatment group	-0.0972	0.0326	0.004
		Age	-0.0005	0.0006	0.444
6	45	Treatment group	-0.0346	0.0561	0.541
		Age	0.0018	0.0015	0.236

[a] Regression Coefficient

[b] Standard Error Regression Coefficient

[c] P value

Table 4.4 Median, minimum, maximum and range values (× 10^{12}/l) for RBC counts (reference interval 5.5-8.5 × 10^{12}/l) over time for the two treatment groups.

Note: the number of dogs evaluated decreased by day as indicated in Table 4.1

Treatment group	Blood transfusion				Non-blood transfusion			
Days	0	1	3	6	0	1	3	6
Median	1.48	2.55	3.27	4.30	4.08	3.26	4.12	5.35
Minimum	0.86	1.57	1.99	2.68	1.28	1.24	2.12	3.31
Maximum	3.47	4.46	5.74	7.19	8.97	36.40	6.95	7.44
Range	2.61	2.89	3.75	4.51	7.69	35.16	4.83	4.13
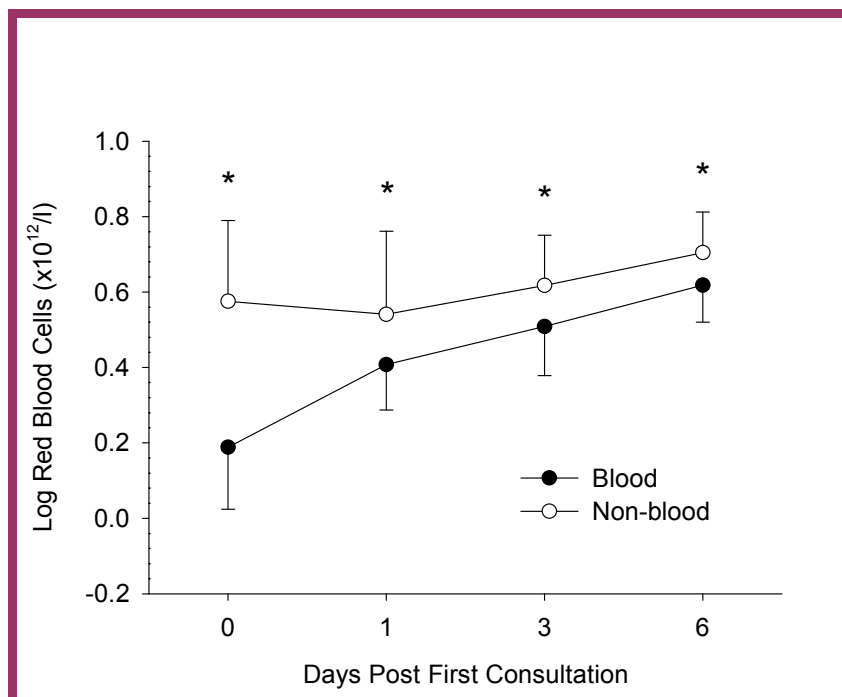
Figure 4.2 Mean log-transformed values for RBC count over time for the two treatment groups.

Asterisks indicate a significant difference between the two groups adjusted for age, using multiple regression models. Error bars represent standard deviation. "Blood" indicates that a blood transfusion was given on Day 0; "Non-blood" indicates no blood transfusions were given. Note: the number of dogs evaluated decreased by day as indicated in Table 4.1

Table 4.5 Effect of treatment (blood transfusion vs. non-blood transfusion) and age on log transformed RBC count: output of multiple regression models for Day 0, 1, 3 and 6. The shaded values indicate significance (p<0.05)

Day post first consultation	n	Predictor	β^a	SE $(\beta)^b$	P^c
0	88	Treatment group	-0.3787	0.0448	< 0.001
		Age	0.0008	0.0007	0.292
1	81	Treatment group	-0.1340	0.0450	0.004
		Age	-0.0001	0.0009	0.052
3	68	Treatment group	-0.1115	0.0332	0.001
		Age	-0.0004	0.0006	0.473
6	45	Treatment group	-0.0857	0.0322	0.011
		Age	0.0001	0.0008	0.863

[a] Regression Coefficient

[b] Standard Error Regression Coefficient

[c] P value

Table 4.6 Median, minimum, maximum and range values (l/l) for HCT (reference interval 0.37-0.55 l/l) over time for the two treatment groups.

Note: the number of dogs evaluated decreased by day as indicated in Table 4.1

Treatment group	Blood transfusion				Non-blood transfusion			
Days	0	1	3	6	0	1	3	6
Median	0.10	0.17	0.24	0.31	0.28	0.21	0.28	0.36
Minimum	0.06	0.10	0.14	0.19	0.08	0.09	0.16	0.22
Maximum	0.23	0.31	0.37	0.47	0.57	0.45	0.46	0.48
Range	0.17	0.21	0.23	0.28	0.49	0.36	0.30	0.26
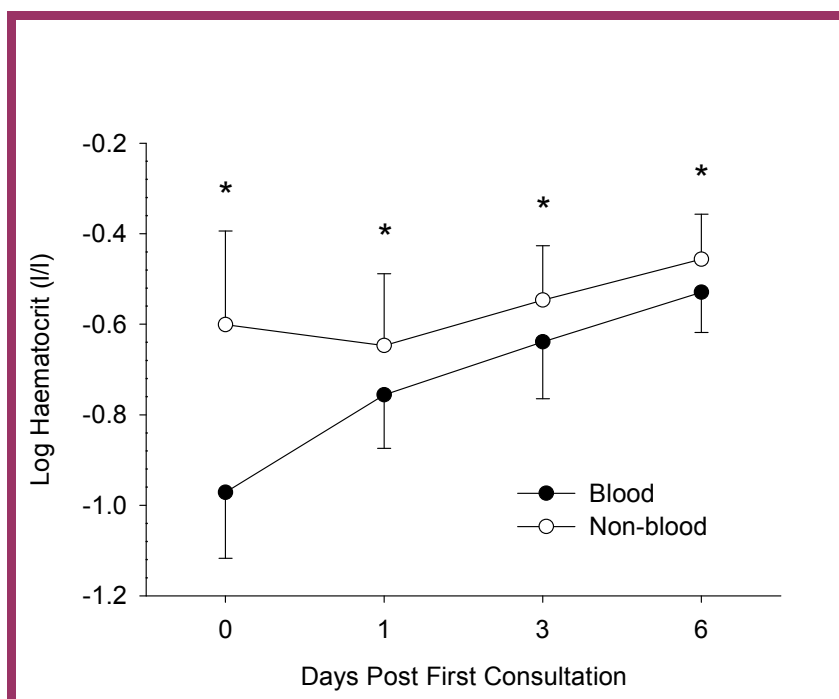
Figure 4.3 Mean log-transformed values for HCT over time for the two treatment groups. Asterisks indicate a significant difference between the two groups adjusted for age, using multiple regression models. Error bars represent standard deviation. "Blood" indicates that a blood transfusion was given on Day 0; "Non-blood" indicates no blood transfusions were given. Note: the number of dogs evaluated decreased by day as indicated in Table 4.1

Table 4.7 Effect of treatment (blood transfusion vs. non-blood transfusion) and age on log transformed HCT: output of multiple regression models for Day 0, 1, 3 and 6. The shaded values indicate significance (p<0.05)

Day post first consultation	n	Predictor	ß[a]	SE (ß)[b]	P[c]
0	88	Treatment group	-0.3606	0.0422	< 0.001
		Age	0.0010	0.0007	0.158
1	79	Treatment group	-0.1085	0.0344	0.002
		Age	0.0000	0.0007	0.948
3	66	Treatment group	-0.0947	0.0310	0.003
		Age	-0.0005	0.0006	0.424
6	45	Treatment group	-0.0705	0.0296	0.022
		Age	0.0004	0.0008	0.596

[a] Regression Coefficient

[b] Standard Error Regression Coefficient

[c] P value

Table 4.8 Median, minimum, maximum and range values (fl) for MCV (reference interval 60-70 fl) over time for the two treatment groups.

Note: the number of dogs evaluated decreased by day as indicated in Table 4.1

Treatment group	Blood transfusion				Non-blood transfusion			
Days	0	1	3	6	0	1	3	6
Median	67.7	66.7	70.0	70.0	66.6	67.7	68.5	69.7
Minimum	60.5	61.3	63.5	65.2	58.8	61.9	61.9	61.8
Maximum	87.3	73.4	79.5	83.1	76.8	76.7	80.2	74.1
Range	26.8	12.1	16.0	17.9	18.0	14.8	18.3	12.3
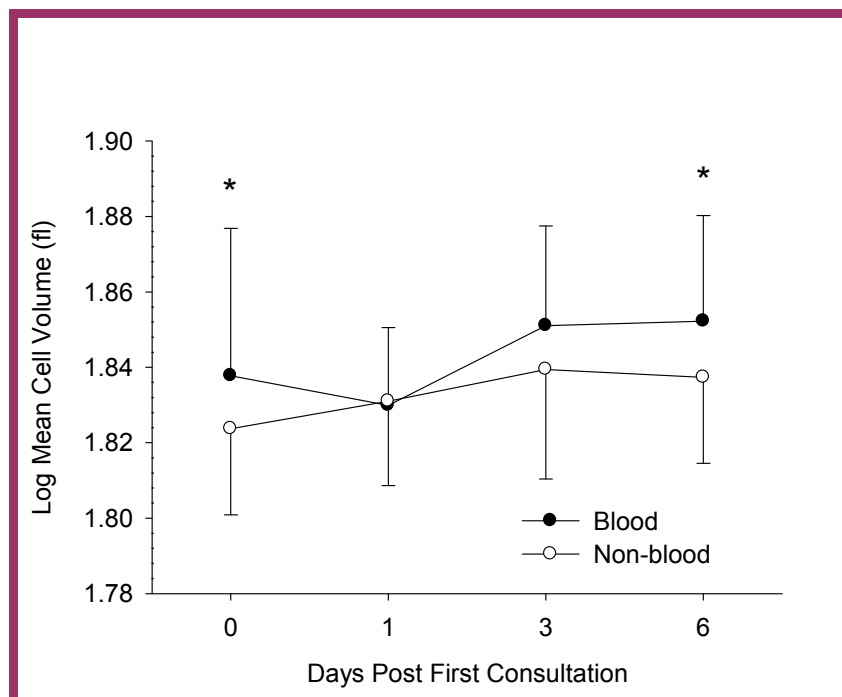
Figure 4.4 Mean log-transformed values for MCV over time for the two treatment groups. Asterisks indicate a significant difference between the two groups adjusted for age, using multiple regression models. Error bars represent standard deviation. "Blood" indicates that a blood transfusion was given on Day 0; "Non-blood" indicates no blood transfusions were given. Note: the number of dogs evaluated decreased by day as indicated in Table 4.1

Table 4.9 Effect of treatment (blood transfusion vs. non-blood transfusion) and age on log transformed MCV: output of multiple regression models for Day 0, 1, 3 and 6. The shaded values indicate significance (p<0.05)

Day post first consultation	n	Predictor	ß[a]	SE (ß)[b]	P[c]
0	88	Treatment group	0.0001	0.0050	0.016
		Age	0.0002	0.0001	0.049
1	81	Treatment group	0.0162	0.0066	0.979
		Age	0.0002	0.0001	0.099
3	68	Treatment group	0.0128	0.0070	0.073
		Age	0.0002	0.0001	0.119
6	45	Treatment group	0.0164	0.0077	0.039
		Age	0.0002	0.0002	0.268

[a] Regression Coefficient

[b] Standard Error Regression Coefficient

[c] P value

Table 4.10 Median, minimum, maximum and range values (g/dl) for mean cell haemoglobin content (MCHC) (reference interval 32-36 g/dl) over time for the two treatment groups.

Note: the number of dogs evaluated decreased by day as indicated in Table 4.1

Treatment group	Blood transfusion				Non-blood transfusion			
Days	0	1	3	6	0	1	3	6
Median	34.5	34.2	33.3	32.6	34.4	34.1	33.7	33.3
Minimum	30.4	31.6	31.1	30.5	31.6	31.6	31.1	31.1
Maximum	41.0	37.4	34.8	34.8	44.4	52.4	43.4	43.9
Range	10.6	5.8	3.7	4.3	12.8	20.8	12.3	12.8
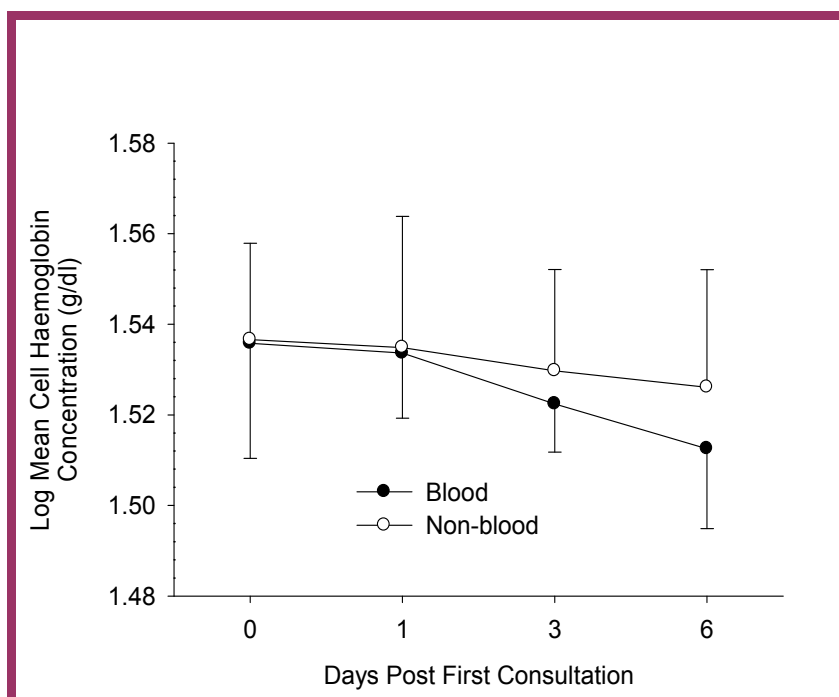
Figure 4.5 Mean log-transformed values for MCHC over time for the two treatment groups.

Error bars represent standard deviation. "Blood" indicates that a blood transfusion was given on Day 0; "Non-blood" indicates no blood transfusions were given. Note: the number of dogs evaluated decreased by day as indicated in Table 4.1

Table 4.11 Effect of treatment (blood transfusion vs. non-blood transfusion) and age on log transformed MCHC: output of multiple regression models for Day 0, 1, 3 and 6.

Day post first consultation	n	Predictor	ß[a]	SE (ß)[b]	P[c]
0	88	Treatment group	-0.0013	0.0052	0.803
		Age	0.0000	0.0001	0.596
1	81	Treatment group	-0.0016	0.0058	0.790
		Age	0.0000	0.0001	0.688
3	68	Treatment group	-0.0075	0.0048	0.125
		Age	0.0000	0.0001	0.698
6	45	Treatment group	-0.0134	0.0072	0.069
		Age	0.0000	0.116	0.909

[a] Regression Coefficient

[b] Standard Error Regression Coefficient

[c] P value

4.8.2 Regenerative response

Table 4.12 and Table 4.14 indicate the medians and ranges for reticulocyte counts and red cell distribution width (RDW) for each treatment group at each time point. The regenerative response (based on reticulocyte counts, see Table 3.3) was moderate on Days 0, 1 and 3 and mild on Day 6 for dogs that received a blood transfusion. There was no regenerative response on Day 0 and Day 1, a moderate regenerative response on Day 3 and a mild regenerative response on Day 6 for dogs that did not receive a blood transfusion. There was a peak response on Day 3 in both treatment groups. The median RDW was within the reference interval in both treatment groups throughout the study period, except for an increase in median RDW on Day 3 in dogs that received a blood transfusion.

Figure 4.6 and Figure 4.7 indicate the mean log-transformed values for reticulocyte count and RDW for each treatment group at each time point.

Table 4.13 and Table 4.15 indicate the predictor (treatment group) that had a significant effect on reticulocyte count and RDW, based on multiple regression models. The duration of illness, history of previous illness due to babesiosis and age had no significant effect on these erythrocyte variables throughout the study period. There was a significant effect of treatment on Day 0 ($p<0.001$) Day 1 ($p<0.001$) and Day 3 ($p=0.031$) for reticulocyte count, with dogs that received a blood transfusion having higher mean reticulocyte counts than those that were not transfused. There was a significant effect of treatment on Day 0 ($p<0.001$) and Day 6 ($p=0.007$) for RDW, with dogs that received a blood transfusion having higher mean RDWs than those that were not transfused.

Table 4.12 Median, minimum, maximum and range values ($\times 10^9$/l) for reticulocyte counts ($< 60 \times 10^9$/l seen as non regenerative if anaemic) over time for the two treatment groups.

Note: the number of dogs evaluated decreased by day as indicated in Table 4.1

Treatment group	Blood transfusion				Non-blood transfusion			
Days	0	1	3	6	0	1	3	6
Median	155.02	187.37	323.78	91.88	33.67	38.85	227.31	130.25
Minimum	15.74	8.80	44.77	20.58	10.45	5.75	6.64	24.94
Maximum	455.07	964.88	1063.23	537.19	500.35	708.76	1377.96	766.34
Range	439.33	956.08	1018.46	516.61	489.90	703.76	1371.32	741.40

Figure 4.6 Mean log-transformed values for reticulocyte count over time for the two treatment groups.

Asterisks indicate a significant difference between the two groups adjusted for age, using multiple regression models. Error bars represent standard deviation. "Blood" indicates that a blood transfusion was given on Day 0; "Non-blood" indicates no blood transfusions were given. Note: the number of dogs evaluated decreased by day as indicated in Table 4.1

Table 4.13 Effect of treatment (blood transfusion vs. non-blood transfusion) and age on log transformed reticulocyte count: output of multiple regression models for Day 0, 1, 3 and 6.

The shaded values indicate significance (p<0.05)

Day post first consultation	n	Predictor	ß[a]	SE (ß)[b]	P[c]
0	87	Treatment group	0.4013	0.0943	< 0.001
		Age	0.0005	0.0015	0.751
1	80	Treatment group	0.4759	0.1240	< 0.001
		Age	0.0000	0.0024	0.985
3	71	Treatment group	0.2610	0.1180	0.031
		Age	-0.0016	0.0022	0.470
6	46	Treatment group	-0.1741	0.1180	0.147
		Age	0.0040	0.0031	0.202

[a] Regression Coefficient

[b] Standard Error Regression Coefficient

[c] P value

Table 4.14   Median, minimum, maximum and range values (%) for RDW (reference interval 15.5-19.5%) over time for the two treatment groups.

Note: the number of dogs evaluated decreased by day as indicated in Table 4.1

Treatment group	Blood transfusion				Non-blood transfusion			
Days	0	1	3	6	0	1	3	6
Median	16.7	16.9	18.6	22.1	15.4	15.5	16.2	17.9
Minimum	14.6	14.2	15.1	17.3	13.6	12.9	13.2	14.8
Maximum	33.5	37.3	39.7	30.5	26.3	29.4	40.2	29.0
Range	18.9	23.1	24.6	13.2	12.7	16.5	27.0	14.2
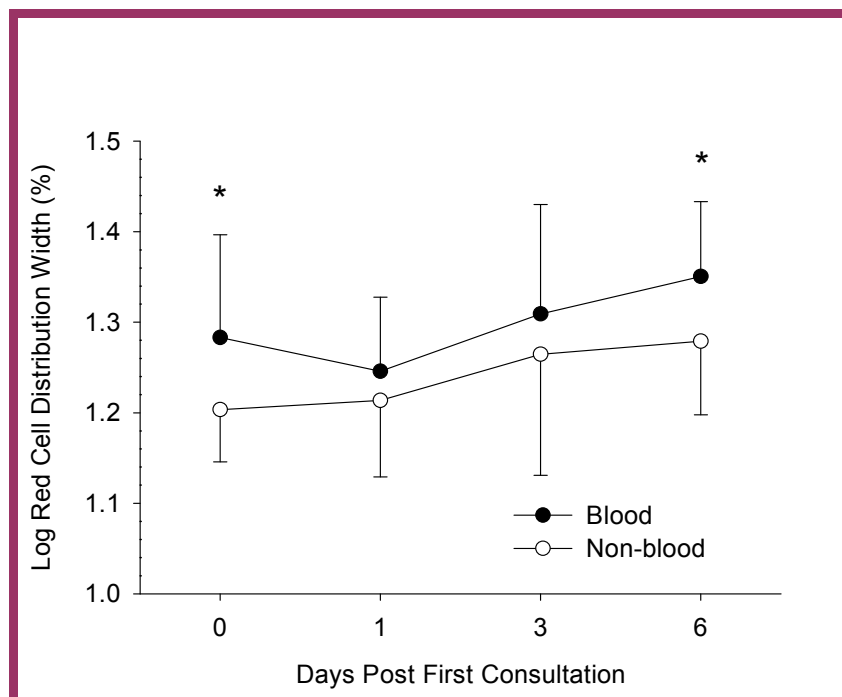
Figure 4.7   Mean log-transformed values for RDW over time for the two treatment groups. Asterisks indicate a significant difference between the two groups adjusted for age, using multiple regression models. Error bars represent standard deviation. "Blood" indicates that a blood transfusion was given on Day 0; "Non-blood" indicates no blood transfusions were given. Note: the number of dogs evaluated decreased by day as indicated in Table 4.1

Table 4.15 Effect of treatment (blood transfusion vs. non-blood transfusion) and age on log transformed RDW width: output of multiple regression models for Day 0, 1, 3 and 6. The shaded values indicate significance (p<0.05)

Day post first consultation	n	Predictor	ß[a]	SE (ß)[b]	P[c]
0	88	Treatment group	0.0819	0.0186	< 0.001
		Age	0.0002	0.0003	0.450
1	81	Treatment group	0.0338	0.0197	0.090
		Age	0.0002	0.0004	0.562
3	70	Treatment group	0.0461	0.0325	0.161
		Age	0.0002	0.0006	0.716
6	46	Treatment group	0.0717	0.0255	0.007
		Age	0.0000	0.0007	0.998

[a] Regression Coefficient

[b] Standard Error Regression Coefficient

[c] P value

4.9 White blood cell responses

4.9.1 Total white blood cell counts

Table 4.16 indicates the medians and ranges for total white blood cell (WBC) counts for each treatment group at each time point. The median total WBC count was within the reference interval for both treatment groups throughout the study period, except on Day 3, when mild leukocytosis was found for both treatment groups, and on Day 0, when very mild leukopaenia was found for dogs that did not receive a blood transfusion.

Figure 4.8 indicates the mean log-transformed values for total WBC counts for each treatment group at each time point.

Table 4.17 indicates that the predictors treatment group and age did not have a significant effect on total WBC count, based on multiple regression models. The duration of illness and history of previous illness due to babesiosis also had no significant effect on this haematological variable throughout the study period.

Table 4.16 Median, minimum, maximum and range values (× 10^9/l) for total WBC counts (reference interval 6.0-15.0 ×10^9/l) over time for the two treatment groups.

Note: the number of dogs evaluated decreased by day as indicated in Table 4.1

Treatment group	Blood transfusion				Non-blood transfusion			
Days	0	1	3	6	0	1	3	6
Median	8.6	12.4	16.6	12.9	5.8	10.6	16.9	13.3
Minimum	2.2	3.9	8.1	7.4	2.4	2.8	6.7	5.8
Maximum	17.5	33.3	56.1	44.6	39.4	37.6	68.3	38.3
Range	15.3	29.4	48.0	37.2	37.0	34.8	61.6	32.5

Figure 4.8 Mean log-transformed values for total WBC count over time day for the two treatment groups.

Error bars represent standard deviation. "Blood" indicates that a blood transfusion was given on Day 0; "Non-blood" indicates no blood transfusions were given. Note: the number of dogs evaluated decreased by day as indicated in Table 4.1

Table 4.17   Effect of treatment (blood transfusion vs. non-blood transfusion) and age on log transformed WBC count: output of multiple regression models for Day 0, 1, 3 and 6.

Day post first consultation	n	Predictor	ß[a]	SE (ß)[b]	P[c]
0	88	Treatment group	0.0931	0.0539	0.088
		Age	0.0010	0.0009	0.242
1	81	Treatment group	0.0831	0.0488	0.093
		Age	0.0002	0.0010	0.867
3	70	Treatment group	0.0031	0.0583	0.958
		Age	-0.0006	0.0011	0.566
6	46	Treatment group	-0.0124	0.0614	0.842
		Age	0.0004	0.0016	0.782

[a] Regression Coefficient

[b] Standard Error Regression Coefficient

[c] P value

4.9.2 Segmented and band neutrophil counts

Table 4.18 and Table 4.20 indicate the medians and ranges for segmented neutrophil counts and band neutrophil counts for each treatment group at each time point. The median segmented neutrophil count was within the reference interval for both treatment groups throughout the study period. The median band neutrophil count was increased throughout the study period for dogs receiving a blood transfusion, with a peak response on Day 3. The median band neutrophil count was within reference interval on Day 0 and Day 6, and increased on Day 1 and Day 3 for dogs that did not receive a blood transfusion.

Figure 4.9 and Figure 4.10 indicate the mean log-transformed values for segmented neutrophil counts and band neutrophil counts for each treatment group at each time point.

Table 4.19 and Table 4.21 indicate the predictors (treatment group and age) that had a significant effect on segmented neutrophil count and band neutrophil count, based on multiple regression models. The duration of illness and history of previous illness due to babesiosis had no significant effect on these haematological variables throughout the study period. There was a significant effect of treatment on Day 0 (p<0.001) and Day 1 (p=0.001) with dogs that received a blood transfusion having higher mean band neutrophil counts than those that were not transfused. There was a significant effect of age on Day 0 (p=0.042), with older dogs having higher mean segmented neutrophil counts than younger dogs.

Table 4.22 and Table 4.23 indicate the percentages of dogs with left shifts and degenerative left shifts for each treatment group at each time point. There was a significant difference between the two treatment groups on Day 0 (p=0.002) and Day 1 (p=0.002), with dogs with more severe anaemia having a higher percentage of dogs with a left shift. The occurrence of degenerative left shifts did not differ significantly between the two treatment groups.

Table 4.18   Median, minimum, maximum and range values (×10^9/l) for segmented neutrophil counts (reference interval 3.0-11.5 × 10^9/l) over time for the two treatment groups. Note: the number of dogs evaluated decreased by day as indicated in Table 4.1

Treatment group	Blood transfusion				Non-blood transfusion			
Days	0	1	3	6	0	1	3	6
Median	4.46	6.82	7.84	7.09	3.11	5.78	9.13	8.29
Minimum	0.62	1.68	2.97	3.44	1.00	1.19	1.69	2.55
Maximum	12.13	18.29	39.83	35.23	28.80	23.31	43.21	29.49
Range	11.51	16.61	36.86	31.79	27.80	22.12	41.52	26.94
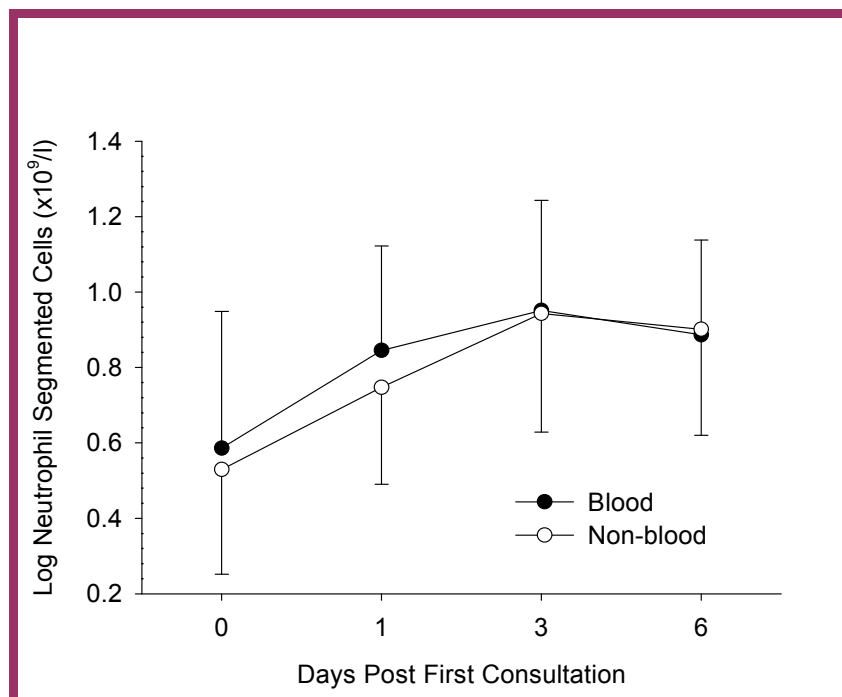
Figure 4.9   Mean log-transformed values for segmented neutrophil count over time for the two treatment groups.

Error bars represent standard deviation. "Blood" indicates that a blood transfusion was given on Day 0; "Non-blood" indicates no blood transfusions were given. Note: the number of dogs evaluated decreased by day as indicated in Table 4.1

Table 4.19 Effect of treatment (blood transfusion vs. non-blood transfusion) and age on log transformed segmented neutrophils: output of multiple regression models for Day 0, 1, 3 and 6.

The shaded value indicate significance (p<0.05)

Day post first consultation	n	Predictor	ß[a]	SE (ß)[b]	P[c]
0	88	Treatment group	0.0804	0.0689	0.247
		Age	0.0024	0.0011	0.042
1	81	Treatment group	0.1113	0.0613	0.073
		Age	0.0018	0.0012	0.129
3	70	Treatment group	0.0139	0.0768	0.857
		Age	0.0009	0.0014	0.538
6	46	Treatment group	0.0056	0.0824	0.946
		Age	0.0029	0.0021	0.190

[a] Regression Coefficient

[b] Standard Error Regression Coefficient

[c] P value

Table 4.20 Median, minimum, maximum and range values ($\times 10^9$/l) for band neutrophil counts (reference interval 0.0-0.5 $\times 10^9$/l) over time for the two treatment groups.

Note: the number of dogs evaluated decreased by day as indicated in Table 4.1

Treatment group	Blood transfusion				Non-blood transfusion			
Days	0	1	3	6	0	1	3	6
Median	1.10	1.07	1.76	0.52	0.46	0.54	1.24	0.30
Minimum	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00
Maximum	6.03	6.82	15.25	4.01	5.91	4.95	23.22	4.90
Range	6.03	6.65	15.25	4.01	5.91	4.95	23.22	4.90
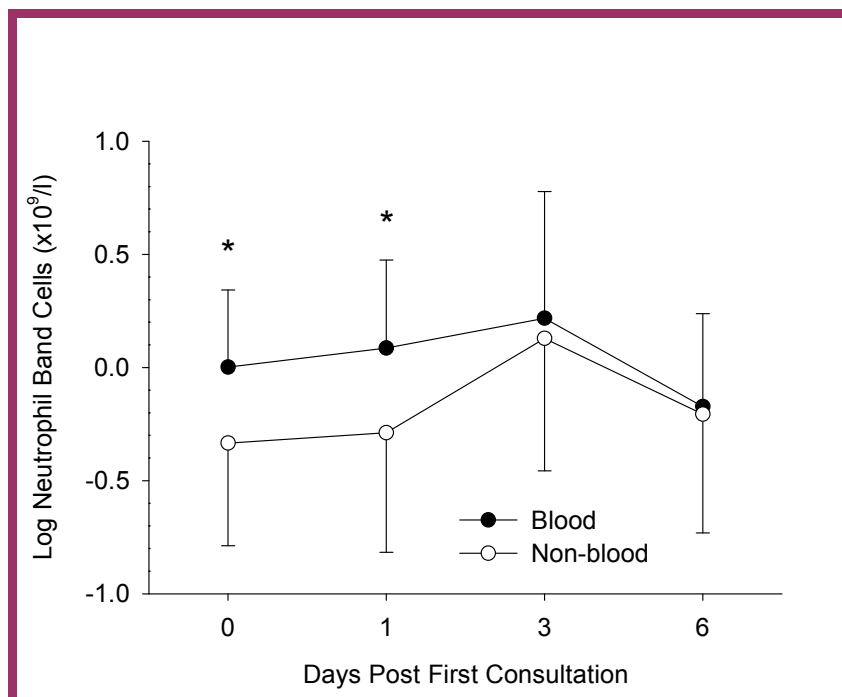
Figure 4.10 Mean log-transformed values for band neutrophil count over time for the two treatment groups.

Asterisks indicate a significant difference between the two groups adjusted for age, using multiple regression models. Error bars represent standard deviation. "Blood" indicates that a blood transfusion was given on Day 0; "Non-blood" indicates no blood transfusions were given. Note: the number of dogs evaluated decreased by day as indicated in Table 4.1

Table 4.21 Effect of treatment (blood transfusion vs. non-blood transfusion) and age on log transformed band neutrophils: output of multiple regression models for Day 0, 1, 3 and 6. The shaded values indicate significance (p<0.05)

Day post first consultation	n	Predictor	ß[a]	SE (ß)[b]	P[c]
0	86	Treatment group	0.3490	0.0961	< 0.001
		Age	0.0013	0.0015	0.394
1	79	Treatment group	0.3868	0.1140	0.001
		Age	0.0017	0.0022	0.437
3	65	Treatment group	0.1001	0.1515	0.511
		Age	0.0016	0.0027	0.567
6	33	Treatment group	0.0470	0.1754	0.791
		Age	0.0030	0.0048	0.547

[a] Regression Coefficient

[b] Standard Error Regression Coefficient

[c] P value

Table 4.22   Percentages of dogs that had a left shift (band neutrophils > 0.5×10^9/l) by day for the two treatment groups.

Treatment group	Days	0	1	3	6
Blood transfusion	n	23/31	26/29	17/26	9/18
	% of dogs	74.0	87.7	65.1	50.0
Non- blood transfusion	n	22/57	28/52	30/44	11/28
	% of dogs	36.8	53.8	68.2	39.3
P[a]		0.002	0.001	1.000	0.550

[a] P value, Fisher exact test. The shaded values indicate significance (p<0.05)

Table 4.23   Percentages of dogs that had a left shift, with a degenerative left shift (segmented neutrophils ≤ 11.5×10^9/l and band neutrophils > 0.5×10^9/l) by day for the two treatment groups.

Treatment group	Days	0	1	3	6
Blood transfusion	n	21/23	17/26	10/17	7/9
	% of dogs	91.3	65.5	58.8	78.0
Non- blood transfusion	n	20/22	22/28	16/30	3/11
	% of dogs	90.9	78.5	53.3	27.0
P[a]		1.000	0.370	0.77	0.070

[a] P value, Fisher exact test.

4.9.3 Lymphocyte counts

Table 4.24 indicates the medians and ranges for lymphocyte counts for each treatment group at each time point. The median lymphocyte count was within the reference interval throughout the study period for both treatment groups.
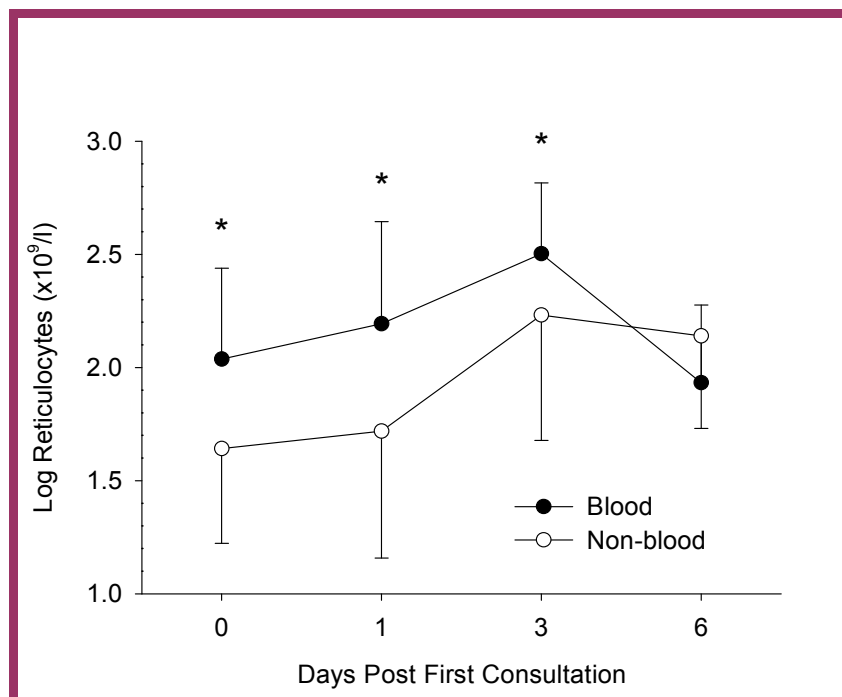
Figure 4.11 indicates the mean log-transformed values for lymphocyte counts for each treatment group at each time point.

Table 4.25 indicates the predictor (age) that had a significant effect on lymphocyte counts, based on multiple regression models. The duration of illness, history of previous illness due to babesiosis and treatment group had no significant effect on this haematological variable throughout the study period. There was a significant effect of age throughout the study period (p<0.001), with older dogs having lower mean lymphocyte counts than younger dogs.

Table 4.24   Median, minimum, maximum and range values ($\times 10^9$/l) for lymphocyte counts (reference interval 1.0-4.8 $\times 10^9$/l) over time for the two treatment groups.

Note: the number of dogs evaluated decreased by day as indicated in Table 4.1

Treatment group	Blood transfusion				Non-blood transfusion			
Days	0	1	3	6	0	1	3	6
Median	1.37	2.20	4.17	2.80	1.06	2.65	4.05	3.08
Minimum	0.11	0.30	1.27	0.27	0.14	0.48	1.01	0.87
Maximum	3.05	14.32	11.25	6.40	4.32	7.52	8.42	6.14
Range	2.94	14.02	9.98	6.13	4.18	7.04	7.41	5.27
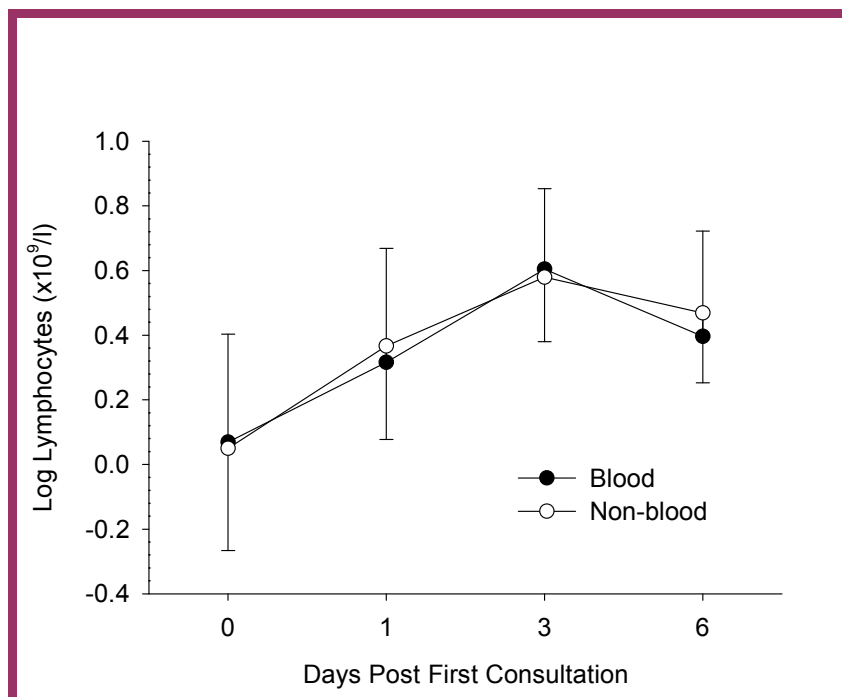
Figure 4.11   Mean log-transformed values for lymphocyte count over time for the two treatment groups.

Error bars represent standard deviation. "Blood" indicates that a blood transfusion was given on Day 0; "Non-blood" indicates no blood transfusions were given. Note: the number of dogs evaluated decreased by day as indicated in Table 4.1

Table 4.25   Effect of treatment (blood transfusion vs. non-blood transfusion) and age on log transformed lymphocyte count: output of multiple regression models for Day 0, 1, 3 and 6.

The shaded values indicate significance (p<0.05)

Day post first consultation	n	Predictor	ß[a]	SE (ß)[b]	P[c]
0	88	Treatment group	-0.0217	0.0684	0.7515
		Age	-0.0040	0.0011	< 0.001
1	81	Treatment group	-0.0910	0.0662	0.1737
		Age	-0.0056	0.0013	< 0.001
3	70	Treatment group	0.0002	0.0500	0.997
		Age	-0.0035	0.0009	< 0.001
6	46	Treatment group	-0.1286	0.0682	0.066
		Age	-0.0078	0.0018	< 0.001

[a] Regression Coefficient

[b] Standard Error Regression Coefficient

[c] P value

4.9.4　Monocyte counts

Table 4.26 indicates the medians and ranges for monocyte counts for each treatment group at each time point. The median monocyte count was within the reference interval on Day 0 and Day 6 for dogs that received a blood transfusion. This treatment group had mild monocytosis on Day 1 and Day 3. The median monocyte count was within the reference interval on Day 0, Day 1 and Day 6 for dogs that did not receive a blood transfusion. This treatment group had mild monocytosis on Day 3.

Figure 4.12 indicates the mean log-transformed values for monocyte count for each treatment group at each time point.

Table 4.27 indicates that the predictors treatment group and age had no significant effect on mean monocyte counts, based on multiple regression models. The duration of illness and history of previous illness due to babesiosis also had no significant effect on this haematological variable throughout the study period.

Table 4.26 Median, minimum, maximum and range values ($\times 10^9$/l) for monocyte counts (reference interval 0.15-1.35 $\times 10^9$/l) over time for the two treatment groups.

Note: the number of dogs evaluated decreased by day as indicated in Table 4.1

Treatment group	Blood transfusion				Non-blood transfusion			
Days	0	1	3	6	0	1	3	6
Median	0.58	1.46	1.71	0.95	0.50	1.14	1.42	0.74
Minimum	0.05	0.12	0.38	0.19	0.04	0.08	0.13	0.15
Maximum	1.75	3.27	4.98	5.69	2.47	3.29	6.20	2.94
Range	1.70	3.15	4.60	5.50	2.43	3.21	6.07	2.79
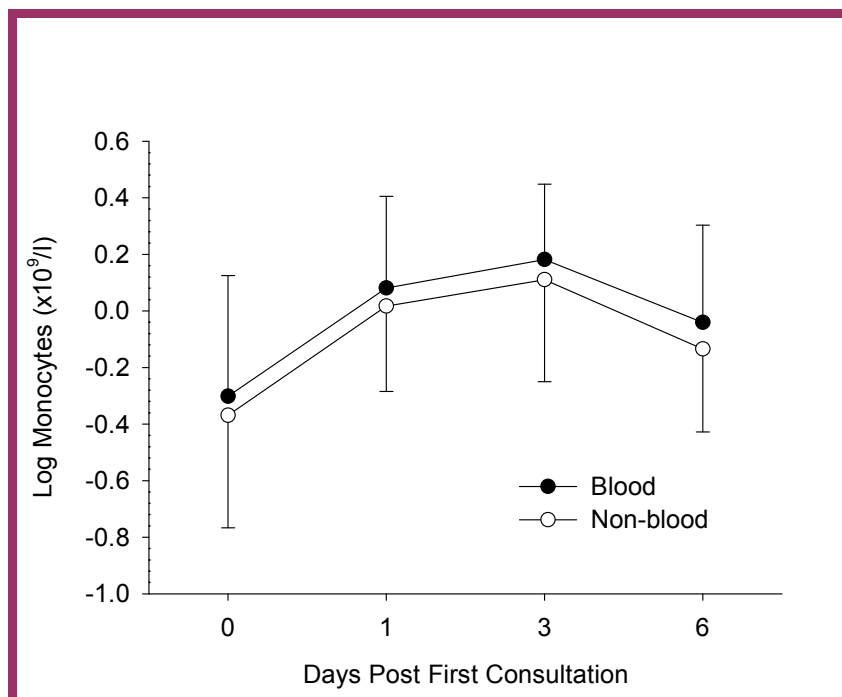
Figure 4.12 Mean log-transformed values for monocyte count over time day for the two treatment groups.

Error bars represent standard deviation. "Blood" indicates that a blood transfusion was given on Day 0; "Non-blood" indicates no blood transfusions were given. Note: the number of dogs evaluated decreased by day as indicated in Table 4.1

Table 4.27   Effect of treatment (blood transfusion vs. non-blood transfusion) and age on log transformed monocyte counts: output of multiple regression models for Day 0, 1, 3 and 6.

Day post first consultation	N	Predictor	ß[a]	SE (ß)[b]	P[c]
0	88	Treatment group	0.0853	0.0922	0.357
		Age	0.0017	0.0015	0.266
1	81	Treatment group	0.0691	0.0730	0.347
		Age	0.0008	0.0014	0.589
3	70	Treatment group	0.0737	0.0825	0.375
		Age	0.0003	0.0015	0.825
6	46	Treatment group	0.0880	0.0973	0.371
		Age	-0.0009	0.0025	0.716

[a] Regression Coefficient

[b] Standard Error Regression Coefficient

[c] P value

4.9.5 Eosinophil counts

Table 4.28 indicates the medians and ranges for eosinophil counts for each treatment group at each time point. The median eosinophil count was within reference interval throughout the study period, except on Day 1 where mild eosinopaenia was found for both treatment groups.
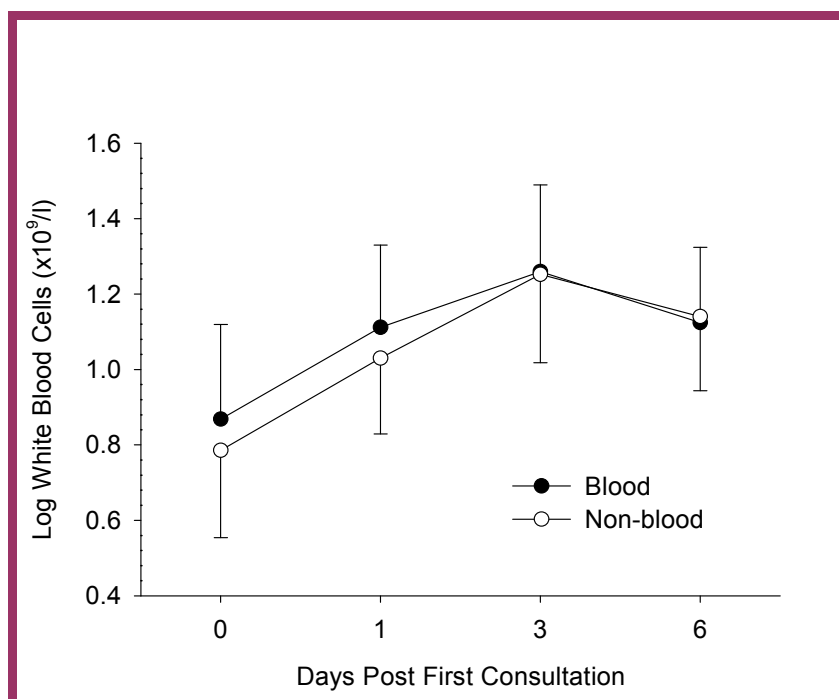
Figure 4.13 indicates the mean log-transformed values for eosinophil count for each treatment group at each time point.

Table 4.29 indicates that the predictors treatment group and age had no significant effect on mean eosinophil counts, based on multiple regression models. The duration of illness and history of previous illness due to babesiosis also had no significant effect on this haematological variable throughout the study period.

Table 4.28   Median, minimum, maximum and range values (× 10^9/l) for eosinophil counts (reference interval 0.10-1.25 × 10^9/l) over time for the two treatment groups.

Note: the number of dogs evaluated decreased by day as indicated in Table 4.1

Treatment group	Blood transfusion				Non-blood transfusion			
Days	0	1	3	6	0	1	3	6
Median	0.00	0.12	0.36	0.59	0.02	0.19	0.56	0.44
Minimum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Maximum	0.27	0.77	2.27	1.30	0.54	0.85	2.59	1.92
Range	0.27	0.77	2.27	1.30	0.54	0.85	2.59	1.92
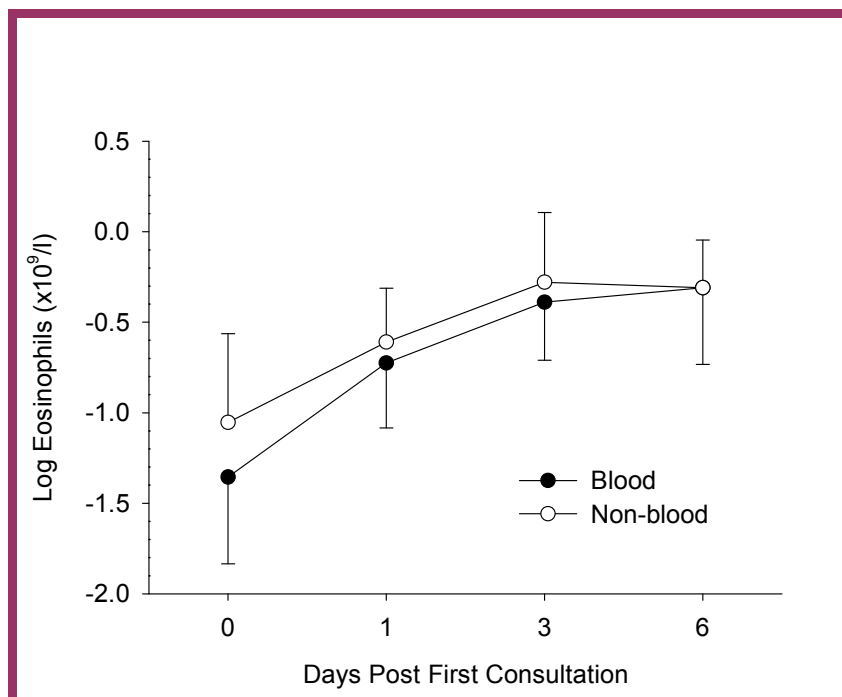
Figure 4.13   Mean log-transformed values for eosinophil count over time for the two treatment groups.

Error bars represent standard deviation. "Blood" indicates that a blood transfusion was given on Day 0; "Non-blood" indicates no blood transfusions were given. Note: the number of dogs evaluated decreased by day as indicated in Table 4.1

Table 4.29 Effect of treatment (blood transfusion vs. non-blood transfusion) and age on log transformed eosinophil counts: output of multiple regression models for Day 0, 1, 3 and 6.

Day post first consultation	N	Predictor	ß[a]	SE (ß)[b]	P[c]
0	46	Treatment group	-0.2997	0.1625	0.072
		Age	0.0003	0.0026	0.901
1	61	Treatment group	-0.1196	0.0875	0.177
		Age	-0.0007	0.0016	0.652
3	62	Treatment group	-0.1127	0.0979	0.254
		Age	-0.0005	0.0017	0.776
6	42	Treatment group	-0.0015	0.1087	0.989
		Age	-0.0002	0.0028	0.942

[a] Regression Coefficient

[b] Standard Error Regression Coefficient

[c] P value

4.9.6   Basophil counts

Table 4.30 indicates the medians and ranges for basophil counts for each treatment group at each time point.   The median basophil count was within the reference interval for both treatment groups throughout the study period.

Table 4.30 indicates that the median basophil count was 0×10^9/l for both treatment groups throughout the study period.   Because the majority of the basophil counts were zero, it was not possible to meaningfully log-transform this data in order to plot line graphs and fit multiple regression models.   No further statistical analysis of this data was done.   The data are provided in Appendix H.

Table 4.30   Median, minimum, maximum and range values (× 10^9/l) for basophil counts (reference interval 0.0-0.1 × 10^9/l) over time by day for the two treatment groups.

Note: the number of dogs evaluated decreased by day as indicated in Table 4.1

Treatment group	Blood transfusion				Non-blood transfusion			
Days	0	1	3	6	0	1	3	6
Median	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Minimum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Maximum	0.20	0.01	0.00	0.12	0.08	0.02	0.38	0.10
Range	0.20	0.01	0.00	0.12	0.08	0.02	0.38	0.10

4.10 Platelet responses

Table 4.31 indicates the medians and ranges for platelet (PLT) counts for each treatment group at each time point. Both treatment groups were severely thrombocytopaenic on Day 1 and Day 2, and moderately thrombocytopaenic on Day 3. The median PLT count was within the normal reference interval on Day 6 for both treatment groups.

Figure 4.14 indicates the mean log-transformed values for PLT counts for each treatment group at each time point.

Table 4.32 indicates the predictor (age) that had a significant effect on PLT counts, based on multiple regression models. The duration of illness, previous illness due to babesiosis and treatment group had no significant effect on this haematological variable throughout the study period. There was a significant effect of age on Day 0 (p=0.037), with older dogs having higher mean PLT counts than younger dogs.

Table 4.31 Median, minimum, maximum and range values ($\times$ 10^9/l) for PLT counts (reference interval 200-500 $\times$ 10^9/l) over time for the two treatment groups.

Note: the number of dogs evaluated decreased by day as indicated in Table 4.1

Treatment group	Blood transfusion				Non-blood transfusion			
Days	0	1	3	6	0	1	3	6
Median	4.1	7.7	122.0	357.5	3.6	5.2	124.0	293.5
Minimum	0.0	0.0	1.5	138.0	0.0	0.0	3.9	152.0
Maximum	92.8	131.0	745.0	538.0	157.0	135.0	397.0	966.0
Range	92.8	131.0	743.5	445.0	157.0	135.0	393.1	814.0
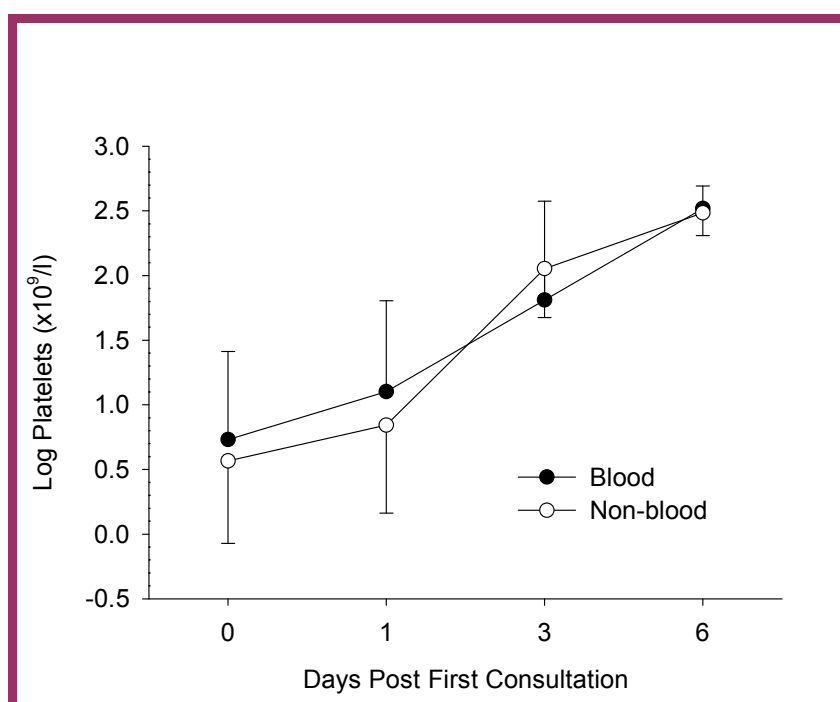
Figure 4.14 Mean log-transformed values for PLT count over time for the two treatment groups.

Error bars represent standard deviation. "Blood" indicates that a blood transfusion was given on Day 0; "Non-blood" indicates no blood transfusions were given. Note: the number of dogs evaluated decreased by day as indicated in Table 4.1

Table 4.32   Effect of treatment (blood transfusion vs. non-blood transfusion) and age on log transformed PLT counts: output of multiple regression models for Day 0, 1, 3 and 6. The shaded value indicates significance (p<0.05)

Day post first consultation	n	Predictor	ß[a]	SE (ß)[b]	P[c]
0	82	Treatment group	0.2064	0.1501	0.173
		Age	0.0062	0.0029	0.037
1	76	Treatment group	0.2963	0.1646	0.076
		Age	0.0052	0.0031	0.101
3	62	Treatment group	-0.2383	0.1496	0.117
		Age	0.0005	0.0027	0.846
6	40	Treatment group	0.0344	0.0592	0.564
		Age	0.0001	0.0015	0.939

[a] Regression Coefficient

[b] Standard Error Regression Coefficient

[c] P value

CHAPTER 5 DISCUSSION

5.1 Introduction

The clinical and haematological profile induced by canine babesiosis varies in different parts of the world depending on the species of babesia involved. The form of canine babesiosis that occurs in South Africa is particularly severe, and is characterized by anorexia, fever, splenomegaly and haemolytic anaemia. South African canine babesiosis is caused by *Babesia rossi*. A lack of knowledge on the haematological kinetics of canine babesiosis in South Africa made it difficult to compare the response to this parasite-induced haemolytic anaemia with the response to other diseases also causing anaemia.

After reviewing the literature it became clear that the data available on the haematological features of canine babesiosis caused by *B rossi,* were limited to one or two reports, despite this South African form of babesiosis causing a life-threatening disease, comparable to human falciparum malaria. No studies reporting on the haematological kinetics after infection with *B rossi* have been done*.*

The present study describes changes in the results of a complete blood count (CBC) of dogs naturally infected with *B rossi* in the Onderstepoort area, during the first six days following diagnosis. A group of dogs that received a blood transfusion was compared to a group that did not. The effect of treatment with a blood transfusion on the CBC of dogs naturally infected with *B rossi,* taking into account the duration of illness before presentation, previous infection with babesiosis and the age of the dogs was also investigated.

The main findings included a slightly to moderately regenerative normocytic normochromic anaemia throughout the study period of one week for both treatment groups. The anaemia induced by babesiosis caused by *B rossi* in this study, was very severe at presentation in dogs that received a blood transfusion and moderate at presentation in dogs that did not receive a blood transfusion. Mild anaemia was still present by the end of the study period, even in dogs that presented with moderate anaemia. The regenerative response was moderate. As expected, a similar but less pronounced regenerative response was found for dogs that did not receive a blood transfusion compared to dogs that did receive a blood transfusion. A mild inflammatory leukocytic response was found in both treatment groups. The median segmented neutrophil count for both treatment groups was within the reference interval throughout the study period of one week. A left shift occurred more commonly in dogs that received a blood transfusion, and was significantly influenced by the degree of

anaemia on Day 0 and 1. In dogs with a left shift, a degenerative left shift was found more commonly. This was not influenced by the degree of anaemia at presentation. A severe thrombocytopaenia for both treatment groups, which resolved within a week in both treatment groups, was found. These responses were not significantly influenced by age, previous infection with babesiosis or duration of illness. Treatment with a blood transfusion reduced the anaemia, but had no significant effect on white blood cell or platelet responses. These findings will be discussed in detail below.

5.2 Red blood cell responses

5.2.1 Anaemia

It is common practice to initially classify an anaemia morphologically on the basis of the mean cell volume (MCV) and mean cell haemoglobin concentration (MCHC), and then to examine the degree of reticulocytosis.[24,46] However, the morphological indices are not sensitive indicators of regeneration, but are useful when considering the pathophysiological mechanisms of anaemia.[20,46] Haemolytic anaemias are usually regenerative, and when the regeneration is marked, may be macrocytic and hypochromic.[33,46] In this study the anaemia was normocytic and normochromic in both treatment groups throughout the study period.

This is the first description of this morphological feature of the anaemia caused by *B rossi*. In spite of the introductory statement by Furlanello *et al.*[30] that the anaemia of babesiosis becomes macrocytic and hypochromic after initially being normocytic and hypochromic, no evidence of macrocytosis could be found in that investigation. In the Furlanello *et al.* study[30] normocytic normochromic anaemia was found based on one blood sample analysed. It is not stated when the blood sample was collected. Only three out of the 23 dogs in the Furlanello *et al.* study had weak regeneration.[30] In the two studies by Reyers *et al.*[81] and Abdullahi *et al.*,[1] there is no mention of the morphological classification of the regenerative anaemia caused by this parasite.

The finding that the anaemia was normocytic and normochromic in this study is not unexpected, as only markedly regenerative anaemias will influence these indices to the extent that a macrocytic hypochromia is found. In this study the regenerative response was never severe. It is thus recommended that the anaemia associated with babesiosis, first be categorised based on regeneration, rather than on morphological indices.

The red cell distribution width (RDW) will increase if there is significant reticulocytosis.[24] The latter was within the reference interval in both treatment groups throughout the study period,

except for a slight increase in RDW on Day 6 in dogs that did not receive a blood transfusion. As reticulocytosis was never severe in this study, it was expected to find the RDW within reference interval. The increase seen on Day 6 was as expected in the more anaemic group of dogs.

Mild anaemia was still present in both treatment groups at the end of the study period of one week. Clinically this had little effect, as by that time all the dogs showed a normal habitus and appetite. However, as all the dogs in this study were from a population of pets, the situation might be different in a group of working dogs. Owners are often cautioned not to overexert their dogs in the first week post treatment of babesiosis, and this can now be substantiated, based on the mild anaemia still present.

5.2.2   Regenerative response

The absolute reticulocyte count is the best indicator of effective erythropoiesis.[20,94] The severity of the regenerative response (based on absolute reticulocyte counts) differed significantly between the two treatment groups. This was expected, as the decision to give a blood transfusion, was largely based on the packed cell volume (PCV) at presentation, with the most anaemic dogs receiving a blood transfusion. The more hypoxic an animal is, the greater is the stimulation of erythropoiesis and the greater the reticulocytosis.[46] The severely anaemic dogs that received a blood transfusion had a moderate regenerative response at presentation and on Day 1 and Day 3, which then decreased significantly to a mild regenerative response by Day 6. This was an expected finding, since transfusion replenishes red blood cell (RBC) mass and decreases the stimulus for erythropoiesis. It is however stated that in cats and dogs, the regenerative response after an initial insult causing haemolytic anaemia peaks at days 4-7 and then returns to normal by day 15 (2-3 weeks), even when slight anaemia is still present.[20] Tvedten[93] also stated that reticulocyte numbers are best interpreted at the time of an expected peak response at 4-7 days, and might be misleading early in the regenerative response (i.e. with pre-regenerative anaemia) or late in the regenerative response (by day 10-14, when reticulocyte numbers decline). The dogs that presented with moderate anaemia, and therefore did not receive a blood transfusion, had no regenerative response at presentation or on Day 1, had a moderate response on Day 3 and a mild response on Day 6. If the median duration of babesial illness is two days, as was found in this study, the regenerative response is understandably lacking or mild in the first two days after diagnosis. Dogs in this study however, never showed a strong regenerative response, not even in the face of very severe anaemia or later in the course of the regenerative response. In healthy dogs with an acute severe anaemia and a functional bone marrow, a strong regenerative response is expected.[20]

In a study on experimental infection of canine babesiosis, Maegraith[62] found a moderate reticulocyte response (based on reticulocyte percentages) one week after infection, which then increased to a marked reticulocyte response by Day 8. (The classification of the regenerative response - adapted for reticulocyte percentages - as used in the current study, was applied to the study by Maegraith). This finding was different to the findings of this study, in which the reticulocyte response was never marked, and significantly declined by Day 6.

The reticulocyte response in the study by Reyers *et al.*[81] was reported as logarithmic mean percentages, and was in fact normal (non-regenerative) for dogs that were non-anaemic and moderately anaemic (corresponding to the group of dogs that did not receive a blood transfusion in this study), and only slightly regenerative for dogs that were severely anaemic (corresponding to the group of dogs that did receive a blood transfusion in this study). No data were available as to the duration of illness in the Reyers *et al.* study, and it is therefore difficult to assess what the response would have been relative to the course of the regenerative response. The Reyers *et al.* study[81] was retrospective and based on only one blood sample collected before treatment. Jacobson[42] stated that the regenerative anaemia described by Reyers *et al.*[81] indicated that dyserythropoiesis (as is described in human malaria[18,100]) is unlikely to play a role. This statement cannot be validated based on one blood sample with no indication of course of the regenerative response and without evaluation of bone marrow.

The strong reticulocyte responses in the study by Maegraith[62] and also in the study by Reyers *et al.*[81] were based on reticulocyte percentages, which can be falsely increased in anaemic animals, especially if the anaemia is severe, resulting in the erroneous interpretation of anaemia as regenerative. The absolute reticulocyte count is the best indicator of effective erythropoiesis,[20,93] as stated before.

A canine model of acute normovolaemic phlebotomy-induced anaemia has been described.[89] For the babesia-infected anaemic dogs that received a blood transfusion in this study, the reticulocyte counts (i.e. the measure of regeneration) throughout the duration of the study were significantly lower compared to the reticulocyte counts[b] (at a comparable severity of anaemia or haematocrit [HCT]) in the normovolaemic phlebotomy-induced anaemia model.[89] This was unexpected, as a more pronounced reticulocyte response is expected following haemolysis compared to a blood loss induced anaemia.[46] In the Spotswood *et al.* study cited above, the reticulocyte counts continued to rise significantly over the first week, and a

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[b] Unpublished data, from dataset of Spotswood TC *et al.*, 2005, Onderstepoort Journal of Veterinary Research, 72(2): 135-143 (with permission)

marked reticulocyte response was seen on Day 6.[89]   This is a clear difference in the regenerative response seen with babesia infection in this study, where the trend was towards a significant decrease by the sixth day post treatment. The HCTs of the babesia infected anaemic dogs and the phlebotomy induced anaemic dogs showed the same trend, by being low initially and then rising over a six day period.

Based on the observations described above, it appears that the regenerative response in babesia induced anaemia is inadequate. Based on this study that followed the regenerative response over a week, it appears that ineffective erythropoiesis may well be present during *B rossi* infections. This ineffective erythropoiesis could be directly parasite induced, or due to secondary immune mediated mechanisms. Evidence for dyserythropoiesis (e.g. basophilic stippling, multiple Howell-Jolly bodies and inappropriate metarubricytosis) was not found in this study, and no bone marrow samples were evaluated.

In summary it can be stated that the severe to moderate normocytic normochromic anaemia caused by *B rossi*, is only mildly to moderately regenerative, and still present a week after treatment. Even though previous authors have described a regenerative response and on the basis of that reasoned that the bone marrow response to canine babesiosis is adequate, this study showed that this response might be inadequate. This may have to be investigated further before definite conclusions can be drawn.

5.3   White blood cell responses

5.3.1   Total white blood cell counts

The median total white blood cell (WBC) count for dogs that did not receive a blood transfusion, was very mildly decreased below the reference interval at presentation. This is most commonly caused by neutropaenia.[55]   The median segmented neutrophil count for dogs in this study that did not receive a blood transfusion was within the reference interval at presentation, but it was at the lower range. Neutropaenia is caused by an increased tissue emigration of neutrophils in excess of bone marrow release, and leukopaenia and neutropaenia have been described in babesiosis in previous studies.[13,30,41,78,84,86,87,97]   However, in this case the neutropaenia at presentation was very mild and transient.

Apart from the finding discussed above, the median total WBC count was within the normal reference interval for both treatment groups throughout the study period, except for a mild leukocytosis present in both treatment groups on Day 3. Total WBC counts are not helpful in diagnosing disease, as changes in WBC subtypes, will offer more information about the

underlying pathophysiology. The only median differential WBC count that was increased in this study, was the band neutrophil count. Total WBC counts are most greatly affected by changes in neutrophil numbers, as neutrophils are the most numerous blood leukocyte in health and disease.[55] As the segmented neutrophil counts were within the reference interval throughout the study period, it is to be expected that the total WBC count therefore would also mostly be within the reference interval. The mild leukocytosis seen on Day 3, was probably due to inflammation caused by babesiosis, based on a left shift. The response appeared to be inadequate, as inflammation present for several days, as was the case here, should present with a leukocytosis with neutrophilia.[86]

Reyers *et al.*[81] also found mild to moderate leukocytosis in dogs that were severely and moderately anaemic, and a normal WBC count in dogs that were mildly anaemic, presumably at presentation, as the blood sample was collected before treatment. The severe leukaemoid reaction (WBC count above 50×10^9/l) described in two cases by Lobetti,[59] was only found in two CBCs (in two dogs on two occasions on Day 3) out of the total of 285 CBCs analysed in this study.

5.3.2   Segmented and band neutrophil counts

The median segmented neutrophil count for both treatment groups was within the reference interval throughout the study period. Neutrophilia, together with lymphopaenia and monocytosis occurs commonly with haemolytic anaemias.[24,31,88] Neutrophilia is caused by the release of inflammatory cytokines, including granulocyte-colony-stimulating factor associated with an immune response, necrosis and erythrophagocytosis by macrophages.[31]

Immune mediated haemolytic anaemia (IMHA) usually causes severe neutrophilia and even leukaemoid reactions.[31,86] While 88% of dogs with babesiosis are Coombs' positive,[81] most of these do not have spherocytosis or agglutination and are not clinically classified as having secondary IMHA.[42] In this study all dogs that tested positive on the in-saline agglutination test were excluded from the study, as they were subsequently given corticosteroid therapy. The dogs in this study therefore were not expected to show the typical leukocyte response associated with immune mediated haemolytic anaemias, but even so it seems that the lack of a neutrophilia, as found in this study, is unexpected. It is also true that the pathophysiology of canine babesiosial haemolysis is not well understood.[42] Both intravascular and extravascular haemolysis occur,[62] and both immune mediated and non immune mediated mechanisms of erythrocyte destruction could occur.[5] Reyers *et al.*[81] stated that the anaemia in severely and moderately anaemic dogs was caused by secondary immune mediated haemolysis. If so, then one would expect to find the severe neutrophilias

which usually accompany immune mediated haemolytic anaemias (although it can be quite variable).[31] This understanding of the pathophysiology of the anaemia of canine babesiosis, then does not explain the lack of severe neutrophilia in dogs with babesiosis in both this study and the study by Reyers *et al*.[81] Another possibility is this is a true reflection of the pathophysiology, but that the bone marrow was not able to mount the appropriate response.

A high percentage of dogs with a left shift were found in this study. This was an expected finding, as haemolysis, as a form of tissue destruction, may be accompanied by an inflammatory leukogram. The most anaemic dogs (i.e. dogs that received a blood transfusion) had a significantly higher percentage of left shifts at presentation and also at Day 1, or 24 hours after a blood transfusion was given. This could indicate that more severe anaemia is also a greater stimulus for the inflammatory leukocyte response.

A very high percentage of dogs with left shifts had degenerative left shifts, with 91% of dogs with a left shifts, in both treatment groups, with specifically a degenerative left shift at presentation. This was not influenced by the degree of anaemia. Both treatment groups had equally high percentages of dogs with degenerative left shifts. Even though degenerative left shifts are considered an ineffective response to inflammation, it is not uncommon to observe degenerative left shifts early or in severe inflammatory responses, as might be the case here. The number of dogs with degenerative shifts declined during the study period in both treatment groups. An exception to this was in dogs with more severe anaemia, where a high percentage of degenerative left shifts were again found on Day 6. This finding, different to the general trend throughout the study period, must be interpreted with caution, as the sample size was very small and the difference between the two treatment groups in this case was not statistically significant. While the occurrence of degenerative left shifts declined over the study period, it still occurred in more than 50% of all dogs that showed a left shift on Day 3. It would have been expected that by this time, about a week into the occurrence of an infectious process causing inflammation, a higher percentage of neutrophilias with a left shift should be present. It is known that the magnitude of a left shift diminishes and perhaps even disappears when the inflammatory process is chronic, as granulocytic hyperplasia results in the replenishment of the neutrophil storage pool, so that the rate of band neutrophil release from the bone marrow is not increased.[90] Given the time span in this study, it is unlikely that chronic inflammation is the cause of the degenerative left shifts or the absence of left shifts. It can again be reasoned that the bone marrow seems to be inadequate in responding to the inflammatory stimulus.

This study was the first to report on the characteristics of neutrophilias with a left shift and degenerative left shifts occurring in canine babesiosis caused by *B rossi*, with large case

numbers. The only other study providing haematological data based on large numbers, i.e. the study by Reyers *et al.*,[81] reported on a severe left shifted neutrophilia occurring in both severely and moderately anaemic dogs. However, when actual mean haematological variables in that study were examined, it was found that the inflammatory response was actually also one of a degenerative left shift, with mean segmented neutrophil counts within reference interval and the mean band neutrophils increased in the three groups of dogs that were described based on severity of anaemia. A clear definition as to left shifts was not given in the study by Reyers *et al.*,[81] but the mean segmented neutrophil counts in that study did not reflect a neutrophilia, which is the hallmark of the term "neutrophilia with a left shift". That study therefore actually concurs with the current study as to the occurrence of degenerative left shifts at presentation, even though it is then discussed as a severe left shift neutrophilia.

5.3.3　Lymphocyte counts

The median lymphocyte count was within the reference interval for both treatment groups throughout the study period, with a peak response on Day 3. Lymphocytosis in canine babesiosis based on smaller case numbers has been described in previous studies.[1,3,30,37,87] Reyers *et al.*[81] also found mean lymphocyte counts to be within the reference interval. The expected inflammatory lymphocytosis is typically mild (i.e. less than twice the upper reference interval).[90] Again it might be reasoned that a stronger response would have been expected, given the inflammatory nature of this disease. It might also be that stress masked a potential lymphocytosis.

It has been stated that lymphopaenia occurs together with haemolytic anaemias.[24,86] Lymphopaenia had been reported in other babesiosis studies with smaller case numbers.[30,41] This was not found in this study.

5.3.4　Monocyte counts

Mild monocytosis was found on Day 1 and Day 3 for dogs that received a blood transfusion and mild monocytosis was found on Day 3 for dogs that did not receive a blood transfusion. There was a peak response on Day 3 in both treatment groups. This was expected, given the inflammatory and haemolytic nature of this disease. An inflammatory monocytosis is typically mild (i.e. less than twice the upper reference interval) but may even exceed 10×10^9/l.[90] It is a fairly common leukogram alteration and can also occur with haemolysis.[24,86,88] Reyers *et al.*[81] also found a mild mean monocytosis in severely anaemic dogs with normal mean monocyte counts in moderately anaemic dogs.

5.3.5 Eosinophil counts

Very mild eosinopaenia was found at presentation in both treatment groups. This was transient, and median eosinophil counts increased in both treatment groups throughout the study period, but stayed within the reference interval. It may be that the eosinopaenia found in this study was stress induced. It is also clear from this study that canine babesiosis does not cause an eosinophilia. This finding is not unexpected, as the most common cause for an eosinophilia in dogs, is ecto- and endoparasitism.[86] It is also true that concurrent infections with ecto- and endoparasites can influence eosinophil counts in dogs with babesiosis. Owners who do not practice tick control, usually also do not practice control of other ecto- and endoparasites either.

As this is the first study with substantial case numbers of babesiosis caused by *B rossi,* to report on eosinophil numbers, it was not possible to compare these counts to previous studies. Reyers *et al.*[81] did not report on eosinophil counts at all. Three other authors[1,13,82] have reported on eosinophil counts, and found the counts to be within the reference interval.

5.3.6 Basophil counts

The median basophil count was 0×10^9/l throughout the study period for both treatment groups. This was within the reference interval, and it can therefore be concluded that canine babesiosis does not cause a basophilia in the week post treatment. Again, as in the case of the findings for eosinophil counts, this finding was not unexpected. This is the only study to date which reports on basophil counts in cases of canine babesiosis.

5.4 Platelet responses

Severe thrombocytopaenia was found at presentation and on Day 1 and moderate thrombocytopaenia was found on Day 3, in both treatment groups in this study. Platelet (PLT) counts were within the reference interval by Day 6.

Thrombocytopaenia is commonly recognised in dogs with babesiosis.[65,82,104] The severe thrombocytopaenia in dogs in this study concurs with findings by Kettner,[49] who found a median PLT count of 14×10^9/l and a mean PLT count of 34.6×10^9/l in 1162 dogs admitted to a veterinary teaching hospital and diagnosed with babesiosis. Reyers *et al.*[81] found a mean PLT count of 48×10^9/l in the severely anaemic group, 52×10^9/l in the moderately anaemic group and 44×10^9/l in the mildly anaemic group. In both these retrospective

studies, it is not known if concurrent disease, such as ehrlichiosis, was conclusively ruled out. In addition, it is unclear whether the CBC in the study by Kettner[49] was taken before or after treatment of the dogs. Dogs are usually treated at admission to this veterinary teaching hospital, while a blood sample for a CBC is usually only taken at various time intervals after admission and treatment. The median PLT count in both the treatment groups in the current study was much lower at presentation and at Day 1 than previously reported by Reyers *et al.*[81] and Kettner.[49] Due to uncertainties regarding the methodology, the reasons for the differences in PLT counts between this study and previous studies could not be surmised. The pathogenesis of the thrombocytopaenia seen in babesiosis is also still unclear.[49] It is known however, that in spite of severe thrombocytopaenia, clinical manifestation of a bleeding tendency, such as petechiae and ecchymoses, does not occur in babesiosis caused by *B (canis) rossi.*[49]

5.5 Multiple regression analysis

5.5.1 Effect of duration of illness, previous infection with babesiosis and age

Reyers *et al.*[81] speculated that disease in mildly or non-anaemic dogs was of such an overwhelming nature that there was no time for an inflammatory response to become evident. From this study it is clear that all dogs had the same median duration of illness (two days) and even if it was different, results of multiple regression analysis showed that duration of illness had no significant effect on any of the haematological variables. It cannot be explained by this study why with the same median duration of illness, one group of dogs became more anaemic more rapidly than the other group. Another hypothesis that can also now be addressed is the supposition that an inappropriate inflammatory response was mounted upon second infection with babesiosis.[81] This study showed that previous infection with babesiosis had no significant effect on any haematological variable, and therefore did not affect the inflammatory response.

Age did have an effect on some of the haematological variables. No logical explanation could be found for the fact that older dogs had higher mean MCVs at presentation. Reticulocytosis, a common cause for an increase in MCV, was not influenced by age. It has been reported that the MCV of newborn pups is 95-100 fl, but that adult levels (reference interval 60-77 fl) are reached by 2-3 months.[68] Breed variation has also been described, with Akitas and Shibas having microcytosis.[68] It must be noted that Poodle macrocytosis[68], is not a breed characteristic, but a hereditary anomaly found in some Poodles. None of these breeds was represented in this study, so breed could not have had a significant effect on MCV. As this finding bordered on non-significance (p=0.049) with a transient occurrence and

no longer present 24 hours later, it is probably not of significance. The same can be said for the influence of age on mean segmented neutrophil and mean PLT counts at presentation. In a study of Beagles less than two months old, fluctuations in total WBC counts and neutrophil counts occurred, but values in general stayed within reference intervals.[68] In another study of Beagles it was found that from 1.5 to 14 years of life, there was no significant trend in any of the leukocyte types.[61] It has been reported that leukocyte numbers in puppies are variable and can be higher than those in adult dogs.[94] No published data could be found on normal variables for young dogs as far as PLT counts go.

The fact that older dogs had lower mean lymphocyte counts was an expected finding. These counts were very significantly ($p<0.001$) and repeatedly lower. It has been reported previously that younger dogs have significantly higher lymphocyte counts.[68,92,94] This might explain why in the Reyers *et al.* study,[81] older dogs from the mild to non-anaemic group had significantly lower mean lymphocyte counts than younger dogs in the moderate and severely anaemic groups.

From the current study it is therefore clear that apart from the expected effect on mean lymphocyte counts, age did not influence the findings of any haematological variable significantly. While it is true that the median age of more anaemic dogs was lower than the median age of less anaemic dogs in this study, and that adult levels of RBC mass are only reached by an age of 6 months-1 year,[68] this study showed that when multiple regression models were fitted to a diseased state, this variation did not bring about significant influences. Reyers *et al.*[81] speculated that older dogs were less anaemic and mounted an overwhelming or inadequate inflammatory response. Based on the findings of the current study there is no significant difference between the erythroid regenerative response, leukocytic inflammatory response and thrombocytic response of younger versus older dogs.

5.5.2 Effect of treatment

When multiple regression models were fitted on the predictor "treatment group", it was in fact in investigation into the effect of degree or severity of anaemia on the haematological variables, as this is what determined the decision to give a blood transfusion or not. The degree of anaemia had a significant effect on haemoglobin (HGB) concentration (with the more anaemic dogs having lower mean HGB concentrations), on RBC counts (with the more anaemic dogs having lower mean RBC counts) on HCT (with the more anaemic dogs having lower mean HCTs), on MCV (with the more anaemic dogs having higher mean MVCs) and on reticulocyte counts (with the more anaemic dogs having higher mean reticulocyte counts). All these findings were expected, as these haematological variables actually define

regenerative anaemia as such. The degree of anaemia also had a significant effect on RDW (with the more anaemic dogs having a higher mean RDW). This was an expected finding, as the occurrence of reticulocytosis will increase the RDW.[24]

The degree of anaemia had a significant effect on band neutrophil counts and left shifts on Day 0 and Day 1 (with dogs with more severe anaemia having higher mean band neutrophil counts and a higher percentage of dogs with left shifts). This can be explained by the fact that haemolysis serves as an inflammatory stimulus on neutrophils, and that a greater degree of haemolytic anaemia will therefore serve as a stronger stimulus. However, once the severity of anaemia decreased with treatment, later in the disease process, this stimulus disappeared and the inflammatory band neutrophil response was then not significantly influenced anymore.

The degree of anaemia had no significant effect on platelet responses. This is the first study to report on this finding.

5.5.3   Comparison to other studies

When the results on age and degree of anaemia were compared to the study done by Reyers *et al.*,[81] some differences were found. Reyers *et al.*[81] found that the younger, more anaemic dogs mounted an inflammatory response (described by the author as a severely left shifted neutrophilia, monocytosis and lymphocyte counts within reference interval) while the older, non-anaemic dogs mounted a mild to no inflammatory response (described by the author as often found leukopaenia and near-lymphopaenia) and that there was a significant difference between these groups. If the mean HCT in the non-anaemic group in the Reyers *et al.* study[81] is considered (0.47 l/l), it is clear that that population probably differed considerably from the less anaemic group of dogs in this study, (where the median (and mean) HCT was 0.28 l/l) The non anaemic group in the Reyers *et al.* study[81] probably included more dogs in the haemo-concentrated category. This hypothesis is supported by the fact that the total Reyers *et al.* population consisted of hospitalized dogs, and hospitalized dogs with higher HCTs are generally seen to be more ill.[42,43] This could have influenced the nature of the inflammatory response in these generally more critical dogs. One of the strengths of the current study, apart from its prospective nature, is the fact that the less anaemic population included a high percentage of dogs that was treated as outpatients, and no data were previously available on the haematological responses of this important babesiosis population. However, this study only had one dog that fell into the HCT-based definition of haemoconcentration, i.e. HCT > 0.50 l/l. Data on this group of dogs were therefore lacking in this study. It should be noted that these dogs have a worse

outcome,[81] and many do not survive for a week post admission. It is stated in the Reyers *et al.* study[81] that multiple regression models were fitted on the haematological (and other variables), but apart form giving significance levels for log transformed mean counts, no other data on results of multiple regression studies were discussed.

Reyers *et al.*[81] also found that the degree of anaemia did not influence the mean PLT count. Kettner[49] in his study on thrombocytopaenia in canine babesiosis, speculated that the severity of thrombocytopaenia might be influenced by the severity of the anaemia. This was not found to be the case in the current study.

Though less obvious, there are more similarities between this study and the study done by Reyers *et al.*[81] It should be noted that the "severely left shifted neutrophilia" in the younger more anaemic dogs referred to by Reyers *et al.*, was in fact a degenerative left shift by definitions used in this study. The older mild to non-anaemic dogs in fact also showed a degenerative left shift. Degenerative left shifts were also found at presentation in both treatment groups in the current study. The "leukopaenia" described by Reyers *et al.* in the older less anaemic dogs, were in fact leukocyte counts within reference interval. WBC counts mainly within reference interval were also found in the current study. The monocytosis found in the study by Reyers *et al.* was mild in all groups of dogs, similar to this study. The mean lymphocyte counts were within reference interval for all groups, similar to this study. The near-lymphopaenia, seen as a weaker inflammatory response in the older less anaemic group, might be explained by the older age distribution of this group. Therefore, when applying the definitions of the current study, the inflammatory response described by Reyers *et al.*[81] may in fact, have been inadequate as well.

5.5.4   Conclusions drawn from multiple regression analysis

Based on multiple regression analysis it was found that the duration of illness before presentation and previous infection with babesiosis did not influence the haematological kinetics in the week after treatment. It therefore appears that the immune system is not primed by a previous infection, resulting in an inadequate or exaggerated inflammatory response. As expected, age only played a role as far as lymphocyte responses were concerned. The degree of anaemia affected the occurrence of an inflammatory cell response in the immediate period after treatment, but did not affect the platelet response.

CHAPTER 6 CONCLUSION

Canine babesiosis caused by *Babesia rossi* is an economically important and potentially life-threatening disease. An understanding of the haemopoietic response is required in order to make decisions and evaluate the appropriateness of the response and effectiveness of treatment. The bone marrow response, as evaluated by peripheral blood findings, to this infectious disease is poorly documented.

The fact that this disease causes anaemia and thrombocytopaenia is well known, but no valid data were available as to the white blood cell responses. This study complements existing knowledge, and also adds new findings.

Research in this study showed that in the week following treatment of canine babesiosis, results of the complete blood count included a mild to moderately regenerative normocytic normochromic anaemia which did not resolve by the end of one week. An inflammatory leukogram was observed, characterized mainly by a degenerative left shift. The severe thrombocytopaenia at presentation resolved within a week after commencing treatment.

It can be concluded from this study of temporal changes in peripheral blood cell counts that the red blood cell and white blood cell response seems inadequate in dogs with babesiosis, given the degree of anaemia and inflammatory stimulus present. Factors such as age, previous infection with babesiosis and duration of illness were shown to have no effect on the subsequent responses.

The reason for the inappropriate blood cell response is unclear at this stage, and warrants further investigation.

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Client Information Sheet

From the clinical examination and laboratory tests so far performed on your dog, it is clear that your dog is suffering from a blood parasite infection called babesiosis or "tick fever", transmitted via a tick bite. This blood parasite causes a blood loss as red blood cells are broken down.

It has been advised that your dog must be treated with anti-babesial medication/should be admitted to the Onderstepoort Veterinary Academic Hospital for intensive treatment, which will include anti-babesial medication and a blood transfusion if required. Other medications to treat possible side effects of babesiosis, such as nausea, low blood glucose, brain involvement, kidney failure and lung damage, will also be given as required.

If your dog has not been admitted, we will request you to keenly observe it for any signs of complications due to babesiosis (such as lack of appetite 24 hours after treatment, collapse, vomition, difficult breathing) occurring, and will request you to return your dog for blood sampling one day, three days and six days after our first consultation.

You will be allowed to withdraw your dog from the study at any stage, should you so wish.

In this study we will collect a blood sample from your dog, which is a painless procedure that will not harm your dog. We wish to measure the various cellular components (complete blood count) one, three and six days from being treated until recovery, and will collect blood samples on those days. We will be comparing this data with the severity of illness of your dog. With this information we hope to learn more about the disease.

This study will cost you no more money that it would usually cost you to treat your dog. We are paying for all the additional blood tests performed on your dog.

The Animal Use and Care Committee of the University of Pretoria have approved this study.

Thank you for allowing your dog to be included in the study. If you have any further questions please feel free to ask the clinician on duty, or myself [Tel: (012) 529 8163 (w)].

Dr Elrien Scheepers BVSc Hons
Senior lecturer, Dept of Companion Animal Clinical Studies

Consent form for canine babesiosis study

I, ……….
(Full Names)

Herewith give permission for the dog under my care

………
(Name of dog)

………
(breed, sex, colour, age)

to participate in the study on whole blood parameters in canine babesiosis in the Department of Companion Animal Clinical Studies, Section Small Animal Medicine, Faculty of Veterinary Science, University of Pretoria.

The study has been explained to me and I understand that the blood samples drawn are routine and safe. I understand, furthermore, that the costs of the additional tests will be borne by the trial fund, and that I will only be liable for costs pertaining the treatment that would in any event be required by my dog, including any complications that may arise as a result of canine babesiosis.

Signed at: …………………………………………………………… (place)

on the ………………… day of ………………………………..……… 20……

…………………………………………....
Signature of owner or authorized person

Blood transfusion therapy - volume to be transfused

Whole blood transfusion therapy will be performed if the patient's PCV is < 15%. The amount to be transfused will be calculated by the following formula (adapted from Kristensen AT, Feldman BF: Blood banking and transfusion medicine. In Ettinger SJ, Feldman BF, eds: Textbook of Veterinary Internal Medicine. Philadelphia, WB Saunders; 1995: 347-360) A PCV value of 25% is desired.

$$\text{Volume (ml) blood needed} = \frac{\text{PCV (desired)} - \text{PCV (patient)}}{\text{PCV (donor)}} \times 90 \times \text{body weight (kg)}$$

Complete Blood Count

A complete blood count was done using the CELL-DYN[®] 3700 System to measure, count, and calculate the haematologic results. The CELL-DYN[®] 3700 System is a multi-parameter, automated haematology analyser designed for in vitro diagnostic use in clinical laboratories, and has been validated for use in the dog.[80]

Variables that were included:
- Haemoglobin (HGB) Concentration
- Red Blood Cell (RBC) Count
- Haematocrit (HCT)
- Mean Cell Volume (MCV)
- Mean Cell Haemoglobin Concentration (MCHC)
- Red Cell Distribution Width (RDW)
- Reticulocyte Count
- Total White Blood Cell (WBC) Count
- Neutrophil (segmented) count
- Neutrophil (bands) count
- Lymphocyte count
- Monocyte count
- Eosinophil count
- Basophil count
- Platelet (PLT) count

Four independent measurements are used in the CELL-DYN[®] 3700 System to obtain the haematologic variables.
- The WBC Optical Count (WOC) and the WBC differential data are measured in the optical flow channel.
- The WBC Impedence Count (WIC) is measured in one electrical impedence channel.
- The RBC and PLT data are measured in a second electrical impedence channel.
- The HGB concentration is measured in the spectrophotometric channel.

During each instrument cycle, the sample is aspirated, diluted and mixed, and each variable is measured.

Manual leukocyte differential counts were performed by an experienced veterinary haematology technologist by counting 100 cells on an air-dried Cam's Quick-Stain-stained thin capillary blood smear. When counting immature neutrophils, band neutrophils only were included in the count.

PCR analysis (as described by Matjila *et al.*[64])

DNA extraction

DNA was extracted from all the blood specimens taken at first consultation. The Qiamp blood and tissue extraction kit (Qiagen, Hilden, Germany) was used for DNA extractions. DNA was extracted from 200 µl of whole blood by adding 500 µl phosphate buffered saline (PBS), mixing and centrifuging (14 000×g) for five minutes, and discarding the supernatant. These steps were repeated 3-5 times until the pellet was white and the supernatant clear. The pellet was resuspended in 100 µl of lysis buffer (50 mM KCl, 0.5% Tween 20, 10 mM Tris-HCl [pH 8.0]) and 1 µl of proteinase K solution (1 µg/µl), mixed and incubated overnight at 56°C and heated at 100°C for 1 min to inactivate the proteinase K.

PCR

Babesia PCR was performed with a set of primers RLB F2 (5'-AC ACA GGG AGG TAG TGA CAA G-3') and RLB R2 (biotin- 5'-CTA AGA ATT TCA CCT CTG ACA GT-3') that amplified a fragment of 460-540 bp of the 18S SSUr RNA gene spanning the V4 region. The Ehrlichia PCR amplified the V1 hypervariable region of the 16S SSU rRNA. All primers were obtained from Isogen BV (Maarsen, The Netherlands).

The PCR reaction (25 µl) contained 2.5 µl of DNA template in 1× PCR buffer (HT Biotechnology, Cambridge, UK), 5 U of SuperTaq (HT Biotechnology, Cambridge, UK), 200 and 100 µM of each of the following deoxynucleoside triphosphate (dATP, dCTP, dGTP) and (dTTP, dUTP), respectively (Pharmacia Biotech, Uppsala, Sweden), 20 pmol of each primer, 5 U Taqstart Antibody (Clontech, California, USA) and 0.4 U Uracil DNA Glycosylase (Invitrogen, Breda, The Netherlands). The reactions were performed in an automated I-Cycler (Biorad, California, USA) with an initial step of 3 min at 37°C, 10 min at 94°C, 10 cycles of 94°C (20 s)-67°C (30 s)-72°C (30 s), with lowering of the annealing step after every second cycle with 2°C (touchdown) then followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 57°C for 30 s and extension at 72°C for 30 s.

Reverse line blot hybridization

Reverse line blot (RLB) was performed using PCR products as described by Gubbels *et al*. (1999) with the following modifications: denatured PCR products were diluted in 2× SSPE-0.1% sodium dodecyl sulphate (SDS), loaded onto the membrane and incubated at 42°C for 1 h. Thereafter the membrane was washed twice at 50°C instead of 42°C.

Sequence analysis

To differentiate between the three large piroplasms of dogs, species-specific oligonucleotides were deduced in the amplified V4 region. The following GenBank accession numbers of the 18S rDNA

sequences were used to deduce the species-specific oligonucleotides: *B. c. rossi*, <u>L19079</u>; *B. c. vogeli*, <u>AY072925</u>; *B. c. canis*, <u>AY072926</u>.

To confirm RLB results and to determine sequence heterogeneity of isolates, two out of 12 *B. c. vogeli* positive samples and one *B. c. rossi* positive sample from Bloemfontein were re-amplified with primers RLB-F2 and 18SEQ2 (5′-GCCCTTCCGTCAATTCCTTTAA-3′). PCR conditions were the same as described above but without dUTP and UDG and there were 30 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 1 min followed by a final step of 7 min at 72°C. Partial sequences (530-540 bp) from the products were sequenced (BaseClear, Leiden, The Netherlands).

A search was performed to examine similarities among sequences in GenBank using the BLASTN program.   Sequence alignment was done using the Multalin on-line interface (<u>http://www.toulouse.inra.fr/multalin.html</u>) and thereafter manually edited in Genedoc (version 2.6.001).

Clinical Examination

Admission/First consultation		Day 1		Day 3		Day 6	
Date:							
Client Name:			Tel no.			F Number:	STICKER
Patient Name:							
Breed:							
Sex:			Age:			Weight:	
Vaccination and deworming status:							
Results faecal flotation:							
History of previous babesiosis?							
Date of Admission to OVAH				Time of Admission			
Number of days depressed		1		2		3	> 3 State:
Number of days anorectic		1		2		3	> 3 State:
Habitus		1+		2+		3+	4+
% Dehydrated		0-5%		5%		10%	> 10%
Mucosae		moist				dry	
		pale		pink		congested	icteric
Temperature							
Pulse rate							
Pulse quality		weak		strong		water hammer	
Pulse rhythm		regular				irregular	
Respiratory rate							
Depth of respiration		normal		laboured		shallow	
Abnormal lung sounds		yes				no	
If yes, describe							
Abdominal palpation		tense				easily palpable	
		painful				not painful	
Peripheral lymphnodes		normal				enlarged	
Blood smear		% parasitaemia				neutrophilia/neutropaenia	
		thrombocytopaenia				reticulocytes	
		metarubricytosis				monocytosis	
PCV		TPP				ISA	
Treatment given							

APPENDIX G

Patient Outcome

Patient:

Died/recovered/euthanised:

Date died/recovered/euthanised:

Time died/recovered/euthanised:

Days to recovery/death:

Complications developed?

If so, describe:

Signalment, history, treatment and outcome

Dog ID	Breed	Sex	Weight Kg	Age months	Days ill	First infect	Repeat	Treatment	Group	Adm	Outcome
1	Rottwei	F	29.0	31	1	yes	no	Fluids	Nonblood	yes	Recovered
2	CB	M	9.4	18	3	yes	no	Dizene	Nonblood	no	Recovered
3	CB	M	6.2	4	4	yes	no	Blood	Blood	yes	Recovered
4	Dachs	M	5.2	60	2	yes	no	Fluids	Nonblood	yes	Recovered
5	Boerboel	M	17.0	5	3	yes	no	Blood	blood	yes	Recovered
6	CB	M	8.0	18		yes	no	Fluids	nonblood	yes	Recovered
7	Jack Russ	M	8.9	24	2	yes	no	Dizene	nonblood	no	Recovered
8	Boerboel							Lin fb			EXCLUDED
9	Ber M dog	M	42.0	16	0	yes	no	Fluids	nonblood	yes	Recovered
10	Malt	F	5.5	183	2	yes	no	Fluids	nonblood	yes	Died
11	Boerboel	M	3.5	16		yes	no	Dizene	nonblood	no	Recovered
12	Eng Bull	M	14.5	11	0	2nd time	yes	Dizene	nonblood	no	Recovered
13	Jack Russ	M	7.3	9	1	yes	no	Blood	blood	yes	Recovered
14	Dachs	F	6.0	45	7	yes	no	Fluids	nonblood	yes	Recovered
15	Sharpei	F	13.6	39	2	yes	no	Blood	blood	yes	Recovered
16	Boerboel	M	49.0	16	3	yes	no	Dizene	nonblood	no	Recovered
17	Pitt Bul	M	24.0	16		unknown		Dizene	nonblood	no	Recovered
18	CB	M	5.3	40	2	unknown		Blood	blood	yes	Recovered
19	French P							E canis			EXCLUDED
20	Jack Russ	M	7.5	167		yes	no	Fluids	nonblood	yes	Recovered
21	CB							E canis			EXCLUDED
22	CB	M	33.0	31		unknown		Fluids	nonblood	yes	Recovered
23	Fox Terr	F	7.0	38	2	yes	no	Fluids	nonblood	yes	Recovered
24	Boerboel	M	38.0	22	2	yes	no	Dizene	nonblood	no	Recovered
25	CB	M	20.0	72	2	unknown		Blood	blood	yes	Recovered
26	Fox Terr	M	11.0	49	0.5	yes	no	Dizene	nonblood	no	Recovered
27	Jack Russ	F	7.8	19	2	yes	no	Dizene	nonblood	no	Got pred
28	Jack Russ	M	11.6	12	3	unknown		Dizene	nonblood	no	Recovered
29	Labrad	M	22.0	9	0	unknown		Dizene	nonblood	no	Recovered
30	Boerboel	F	24.0	13	2	yes	no	Fluids	nonblood	yes	Recovered
31	Jack Russ	F	6.0	48	2	yes	no	Dizene	nonblood	no	Recovered
32	GSD	M	32.0	10	2	yes	no	Blood	blood	yes	Recovered
33	Dachs	M	7.0	48	2	yes	no	Dizene	nonblood	no	Recovered
34	Boxer	M	25.0	36	3	yes	no	Dizene	nonblood	no	Recovered
35	Basset	F	22.0	30	2	yes	no	Dizene	nonblood	no	Recovered
36	GSD	M	31.5	36	3	unknown		Dizene	nonblood	no	Recovered
37	Schnauz	M	11.7	13		yes	no	Dizene	nonblood	no	Recovered
38	Boerboel							Paralyse			EXCLUDED
39	CB	F	28.0	21		unknowm		Fluids	nonblood	yes	Recovered
40	Rottwei	F	16.0	6	2	yes	no	Dizene	nonblood	no	Recovered
41	CB	F	4.7	72	3	yes	no	Blood	blood	yes	Recovered
42	Rottwei	F	26.0	12	1	2nd time	yes	Dizene	nonblood	no	Recovered
43	Boerboel	M	38.3	22	1	2nd time	yes	Fluids	nonblood	yes	Euthan

Signalment, history, treatment and outcome

Dog ID	Breed	Sex	Weight Kg	Age months	Days ill	First infect	Repeat	Treatment	Group	Adm	Outcome
44	Labrad	M	8.7	4	0	yes	no	Blood	blood	yes	Recovered
45	CB	M	3.0	21	2	yes	no	Blood	blood	yes	Recovered
46	Sharpei	M	18.0	7	3	yes	no	Blood	blood	yes	Recovered
47	Jack Russ	M	7.0	22		unknown		Dizene	nonblood	no	Got pred
48	GSD	M	29.0	28	2	before	yes	Fluids	nonblood	yes	Recovered
49	Jack Russ	M	6.4	39	1	yes	no	Fluids	nonblood	yes	Recovered
50	Malt	F	7.0	84	4	yes	no	Dizene	nonblood	no	Recovered
51	Sharpei	M	17.6	25	1	yes	no	Dizene	nonblood	no	Recovered
52	Bull Terr	M	20.8	18	1	yes	no	Fluids	nonblood	yes	Recovered
53	Malt	F	4.0	6	0	yes	no	Blood	blood	yes	Died
54	Boerboel	F	20.0	8		yes	no	Dizene	nonblood	no	Recovered
55	Dalmat	F	12.2	4	1	unknown		Blood	blood	yes	Died
56	Malt	F	6.5	24	0.5	yes	no	Fluids	nonblood	yes	Recovered
57	Boxer	M	9.9	5	1	2nd time	yes	Blood	blood	yes	Recovered
58	Labrad	F	34.0	24	0	yes	no	Dizene	nonblood	no	Recovered
59	Boerboel	M	7.4	7	3	yes	no	Dizene	nonblood	no	Recovered
60	CB							E canis	B vogeli		EXCLUDED
61	Fox Terr	M	6.6	7	1	yes	no	Blood	blood	yes	Recovered
62	Rottwei	M	32.2	10	10	yes	no	Blood	blood	yes	Recovered
63	Boerboel	F	40.0	36	1	yes	no	Fluids	nonblood	yes	Died
64	Fox Terr	M	9.0	72	2	yes	no	Dizene	nonblood	no	Recovered
65	Bouvier	F	27.5	14	4	yes	no	Dizene	nonblood	no	Recovered
66	Jack Russ	M	3.6	4	2	yes	no	Blood	blood	yes	Recovered
67	Labrad	M	19.0	12	1	unknown		Dizene	nonblood	no	Recovered
68	Husky	F	21.0	7	1	3rd time	yes	Dizene	nonblood	no	Recovered
69	CB	M	4.6	36	3	yes	no	Blood	blood	yes	Recovered
70	Labrad	M	12.2	8	0	yes	no	Dizene	nonblood	no	Recovered
71	Dachs	M	5.6	15	5	unknown		Blood	blood	yes	Got pred
72	Rottwei	F	34.0	15	1	2nd time	yes	Dizene	nonblood	no	Recovered
73	CB	M	9.0	15		before	yes	Dizene	nonblood	no	Recovered
74	GSD	F	6.8	6		yes	no	Blood	blood	yes	Recovered
75	CB	F	24.2	36		unsure		Blood	blood	yes	Recovered
76	Husky	F	17.0	5	1	yes	no	Fluids	nonblood	yes	Died
77	GSD	M	30.0	72	1	yes	no	Blood	blood	yes	Recovered
78	Pitt Bul	F	25.0	24	2	yes	no	Fluids	nonblood	yes	Died
79	Husky	F	5.0	3	0.5	yes	no	Blood	blood	yes	Recovered
80	Jack Russ	M	10.4	48	1	2nd time	yes	Blood	blood	yes	Recovered
81	Rottwei	F	47.8	48	3	unknown		Fluids	nonblood	yes	Recovered
82	Toy Pom	F	3.0	25	1	before	yes	Fluids	nonblood	yes	Recovered
83	Great Dan	F	22.2	6	1	yes	no	Dizene	nonblood	no	Recovered
84	Pitt Bul							Parvo			EXCLUDED
85	Boerboel	F	48.7	34	2	before	yes	Blood	blood	yes	Recovered
86	Boerboel	M	33.9	12	1	unknown		Blood	blood	yes	Recovered
87	CB	M	16.3	6		yes	no	Dizene	nonblood	no	Recovered
88	Min Pin	M	4.0	11		unknown		Blood	blood	yes	Recovered
89	Boerboel	M	17.0	4	1	yes	no	Blood	blood	yes	Recovered

Signalment, history, treatment and outcome

Dog ID	Breed	Sex	Weight Kg	Age months	Days ill	First infect	Repeat	Treatment	Group	Adm	Outcome
90	Rottwei	F	36.0	18		yes	no	Fluids	nonblood	yes	Recovered
91	Bull Mast	M	34.0	6	2	yes	no	Blood	blood	yes	Recovered
92	Husky	F	13.8	9	0.5	2nd time	yes	Blood	blood	yes	Recovered
93	GSD	M	24.0	19	5	yes	no	Dizene	nonblood	no	Recovered
94	Rottwei	M	15.0	5	0	yes	no	Dizene	nonblood	no	Recovered
95	CB	M	20.5	12	7	unknown		Blood	blood	yes	Recovered
96	Jack Russ	M	6.0	8	3	unknown		Blood	blood	yes	Recovered

Red blood cell variables

Dog ID	Treatment	Group	Day	Hb g/l	RCC x10^12/l	Ht l/l	MCV fl	MCHC g/dl cells	RDW %	Retics %	Abs ret x10^9/l
3	Blood	blood	0	23	1.01	0.070	69.40	33.10	16.10	15.80	159.58
3	Blood	blood	1	71	3.03	0.215	70.90	33.10	20.10	14.00	424.20
3	Blood	blood	6	92	4.36	0.295	67.60	31.20	25.80	1.08	47.09
5	Blood	blood	0	33	1.49	0.091	61.00	35.90	16.10	5.91	88.06
5	Blood	blood	1	56	2.52	0.160	63.80	34.60	17.20	5.43	136.84
5	Blood	blood	3	81	3.49	0.245	70.30	33.20	21.10	17.95	626.46
5	Blood	blood	6	93	4.05	0.269	66.20	34.70	17.50	1.43	57.92
13	Blood	blood	0	68	3.05	0.198	64.90	34.40	16.00	0.83	25.32
13	Blood	blood	1	87	4.00	0.260	64.90	33.30	16.50	0.22	8.80
13	Blood	blood	3	124	5.74	0.369	64.30	33.50	17.40	0.78	44.77
13	Blood	blood	6	152	7.19	0.469	65.20	32.40	20.30	1.19	85.56
15	Blood	blood	0								
15	Blood	blood	1	33	1.57	0.103	65.80	31.60	37.30	13.49	211.79
15	Blood	blood	3	58	2.55	0.186	72.90	31.10	39.70	8.85	225.68
15	Blood	blood	6	78	3.53	0.249	70.50	31.20	30.50	0.91	32.12
18	Blood	blood	0	32	1.39	0.093	67.30	34.50	19.20	16.20	225.18
18	Blood	blood	1	41	1.77	0.177	66.00	35.10	16.90	22.79	403.38
18	Blood	blood	3	47	1.99	0.145	72.70	32.40	17.30	13.40	266.66
18	Blood	blood	6	66	2.68	0.203	75.90	32.50	22.90	5.02	134.54
25	Blood	blood	0	36	1.45	0.106	73.10	33.80	28.60	10.12	146.74
25	Blood	blood	1	52	2.18	0.155	70.90	33.80	22.60	5.18	112.92
25	Blood	blood	3	81	3.24	0.242	74.70	33.30	32.10	5.74	185.98
25	Blood	blood	6	104	4.43	0.324	73.10	32.10	28.30	0.82	36.33
32	Blood	blood	0	67	2.92	0.188	64.40	34.50	15.00	0.80	23.36
32	Blood	blood	1	80	3.64	0.238	65.40	33.40	16.50	1.57	57.15
32	Blood	blood	3	105	4.60	0.307	66.90	34.30	17.40	8.95	411.70
32	Blood	blood	7	119	5.29	0.363	68.60	32.90	19.80	1.35	71.42
41	Blood	blood	0	35	1.48	0.098	66.30	35.30	15.60	17.46	258.41
41	Blood	blood	1	50	2.22	0.148	66.60	33.60	15.30	7.47	165.83
41	Blood	blood	3	58	2.41	0.172	71.50	33.50	15.70	8.50	204.85
44	Blood	blood	0	34	1.48	0.095	64.00	35.50	18.00	10.86	160.73
44	Blood	blood	1	72	3.02	0.207	68.60	34.50	16.90	5.03	151.91
44	Blood	blood	3	101	4.22	0.293	69.30	34.40	19.50	14.73	621.61
44	Blood	blood	6	110	4.93	0.331	67.20	33.10	19.20	1.87	92.19
45	Blood	blood	0	45	1.75	0.124	70.90	36.20	16.70	14.84	259.70
45	Blood	blood	1	61	2.61	0.173	66.20	35.40	14.20	8.61	224.72
45	Blood	blood	3	70	3.01	0.214	71.00	33.00	15.60	29.31	882.23
45	Blood	blood	6	106	4.46	0.335	75.10	31.50	29.70	3.68	164.13
46	Blood	blood	0	48	2.11	0.139	66.00	34.60	15.60	2.19	46.21
46	Blood	blood	1	44	2.09	0.128	61.30	34.30	14.60	2.65	55.39
46	Blood	blood	3	56	2.61	0.173	66.40	32.60	15.10	16.88	440.57
46	Blood	blood	6	89	3.94	0.274	69.40	32.60	28.20	2.94	115.84
53	Blood	blood	0	24	0.99	0.078	78.40	31.10	24.30	13.00	128.70
55	Blood	blood	0	23	0.86	0.061	71.10	38.40	30.80	1.83	15.74
57	Blood	blood	0	34	1.46	0.103	70.70	33.20	19.20	8.51	124.25

Red blood cell variables

Dog ID	Treatment	Group	Day	Hb g/l	RCC x10^12/l	Ht l/l	MCV fl	MCHC g/dl cells	RDW %	Retics %	Abs ret x10^9/l
57	Blood	blood	1	39	1.65	0.113	68.40	34.50	18.30	12.56	207.24
57	Blood	blood	3	61	2.43	0.184	75.60	33.30	28.50	17.00	413.10
57	Blood	blood	6	92	4.26	0.302	71.00	30.50	25.10	0.99	42.17
61	Blood	blood	0	28	1.04	0.091	87.30	30.40	28.30	20.26	210.70
61	Blood	blood	1	101	4.15	0.305	73.40	33.10	17.90	23.25	964.88
61	Blood	blood	3	115	4.74	0.350	73.70	33.00	27.50	5.40	255.96
61	Blood	blood	6	117	5.02	0.357	71.10	32.90	21.40	0.41	20.58
62	Blood	blood	0	38	1.78	0.108	60.50	35.10	16.30	2.84	50.55
62	Blood	blood	1	73	3.43	0.217	63.30	33.50	17.20	4.65	159.50
62	Blood	blood	3	103	4.52	0.301	66.60	34.30	18.70	17.86	807.27
62	Blood	blood	6	98	4.34	0.284	65.50	34.50	18.70	2.11	91.57
66	Blood	blood	0	41	1.81	0.121	67.00	34.10	16.20	5.60	101.36
66	Blood	blood	1	86	3.67	0.245	66.70	35.00	15.30	2.01	73.77
66	Blood	blood	3	110	4.64	0.317	68.30	34.80	15.60	11.22	520.61
66	Blood	blood	6	112	4.72	0.322	68.30	34.80	18.60	2.01	94.87
69	Blood	blood	0	57	2.21	0.139	62.90	41.00	16.80	3.16	69.84
69	Blood	blood	1	64	2.72	0.175	64.10	36.80	18.30	3.16	85.95
69	Blood	blood	3	47	2.14	0.136	63.50	34.40	16.60	15.13	323.78
69	Blood	blood	6	64	2.69	0.193	71.60	33.40	17.30	19.97	537.19
71	Blood	blood	0	34	1.54	0.099	64.30	34.60	15.90	29.55	455.07
74	Blood	blood	0	33	1.60	0.097	60.70	33.70	15.00	11.34	181.44
74	Blood	blood	1	48	2.26	0.143	63.40	33.80	16.30	8.33	188.26
74	Blood	blood	3	87	3.84	0.259	67.50	33.50	21.20	13.60	522.24
75	Blood	blood	0	23	0.87	0.069	78.50	33.40	27.10	21.91	190.62
75	Blood	blood	1	54	2.23	0.163	73.20	33.10	23.00	19.80	441.54
75	Blood	blood	4	74	3.00	0.231	76.80	32.30	33.00	9.47	284.10
77	Blood	blood	0	43	1.37	0.098	71.70	34.90	21.20	10.51	143.99
77	Blood	blood	1	49	2.01	0.140	69.80	35.30	17.10	15.72	315.97
77	Blood	blood	3	68	2.61	0.202	77.30	33.90	25.10	8.90	232.29
79	Blood	blood	0	25	1.08	0.071	65.80	35.50	16.00	18.78	202.82
79	Blood	blood	1	58	2.56	0.172	67.00	33.90	16.00	19.37	495.87
80	Blood	blood	0	26	1.03	0.084	80.80	31.20	25.40	15.05	155.02
80	Blood	blood	1	67	2.83	0.200	70.80	33.60	17.00	7.99	226.12
80	Blood	blood	3	69	2.84	0.205	72.20	33.70	18.40	5.18	147.11
80	Blood	blood	6	83	3.44	0.252	73.10	33.10	23.60	4.10	141.04
85	Blood	blood	0	77	3.29	0.225	68.20	34.20	14.60	10.05	330.65
85	Blood	blood	1	42	1.78	0.123	68.90	34.20	15.70	15.32	272.70
85	Blood	blood	3	73	2.85	0.226	79.10	32.30	21.00	8.40	239.40
86	Blood	blood	0	32	1.34	0.094	70.60	33.70	33.50	16.24	217.62
86	Blood	blood	1	46	1.91	0.136	71.30	34.10	23.10	9.81	187.37
86	Blood	blood	3	52	2.00	0.158	79.50	32.60	34.30	9.16	183.11
86	Blood	blood	6	97	3.75	0.312	83.10	31.10	18.40	1.54	57.75
88	Blood	blood	0	40	1.82	0.127	70.00	31.80	19.80	13.58	247.16
88	Blood	blood	1	54	2.32	0.163	70.40	33.00	16.90	11.83	274.46
88	Blood	blood	3		3.30				29.10	19.60	646.80
88	Blood	blood	6	103	4.24	0.334	78.60	31.00	25.20	6.42	272.21

Red blood cell variables

Dog ID	Treatment	Group	Day	Hb g/l	RCC x10^12/l	Ht l/l	MCV fl	MCHC g/dl cells	RDW %	Retics %	Abs ret x10^9/l
89	Blood	blood	0	26	1.00	0.082	81.60	32.30	33.30	16.60	166.00
89	Blood	blood	1	73	2.93	0.212	72.40	34.30	15.50	25.68	752.42
89	Blood	blood	3	86	3.38	0.257	75.90	33.40	19.40	13.40	452.92
91	Blood	blood	0	76	3.47	0.220	63.50	34.70	15.40	1.31	45.46
91	Blood	blood	1	102	4.46	0.295	66.10	34.50	16.30	0.51	22.75
91	Blood	blood	3	112	5.01	0.340	67.80	33.10	16.00	1.66	83.17
92	Blood	blood	0	47	1.71	0.135	68.00	34.40	16.60	1.20	20.52
92	Blood	blood	1	73	2.75	0.194	70.50	37.40	16.50	1.35	37.13
92	Blood	blood	3	92	3.52	0.280	72.40	33.00	16.40	4.87	171.42
92	Blood	blood	6	107	4.48	0.318	71.00	33.70	19.90	2.37	106.18
95	Blood	blood	0	45	1.95	0.128	65.60	35.10	17.20	8.43	164.39
95	Blood	blood	1	58	2.55	0.169	66.30	34.30	16.50	6.62	168.81
95	Blood	blood	3	86	3.66	0.255	69.70	33.50	17.40	29.05	1063.23
96	Blood	blood	0	28	1.16	0.078	67.70	35.70	16.70	1.47	17.05
96	Blood	blood	1	50	2.20	0.145	66.10	34.30	16.20	2.37	52.14
96	Blood	blood	3	52	2.33	0.159	68.30	32.60	15.60	17.68	411.94
1	Fluids	nonblood	0	111	5.18	0.321	61.80	34.70	15.20	0.27	13.99
1	Fluids	nonblood	1	73	3.38	0.210	62.10	34.70	15.20	0.17	5.75
1	Fluids	nonblood	3	98	4.51	0.280	62.10	35.00	16.40	1.42	64.04
2	Dizene	nonblood	0	31	1.28	0.090	70.10	34.90	14.10	11.64	148.99
2	Dizene	nonblood	1	31	1.24	0.089	71.70	34.50	14.70	17.63	218.61
2	Dizene	nonblood	3	52	2.12	0.162	76.50	31.80	32.50	19.60	415.52
2	Dizene	nonblood	7	93	3.96	0.296	74.80	31.50	19.90	6.82	270.07
4	Fluids	nonblood	0	196	8.84	0.558	63.10	35.10	16.40	0.14	12.38
4	Fluids	nonblood	1	131	5.85	0.376	64.50	34.80	15.50	0.16	9.36
4	Fluids	nonblood	3	137	6.07	0.402	66.30	34.00	16.80	0.32	19.42
4	Fluids	nonblood	6	141	6.14	0.403	65.70	34.90	14.80	1.51	92.71
6	Fluids	nonblood	0	110	5.01	0.328	65.50	33.60	14.70	0.58	29.06
6	Fluids	nonblood	1	97	4.35	0.286	65.60	33.90	14.10	0.51	22.19
6	Fluids	nonblood	3	121	5.42	0.358	66.00	33.70	14.00	1.79	97.02
6	Fluids	nonblood	6	135	6.14	0.434	70.70	31.10	15.80	2.45	150.43
7	Dizene	nonblood	0	93	4.35	0.277	63.60	33.70	14.40	0.76	33.06
7	Dizene	nonblood	1	86	4.03	0.257	63.80	33.70	14.50	0.63	25.39
7	Dizene	nonblood	3	108	4.86	0.314	64.70	43.40	14.90	3.13	152.12
7	Dizene	nonblood	6	123	5.60	0.363	64.90	33.70	15.50	1.80	100.80
9	Fluids	nonblood	0	177	7.63	0.493	64.70	35.80	16.50	0.33	25.18
9	Fluids	nonblood	1	151	6.51	0.443	68.00	34.10	15.80	0.25	16.28
9	Fluids	nonblood	3	159	6.86	0.458	66.70	34.70	15.80	0.11	7.55
9	Fluids	nonblood	6	151	6.52	0.433	66.40	43.90	16.50	0.87	56.72
10	Fluids	nonblood	0	144	6.12	0.418	68.30	34.60	14.90	0.52	31.82
11	Dizene	nonblood	0	130	4.18	0.293	70.10	44.40	14.80	0.25	10.45
11	Dizene	nonblood	1	125	3.36	0.240	71.30	52.40	16.20	0.52	17.47
11	Dizene	nonblood	3	154	6.16	0.430	69.90	35.80	16.40	1.18	72.69
11	Dizene	nonblood	6	153	6.74	0.447	66.30	34.30	17.40	0.37	24.94
12	Dizene	nonblood	0	67	2.79	0.178	63.60	37.70	14.80	2.18	60.82

Red blood cell variables

Dog ID	Treatment	Group	Day	Hb g/l	RCC x10^12/l	Ht l/l	MCV fl	MCHC g/dl cells	RDW %	Retics %	Abs ret x10^9/l
12	Dizene	nonblood	1	50	2.24	0.145	64.60	34.40	14.20	6.96	155.90
12	Dizene	nonblood	3	59	2.61	0.172	66.00	34.20	16.10	22.14	577.85
12	Dizene	nonblood	6	88	3.64	0.265	72.80	33.20	27.30	11.31	411.68
14	Fluids	nonblood	0								
14	Fluids	nonblood	1	70	3.08	0.209	68.00	33.50	15.00	10.97	337.88
14	Fluids	nonblood	3	109	4.66	0.340	73.10	32.10	27.90	29.57	1377.96
14	Fluids	nonblood	6	128	5.71	0.401	70.30	31.80	24.00	1.37	78.23
16	Dizene	nonblood	0	74	3.39	0.215	63.40	34.40	16.30	1.38	46.78
16	Dizene	nonblood	1	69	3.19	0.210	65.70	33.00	15.70	2.83	90.28
16	Dizene	nonblood	3	97	4.34	0.287	66.20	33.80	17.60	8.01	347.63
16	Dizene	nonblood	6	119	5.42	0.356	65.60	33.40	19.00	1.22	66.12
17	Dizene	nonblood	0	89	4.08	0.264	64.60	33.90	15.50	0.72	29.38
20	Fluids	nonblood	0	45	1.82	0.140	76.80	32.30	23.30	8.71	158.52
20	Fluids	nonblood	1	61	2.46	0.185	75.30	33.20	18.90	8.43	207.38
20	Fluids	nonblood	3	96	3.74	0.291	77.80	33.10	23.60	8.71	325.75
20	Fluids	nonblood	7	104	4.15	0.318	76.60	32.60	16.50	1.40	58.10
22	Fluids	nonblood	0	108	4.67	0.315	67.40	34.30	15.30	0.35	16.35
22	Fluids	nonblood	1	85	36.40	0.251	68.80	34.00	15.80	0.45	163.80
22	Fluids	nonblood	3	144	6.19	0.419	67.70	34.40	16.00	3.14	194.37
22	Fluids	nonblood	6	150	6.46	0.451	69.90	33.30	18.90	1.74	112.40
23	Fluids	nonblood	0	163	7.39	0.474	64.00	34.30	15.30	0.16	11.82
23	Fluids	nonblood	1	127	5.73	0.369	64.40	34.50	16.10	0.19	10.89
23	Fluids	nonblood	4	174	7.89	0.510	64.70	34.10	16.60	1.73	136.50
23	Fluids	nonblood	6	163	7.44	0.483	64.90	33.80	17.70	4.57	340.01
24	Dizene	nonblood	0	80	3.38	0.229	67.80	34.80	14.70	0.51	17.24
24	Dizene	nonblood	1	83	3.49	0.238	68.10	34.70	15.40	0.86	30.01
24	Dizene	nonblood	3	140	5.89	0.399	67.80	35.10	17.30	5.40	318.06
24	Dizene	nonblood	8	138	5.79	0.398	68.70	34.70	16.60	1.85	107.12
26	Dizene	nonblood	0	165	7.09	0.477	67.30	34.50	15.40	0.31	21.98
26	Dizene	nonblood	1	149	6.32	0.428	67.70	34.80	14.80	0.36	22.75
26	Dizene	nonblood	3	145	6.17	0.422	68.40	34.30	15.00	0.99	61.08
26	Dizene	nonblood	6	150	6.23	0.438	70.30	34.30	16.90	2.87	178.80
27	Dizene	nonblood	0	90	4.07	0.265	65.20	33.80	13.80	2.52	102.56
27	Dizene	nonblood	1	69	3.09	0.202	65.40	34.30	12.90	0.59	18.23
28	Dizene	nonblood	0	134	5.51	0.385	69.90	34.80	14.40	0.34	18.73
28	Dizene	nonblood	1	122	5.18	0.360	69.50	34.00	15.30	0.75	38.85
28	Dizene	nonblood	3	146	5.96	0.418	70.10	35.00	14.70	2.33	138.87
28	Dizene	nonblood	7	152	6.09	0.432	71.10	35.10	16.70	4.54	276.49
29	Dizene	nonblood	0	66	2.87	0.194	67.40	34.30	15.50	1.20	34.44
29	Dizene	nonblood	1	77	3.31	0.222	67.10	34.60	15.70	3.00	99.30
29	Dizene	nonblood	3	101	4.16	0.293	70.30	34.50	15.50	12.41	516.26
29	Dizene	nonblood	6	106	4.51	0.322	71.40	32.90	20.40	5.53	249.40
30	Fluids	nonblood	0	44	1.74	0.128	73.60	34.30	17.40	23.12	402.29
30	Fluids	nonblood	1	39	1.54	0.117	76.10	33.50	28.00	17.61	271.19
30	Fluids	nonblood	3	68	2.55	0.204	80.20	33.40	40.20	16.72	426.36
30	Fluids	nonblood	6	85	3.61	0.266	73.80	32.10	29.00	3.76	135.74

Red blood cell variables

Dog ID	Treatment	Group	Day	Hb g/l	RCC x10^12/l	Ht l/l	MCV fl	MCHC g/dl cells	RDW %	Retics %	Abs ret x10^9/l
31	Dizene	nonblood	0	116	5.32	0.357	67.20	32.60	14.70	0.78	41.50
31	Dizene	nonblood	1	108	4.82	0.307	63.70	35.00	14.20	0.71	34.22
31	Dizene	nonblood	3	118	5.18	0.343	66.30	34.50	15.30	5.30	274.54
31	Dizene	nonblood	6	156	6.80	0.452	66.40	34.50	15.60	2.80	190.40
33	Dizene	nonblood	0	102	4.34	0.300	69.30	33.80	15.10	0.73	31.68
33	Dizene	nonblood	1	63	2.71	0.190	70.30	33.10	15.70	0.39	10.57
33	Dizene	nonblood	3	72	3.10	0.199	64.30	36.00	13.50	0.34	10.54
33	Dizene	nonblood	6	85	3.52	0.261	74.10	32.70	16.80	17.39	612.13
34	Dizene	nonblood	0	121	5.22	0.349	66.80	34.60	15.70	0.41	21.40
34	Dizene	nonblood	1	106	4.48	0.308	68.60	34.40	17.50	0.30	13.44
34	Dizene	nonblood	4	119	5.02	0.353	70.30	33.70	16.60	3.03	152.11
34	Dizene	nonblood	7	123	5.20	0.365	70.20	33.60	17.00	3.32	172.64
35	Dizene	nonblood	0	96	4.20	0.281	66.80	34.30	14.20	0.30	12.60
35	Dizene	nonblood	1	84	3.60	0.247	68.60	33.90	15.70	0.47	16.92
35	Dizene	nonblood	4	119	5.11	0.353	69.10	33.70	16.40	2.47	126.22
35	Dizene	nonblood	7	122	5.21	0.362	69.40	33.80	15.30	1.59	82.84
36	Dizene	nonblood	0	79	3.48	0.226	64.90	34.80	14.70	1.56	54.29
36	Dizene	nonblood	1	58	2.59	0.171	66.10	34.10	14.80	2.69	69.67
36	Dizene	nonblood	3	62	2.73	0.180	66.00	34.10	15.00	4.52	123.40
36	Dizene	nonblood	6	90	3.79	0.275	72.40	32.80	19.90	20.22	766.34
37	Dizene	nonblood	0	92	3.95	0.276	69.80	33.30	13.60	1.88	74.26
37	Dizene	nonblood	1	63	2.75	0.190	69.00	33.30	14.00	0.72	19.80
37	Dizene	nonblood	3	87	3.69	0.261	70.80	33.30	14.00	5.42	200.00
37	Dizene	nonblood	6	108	4.43	0.325	73.30	33.10	19.80	2.12	93.92
39	Fluids	nonblood	0	51	2.33	0.159	68.20	31.90	17.40	15.50	361.15
39	Fluids	nonblood	1	59	2.60	0.183	70.40	32.40	16.30	27.26	708.76
39	Fluids	nonblood	3	83	3.63	0.265	73.20	31.10	30.60	19.40	704.22
40	Dizene	nonblood	0	67	3.07	0.196	63.70	34.00	16.60	5.99	183.89
40	Dizene	nonblood	1	66	3.01	0.192	64.00	34.40	15.70	3.21	96.62
40	Dizene	nonblood	3	89	3.85	0.259	76.10	34.50	17.00	18.49	711.87
40	Dizene	nonblood	6	101	4.54	0.307	67.60	33.00	19.60	3.87	175.70
42	Dizene	nonblood	0	129	5.88	0.377	64.10	34.10	15.00	0.29	17.05
42	Dizene	nonblood	1	101	4.16	0.277	66.60	36.50	14.10	0.55	22.88
42	Dizene	nonblood	3	121	5.23	0.345	66.00	34.90	13.90	1.94	101.46
42	Dizene	nonblood	6	119	5.27	0.356	67.70	33.40	18.00	3.46	182.34
43	Fluids	nonblood	0	164	7.00	0.468	66.90	34.90	15.10	0.45	31.50
43	Fluids	nonblood	1	154	6.61	0.448	67.70	34.40	14.50	0.29	19.17
47	Dizene	nonblood	0	141	6.11	0.403	65.90	35.10	14.80	0.41	25.05
47	Dizene	nonblood	1	119	5.13	0.343	66.90	34.70	15.10	0.52	26.68
48	Fluids	nonblood	0	127	5.52	0.365	66.10	34.70	15.10	0.41	22.63
48	Fluids	nonblood	1	112	4.86	0.319	65.60	35.00	14.70	0.91	44.23
48	Fluids	nonblood	3	128	5.62	0.374	66.50	34.30	14.90	2.33	130.95
48	Fluids	nonblood	7	140	6.10	0.410	67.20	34.30	18.00	2.10	128.10
49	Fluids	nonblood	0	73	3.06	0.217	71.00	33.50	15.00	1.12	34.27
49	Fluids	nonblood	1	71	2.93	0.210	71.50	33.80	15.10	1.07	31.35
49	Fluids	nonblood	3	82	3.60	0.254	70.50	32.20	15.80	9.50	342.00

Red blood cell variables

Dog ID	Treatment	Group	Day	Hb g/l	RCC x10^12/l	Ht l/l	MCV fl	MCHC g/dl cells	RDW %	Retics %	Abs ret x10^9/l
49	Fluids	nonblood	6		4.56				24.80	11.60	528.96
50	Dizene	nonblood	0	105	4.60	0.304	66.10	34.60	13.60	0.61	28.06
50	Dizene	nonblood	1	79	3.40	0.231	67.90	34.00	13.80	0.66	22.44
50	Dizene	nonblood	3	94	4.08	0.279	68.50	33.70	13.20	5.35	218.28
50	Dizene	nonblood	7	99	4.09	0.316	77.30	31.20	18.60	13.93	569.74
51	Dizene	nonblood	0	51	2.69	0.158	58.80	32.10	16.80	2.22	59.72
51	Dizene	nonblood	1	58	2.97	0.184	61.90	31.70	17.70	4.56	135.43
51	Dizene	nonblood	3	86	4.31	0.267	61.90	32.40	17.70	22.31	961.56
52	Fluids	nonblood	0	149	6.50	0.442	68.00	33.70	16.80	0.47	30.55
52	Fluids	nonblood	1	92	4.00	0.275	68.70	33.50	16.20	0.26	10.40
52	Fluids	nonblood	3	106	4.79	0.314	65.50	33.70	15.30	0.53	25.39
52	Fluids	nonblood	7	107	4.83	0.321	66.40	33.40	16.30	4.86	234.74
54	Dizene	nonblood	0	38	1.79	0.119	66.20	31.60	19.70	6.70	119.93
54	Dizene	nonblood	1	40	1.82	0.119	65.20	33.40	18.20	10.24	186.37
54	Dizene	nonblood	3	70	3.03	0.208	68.70	33.60	21.70	7.80	236.34
54	Dizene	nonblood	6	70	3.31	0.218	65.80	32.10	27.50	1.00	33.10
56	Fluids	nonblood	0	128	6.01	0.374	62.20	34.30	15.50	0.25	15.03
56	Fluids	nonblood	1	111	5.14	0.319	62.10	34.70	15.90	0.22	11.31
56	Fluids	nonblood	3	79	3.69	0.230	62.30	34.50	16.30	0.18	6.64
56	Fluids	nonblood	6	90	4.24	0.262	61.80	34.30	16.30	0.76	32.22
58	Dizene	nonblood	0	46	1.92	0.135	70.40	34.00	15.90	26.06	500.35
58	Dizene	nonblood	1	60	2.43	0.184	75.70	32.70	29.40	26.25	637.88
58	Dizene	nonblood	3	74	2.97	0.228	76.90	32.40	35.90	16.46	488.86
58	Dizene	nonblood	6	89	3.64	0.267	73.50	33.30	23.00	0.91	33.12
59	Dizene	nonblood	0	78	3.37	0.221	65.40	35.50	16.70	3.08	103.80
59	Dizene	nonblood	1	83	3.99	0.261	65.50	31.60	18.30	6.06	241.79
59	Dizene	nonblood	3	104	4.88	0.313	64.10	33.20	20.50	10.32	503.62
59	Dizene	nonblood	6	116	5.49	0.353	64.20	33.00	19.90	1.79	98.27
63	Fluids	nonblood	0	205	8.97	0.571	63.70	35.80	17.10	0.22	19.73
64	Dizene	nonblood	0	145	6.15	0.417	67.80	34.80	14.10	0.72	44.28
64	Dizene	nonblood	1	145	6.08	0.415	68.20	34.90	14.80	1.41	85.73
64	Dizene	nonblood	3		6.62				14.50	6.34	419.71
64	Dizene	nonblood	6	154	6.42	0.447	69.70	34.30	17.50	4.05	260.01
65	Dizene	nonblood	0	56	2.28	0.160	70.40	35.10	14.90	4.56	103.97
65	Dizene	nonblood	1	63	2.57	0.182	70.80	34.90	16.10	7.67	197.12
67	Dizene	nonblood	0	84	3.42	0.237	69.10	35.70	13.80	0.54	18.47
67	Dizene	nonblood	1	71	2.94	0.205	69.50	34.70	15.50	0.33	9.70
67	Dizene	nonblood	3	72	3.05	0.214	70.30	33.40	14.50	1.86	56.73
67	Dizene	nonblood	6	95	3.95	0.293	74.10	32.60	16.60	15.41	608.70
68	Dizene	nonblood	0	117	5.38	0.328	60.90	35.80	18.00	1.22	65.64
70	Dizene	nonblood	0	62	2.76	0.180	65.20	34.60	16.60	0.93	25.67
70	Dizene	nonblood	1	64	2.80	0.184	65.50	34.60	14.70	1.76	49.28
70	Dizene	nonblood	3	93	4.01	0.268	66.80	34.70	16.60	9.65	386.97
70	Dizene	nonblood	7	112	4.94	0.340	68.90	33.00	19.50	2.93	144.74
72	Dizene	nonblood	0	110	4.75	0.341	71.80	32.20	17.10	0.35	16.63
72	Dizene	nonblood	1	77	3.23	0.213	66.10	35.90	13.70	0.42	13.57

Red blood cell variables

Dog ID	Treatment	Group	Day	Hb g/l	RCC x10^12/l	Ht l/l	MCV fl	MCHC g/dl cells	RDW %	Retics %	Abs ret x10^9/l
72	Dizene	nonblood	3	73	3.11	0.224	72.30	32.40	14.90	1.75	54.43
73	Dizene	nonblood	0	49	2.06	0.156	75.50	31.60	26.30	6.20	127.72
73	Dizene	nonblood	1	81	3.28	0.252	76.70	32.10	25.40	7.90	259.12
73	Dizene	nonblood	3	115	4.65	0.353	75.90	32.60	21.80	2.45	113.93
76	Fluids	nonblood	0	30	1.37	0.084	61.30	35.90	18.40	2.9	39.73
78	Fluids	nonblood	0	78	3.30	0.216	65.60	35.90	15.90	0.74	24.42
78	Fluids	nonblood	1	44	1.88	0.125	66.60	35.20	14.90	1	18.80
81	Fluids	nonblood	0	47	2.01	0.140	69.60	33.60	17.10	11.26	226.33
81	Fluids	nonblood	1	49	2.06	0.147	71.30	33.40	23.90	13.72	282.63
81	Fluids	nonblood	3	70	2.89	0.216	74.70	32.30	30.90	8.34	241.03
82	Fluids	nonblood	0	54	2.38	0.158	66.60	33.90	14.60	1.83	43.55
82	Fluids	nonblood	1	51	2.22	0.149	67.20	33.80	15.00	4.78	106.12
82	Fluids	nonblood	3	69	2.95	0.205	69.50	33.70	16.00	14.43	425.69
82	Fluids	nonblood	7	102	4.35	0.307	70.70	33.30	19.10	5.72	248.82
83	Dizene	nonblood	0	40	1.78	0.123	69.20	32.70	19.70	13.39	238.34
83	Dizene	nonblood	1	55	2.33	0.171	73.50	32.00	26.50	18.94	441.30
83	Dizene	nonblood	3	70	2.99	0.218	72.90	32.10	31.90	6.63	198.24
83	Dizene	nonblood	7	94	4.20	0.284	67.40	33.00	27.90	1.86	78.12
87	Dizene	nonblood	0	53	2.27	0.160	70.40	33.20	15.30	1.85	42.00
87	Dizene	nonblood	1	62	2.60	0.181	69.70	34.00	14.90	5.93	154.18
87	Dizene	nonblood	3	94	3.90	0.294	76.40	32.10	19.30	17.81	694.59
87	Dizene	nonblood	6	112	4.78	0.338	70.70	33.00	17.20	2.61	124.76
90	Fluids	nonblood	0	143	6.50	0.401	61.70	35.80	15.70	0.33	21.45
90	Fluids	nonblood	1	117	5.42	0.341	62.90	34.20	14.90	0.34	18.43
90	Fluids	nonblood	3	152	6.95	0.433	62.30	35.00	15.20	0.58	40.31
90	Fluids	nonblood	6	155	7.12	0.461	64.80	33.50	16.10	1.16	82.59
93	Dizene	nonblood	0	43	1.92	0.127	66.00	34.10	24.50	5.35	102.72
93	Dizene	nonblood	1	47	2.12	0.142	66.80	33.20	27.60	4.71	99.85
93	Dizene	nonblood	3	69	3.01	0.280	68.90	33.10	35.70	10.87	327.19
94	Dizene	nonblood	0	106	4.81	0.302	62.80	35.20	15.50	1.29	62.05

White blood cell and platelet variables

Dog ID	Treatment	Group	Day	WCC x10^9/l	AbNmat x10^9/l	AbNimm x10^9/l	AbLymph x10^9/l	AbMono x10^9/l	AbEos x10^9/l	AbBaso x10^9/l	Thr C x10^9/l
3	Blood	blood	0	8.00	4.40	1.20	1.84	0.56	0.00	0.00	3.70
3	Blood	blood	1	8.10	3.52	1.22	2.40	0.96	0.00	0.00	38.90
3	Blood	blood	6	8.20	3.44	0.33	3.36	0.82	0.25	0.00	443.00
5	Blood	blood	0	7.50	5.33	0.30	1.80	0.08	0.00	0.00	0.20
5	Blood	blood	1	11.10	6.11	0.89	3.22	0.89	0.00	0.00	7.80
5	Blood	blood	3	13.60	4.22	0.27	8.02	0.82	0.27	0.00	146.00
5	Blood	blood	6	13.80	5.66	0.28	5.93	1.10	0.83	0.00	327.00
13	Blood	blood	0	2.20	0.68	0.68	0.62	0.22	0.00	0.00	0.00
13	Blood	blood	1	3.90	1.68	0.70	1.09	0.43	0.00	0.00	2.22
13	Blood	blood	3	9.00	2.97	1.89	2.97	0.81	0.36	0.00	3.63
13	Blood	blood	6	8.70	4.35	0.70	3.05	0.44	0.17	0.00	
15	Blood	blood	0								
15	Blood	blood	1	12.80	8.45	0.26	2.18	1.15	0.77	0.00	131.00
15	Blood	blood	3	14.60	10.07	1.02	2.34	1.02	0.15	0.00	296.00
15	Blood	blood	6	9.80	7.25	0.00	1.27	0.59	0.69	0.00	347.00
18	Blood	blood	0	10.20	7.57	0.82	0.65	0.95	0.21	0.00	38.90
18	Blood	blood	1	22.40	16.58	3.36	1.12	1.34	0.00	0.00	44.80
18	Blood	blood	3	28.60	19.33	3.15	3.69	1.97	0.49	0.00	86.40
18	Blood	blood	6	13.10	7.07	2.75	1.44	1.05	0.79	0.00	247.00
25	Blood	blood	0	3.70	2.66	0.44	0.37	0.22	0.00	0.00	67.30
25	Blood	blood	1	8.20	5.25	0.74	0.33	1.80	0.08	0.00	75.90
25	Blood	blood	3	11.60	7.05	0.23	1.57	1.58	1.16	0.00	297.00
25	Blood	blood	6	7.40	4.29	0.22	0.96	1.11	0.81	0.00	485.00
32	Blood	blood	0	4.10	2.83	0.37	0.41	0.49	0.00	0.00	1.41
32	Blood	blood	1	9.10	4.55	0.55	2.46	1.46	0.09	0.00	4.60
32	Blood	blood	3	15.50	9.30	1.71	2.33	1.71	0.47	0.00	136.00
32	Blood	blood	7	15.00	10.35	0.15	2.85	1.20	0.45	0.00	159.00
41	Blood	blood	0	9.30	6.52	1.21	1.05	0.50	0.02	0.00	0.80
41	Blood	blood	1	9.60	6.82	1.06	1.15	0.48	0.10	0.00	1.10
41	Blood	blood	3	14.90	8.00	1.94	3.32	1.43	0.21	0.00	4.80
44	Blood	blood	0	14.20	8.38	1.70	2.56	1.56	0.00	0.00	14.60
44	Blood	blood	1	12.20	7.32	2.44	2.20	0.12	0.12	0.00	69.10
44	Blood	blood	3	11.70	5.37	0.70	3.59	1.71	0.33	0.00	355.00
44	Blood	blood	6	12.70	7.11	0.76	3.05	1.14	0.64	0.00	
45	Blood	blood	0	13.40	3.62	6.03	2.68	0.80	0.27	0.00	3.50
45	Blood	blood	1	31.00	18.29	6.82	2.17	3.10	0.62	0.00	
45	Blood	blood	3	49.20	26.08	15.25	4.92	1.97	0.98	0.00	10.60
45	Blood	blood	6	13.30	10.41	0.13	2.18	0.55	0.03	0.00	138.00
46	Blood	blood	0	3.80	1.29	1.06	1.37	0.08	0.00	0.00	7.30
46	Blood	blood	1	15.40	5.85	3.39	3.39	2.62	0.15	0.00	3.10
46	Blood	blood	3	45.30	22.65	9.51	5.89	4.98	2.27	0.00	71.40
46	Blood	blood	6	16.70	9.02	0.84	4.84	0.84	1.17	0.00	258.00
53	Blood	blood	0	2.60	0.62	0.36	1.56	0.05	0.00	0.00	6.40
55	Blood	blood	0	9.60	6.14	0.67	2.11	0.67	0.00	0.00	5.80
57	Blood	blood	0	8.70	4.52	0.87	3.05	0.26	0.00	0.00	5.40

White blood cell and platelet variables

Dog ID	Treatment	Group	Day	WCC x10^9/l	AbNmat x10^9/l	AbNimm x10^9/l	AbLymph x10^9/l	AbMono x10^9/l	AbEos x10^9/l	AbBaso x10^9/l	Thr C x10^9/l
57	Blood	blood	1	15.20	7.60	2.74	3.95	0.91	0.00	0.00	34.50
57	Blood	blood	3	22.50	10.82	3.15	6.48	1.94	0.11	0.00	162.00
57	Blood	blood	6	10.00	6.00	0.70	2.50	0.40	0.40	0.00	405.00
61	Blood	blood	0	9.70	4.46	2.43	1.84	0.97	0.00	0.00	92.80
61	Blood	blood	1	8.20	3.29	1.07	2.42	1.38	0.04	0.00	110.00
61	Blood	blood	3	8.10	3.81	0.16	2.92	1.22	0.00	0.00	241.00
61	Blood	blood	6	13.50	8.64	0.81	2.30	1.76	0.00	0.00	255.00
62	Blood	blood	0	13.50	7.97	1.49	2.97	0.95	0.14	0.00	4.60
62	Blood	blood	1	33.30	14.99	0.67	14.32	2.66	0.67	0.00	54.20
62	Blood	blood	3	20.90	14.94	0.00	4.64	0.38	0.94	0.00	204.00
62	Blood	blood	6	16.10	10.19	0.00	4.43	0.19	1.30	0.00	491.00
66	Blood	blood	0	4.70	2.99	0.61	1.03	0.06	0.01	0.00	0.00
66	Blood	blood	1	12.90	6.32	0.26	5.03	1.17	0.13	0.00	3.80
66	Blood	blood	3	21.20	6.95	0.21	11.00	2.67	0.36	0.00	82.20
66	Blood	blood	6	11.60	5.10	0.00	4.41	0.81	1.16	0.12	192.00
69	Blood	blood	0	6.30	1.01	2.39	2.08	0.76	0.06	0.00	9.40
69	Blood	blood	1	21.40	12.01	4.92	2.16	1.80	0.51	0.00	4.90
69	Blood	blood	3	33.20	21.12	7.30	3.69	0.43	0.70	0.00	6.10
69	Blood	blood	6	44.60	35.23	4.01	3.12	1.78	0.45	0.00	
71	Blood	blood	0	6.40	3.01	2.11	0.77	0.50	0.01	0.00	4.40
74	Blood	blood	0	7.30	3.65	1.46	0.73	1.46	0.00	0.00	0.00
74	Blood	blood	1	11.60	5.49	0.70	3.48	1.65	0.28	0.00	1.60
74	Blood	blood	3	22.50	7.20	1.80	11.25	2.03	0.23	0.00	164.00
75	Blood	blood	0	8.60	6.36	0.95	1.20	0.52	0.00	0.00	59.70
75	Blood	blood	1	8.90	5.96	0.80	0.53	1.51	0.09	0.00	97.70
75	Blood	blood	4	9.50	7.13	0.57	0.67	1.14	0.00	0.00	308.00
77	Blood	blood	0	12.90	9.03	1.81	0.52	1.55	0.00	0.00	37.80
77	Blood	blood	1	20.50	15.79	2.05	1.03	1.64	0.00	0.00	113.00
77	Blood	blood	3	12.80	7.81	1.41	1.92	1.02	0.64	0.00	745.00
79	Blood	blood	0	16.90	12.13	1.18	2.13	1.35	0.10	0.00	2.60
79	Blood	blood	1	12.40	7.58	0.62	1.96	1.62	0.62	0.00	5.00
80	Blood	blood	0	11.30	8.81	1.24	0.11	1.13	0.00	0.00	69.90
80	Blood	blood	1	7.50	4.43	1.50	0.30	1.13	0.15	0.00	45.00
80	Blood	blood	3	11.50	7.71	0.00	1.27	2.07	0.46	0.00	105.00
80	Blood	blood	6	27.20	22.58	1.09	0.27	2.72	0.54	0.00	368.00
85	Blood	blood	0	17.50	11.69	2.45	2.47	0.82	0.07	0.00	5.10
85	Blood	blood	1	22.20	14.74	4.00	1.64	1.62	0.20	0.01	42.30
85	Blood	blood	3	28.20	11.84	8.46	4.79	2.54	0.56	0.00	122.00
86	Blood	blood	0	4.30	2.53	0.22	1.21	0.31	0.02	0.00	1.20
86	Blood	blood	1	6.60	3.04	0.79	2.38	0.26	0.13	0.00	2.50
86	Blood	blood	3	11.60	7.19	0.81	1.86	1.74	0.00	0.00	263.00
86	Blood	blood	6	10.20	6.22	0.00	2.55	1.22	0.20	0.00	398.00
88	Blood	blood	0	2.90	1.04	1.10	0.41	0.35	0.00	0.00	4.10
88	Blood	blood	1	18.50	11.29	2.96	1.67	2.59	0.00	0.00	18.20
88	Blood	blood	3	26.10	16.97	2.09	3.13	3.92	0.00	0.00	1.50
88	Blood	blood	6	23.70	10.90	0.71	6.40	5.69	0.00	0.00	

White blood cell and platelet variables

Dog ID	Treatment	Group	Day	WCC x10^9/l	AbNmat x10^9/l	AbNimm x10^9/l	AbLymph x10^9/l	AbMono x10^9/l	AbEos x10^9/l	AbBaso x10^9/l	Thr C x10^9/l
89	Blood	blood	0	11.60	6.15	2.20	2.67	0.58	0.00	0.00	2.10
89	Blood	blood	1	21.20	12.72	1.06	5.51	1.70	0.21	0.00	1.20
89	Blood	blood	3	12.60	5.19	0.00	5.83	1.44	0.13	0.00	62.90
91	Blood	blood	0	5.50	3.58	0.33	0.99	0.61	0.00	0.00	0.30
91	Blood	blood	1	8.40	4.45	0.17	2.94	0.59	0.08	0.00	4.40
91	Blood	blood	3	9.00	2.97	0.45	4.68	0.63	0.27	0.00	1.80
92	Blood	blood	0	3.40	1.12	0.34	1.56	0.34	0.03	0.00	2.50
92	Blood	blood	1	9.10	2.00	1.27	4.82	0.73	0.27	0.00	7.50
92	Blood	blood	3	17.70	4.78	2.48	7.08	3.01	0.35	0.00	209.00
92	Blood	blood	6	9.00	5.04	0.00	2.52	0.36	1.08	0.00	583.00
95	Blood	blood	0	13.70	8.10	2.06	2.07	1.45	0.03	0.00	2.70
95	Blood	blood	1	19.20	13.06	1.92	1.73	2.30	0.19	0.00	1.50
95	Blood	blood	3	18.30	7.87	4.03	4.76	1.28	0.37	0.00	41.80
96	Blood	blood	0	10.90	8.24	0.00	0.69	1.75	0.02	0.20	2.30
96	Blood	blood	1	21.80	13.52	1.74	3.27	3.27	0.00	0.00	0.00
96	Blood	blood	3	56.10	39.83	6.17	7.85	2.24	0.00	0.00	
1	Fluids	nonblood	0	3.10	1.09	0.47	1.05	0.50	0.04	0.00	1.20
1	Fluids	nonblood	1	6.10	2.93	0.12	1.34	0.85	0.85	0.00	1.40
1	Fluids	nonblood	3	20.20	9.90	1.01	5.66	2.83	0.81	0.00	142.00
2	Dizene	nonblood	0	9.10	4.91	1.27	2.18	0.55	0.18	0.00	1.20
2	Dizene	nonblood	1	21.50	10.97	4.95	4.30	1.08	0.22	0.00	10.60
2	Dizene	nonblood	3	34.50	17.42	12.77	3.86	0.41	0.03	0.00	60.80
2	Dizene	nonblood	7	9.80	5.00	0.20	3.63	0.59	0.39	0.00	283.00
4	Fluids	nonblood	0	2.80	2.41	0.17	0.14	0.08	0.00	0.00	3.60
4	Fluids	nonblood	1	2.80	1.51	0.03	0.78	0.34	0.14	0.00	8.90
4	Fluids	nonblood	3	6.70	2.68	0.40	1.94	1.07	0.60	0.00	95.90
4	Fluids	nonblood	6	8.10	4.86	0.65	1.38	0.15	1.07	0.00	257.00
6	Fluids	nonblood	0	5.40	3.34	0.32	0.98	0.73	0.02	0.00	3.80
6	Fluids	nonblood	1	7.00	3.01	0.14	2.94	0.63	0.28	0.00	8.40
6	Fluids	nonblood	3	13.30	9.98	0.53	2.53	0.13	0.13	0.00	113.00
6	Fluids	nonblood	6	8.60	5.76	0.43	1.38	0.60	0.43	0.00	226.00
7	Dizene	nonblood	0	3.80	2.47	0.46	0.61	0.23	0.04	0.00	0.20
7	Dizene	nonblood	1	8.30	4.97	0.33	1.60	0.87	0.51	0.00	5.90
7	Dizene	nonblood	3	17.50	7.70	3.15	4.55	0.88	1.23	0.00	84.50
7	Dizene	nonblood	6	18.10	12.67	1.81	2.17	0.72	0.72	0.00	257.00
9	Fluids	nonblood	0	8.00	5.28	0.16	1.28	0.96	0.24	0.08	157.00
9	Fluids	nonblood	1	10.70	8.56	0.64	1.07	0.32	0.11	0.00	78.10
9	Fluids	nonblood	3	17.50	9.28	0.18	4.38	2.98	0.70	0.00	
9	Fluids	nonblood	6	14.80	8.44	0.30	4.44	0.89	0.74	0.00	332.00
10	Fluids	nonblood	0	8.70	7.05	0.35	0.70	0.61	0.00	0.00	
11	Dizene	nonblood	0	3.70	2.04	0.04	1.55	0.04	0.04	0.00	0.60
11	Dizene	nonblood	1	5.80	2.15	0.00	2.96	0.41	0.29	0.00	1.20
11	Dizene	nonblood	3	7.31	2.56	0.00	3.58	0.51	0.66	0.00	89.00
11	Dizene	nonblood	6	6.60	2.57	0.13	3.10	0.46	0.33	0.00	224.00
12	Dizene	nonblood	0	3.38	1.79	0.34	0.91	0.34	0.00	0.00	4.81

White blood cell and platelet variables

Dog ID	Treatment	Group	Day	WCC x10^9/l	AbNmat x10^9/l	AbNimm x10^9/l	AbLymph x10^9/l	AbMono x10^9/l	AbEos x10^9/l	AbBaso x10^9/l	Thr C x10^9/l
12	Dizene	nonblood	1	13.60	10.20	0.95	1.63	0.82	0.00	0.00	2.38
12	Dizene	nonblood	3	56.90	27.31	18.21	5.12	5.12	1.14	0.00	
12	Dizene	nonblood	6	22.30	16.50	2.01	2.45	0.89	0.45	0.00	163.00
14	Fluids	nonblood	0								
14	Fluids	nonblood	1	12.90	6.71	3.23	1.55	1.03	0.39	0.00	135.00
14	Fluids	nonblood	3	13.00	7.31	1.04	2.33	1.86	0.48	0.00	227.00
14	Fluids	nonblood	6	5.80	3.21	0.00	1.65	0.73	0.21	0.00	461.00
16	Dizene	nonblood	0	3.90	1.56	0.94	0.98	0.43	0.00	0.00	2.22
16	Dizene	nonblood	1	10.70	5.65	1.07	3.00	0.86	0.11	0.00	4.00
16	Dizene	nonblood	3	20.30	9.54	1.83	7.51	1.22	0.20	0.00	133.00
16	Dizene	nonblood	6	9.80	2.55	0.59	6.08	0.29	0.29	0.00	380.00
17	Dizene	nonblood	0	10.80	6.48	1.84	1.08	1.08	0.32	0.00	0.30
20	Fluids	nonblood	0	18.10	13.58	1.27	1.09	1.99	0.18	0.00	45.50
20	Fluids	nonblood	1	18.30	11.53	2.01	1.28	3.29	0.18	0.00	87.10
20	Fluids	nonblood	3	14.00	8.40	1.96	2.24	1.26	0.14	0.00	288.00
20	Fluids	nonblood	7	6.90	4.28	0.35	1.17	0.62	0.41	0.07	710.00
22	Fluids	nonblood	0	7.40	2.52	0.89	3.85	0.15	0.00	0.00	8.92
22	Fluids	nonblood	1	13.70	7.40	1.51	3.84	0.69	0.27	0.00	7.76
22	Fluids	nonblood	3	17.10	9.23	1.54	4.96	0.68	0.68	0.00	
22	Fluids	nonblood	6	10.50	6.65	0.11	2.61	0.50	0.63	0.00	307.00
23	Fluids	nonblood	0	3.90	3.35	0.08	0.39	0.08	0.00	0.00	3.36
23	Fluids	nonblood	1	4.30	3.10	0.09	0.86	0.26	0.00	0.00	6.40
23	Fluids	nonblood	4	6.80	2.92	0.00	3.67	0.14	0.07	0.00	59.90
23	Fluids	nonblood	6	8.00	4.59	0.16	2.47	0.54	0.24	0.00	242.00
24	Dizene	nonblood	0	2.50	1.00	0.00	1.15	0.35	0.00	0.00	5.60
24	Dizene	nonblood	1	6.00	2.52	0.30	2.28	0.84	0.06	0.00	4.51
24	Dizene	nonblood	3	8.50	4.00	0.17	3.74	0.26	0.34	0.00	
24	Dizene	nonblood	8	4.60	2.62	0.00	1.70	0.09	0.18	0.00	344.00
26	Dizene	nonblood	0	4.90	3.43	0.15	1.03	0.15	0.15	0.00	3.49
26	Dizene	nonblood	1	7.70	4.62	0.00	2.77	0.08	0.23	0.00	3.00
26	Dizene	nonblood	3	10.40	5.93	0.10	2.39	1.03	0.95	0.00	106.00
26	Dizene	nonblood	6	15.90	9.06	0.00	5.72	0.32	0.80	0.00	405.00
27	Dizene	nonblood	0	6.80	4.01	0.27	1.16	0.82	0.54	0.00	5.70
27	Dizene	nonblood	1	7.90	4.42	0.16	2.05	0.79	0.47	0.00	55.30
28	Dizene	nonblood	0	3.40	2.00	0.10	0.54	0.66	0.10	0.00	3.10
28	Dizene	nonblood	1	11.40	5.81	0.11	1.89	2.99	0.59	0.00	4.00
28	Dizene	nonblood	3	15.60	6.71	0.47	5.62	1.25	1.56	0.00	84.30
28	Dizene	nonblood	7	13.30	8.65	0.53	1.20	0.53	2.13	0.27	201.00
29	Dizene	nonblood	0	6.00	2.40	0.12	3.00	0.24	0.24	0.00	0.60
29	Dizene	nonblood	1	9.40	4.14	0.09	4.14	0.85	0.19	0.00	1.60
29	Dizene	nonblood	3	14.10	6.06	0.42	5.64	1.83	0.14	0.00	69.40
29	Dizene	nonblood	6	10.60	4.98	0.00	4.35	0.74	0.53	0.00	187.00
30	Fluids	nonblood	0	4.50	2.61	0.54	1.17	0.09	0.09	0.00	18.60
30	Fluids	nonblood	1	13.40	7.77	1.21	2.14	1.61	0.67	0.00	61.70
30	Fluids	nonblood	3	21.50	16.56	0.43	3.44	0.43	0.65	0.00	297.00
30	Fluids	nonblood	6	13.90	10.43	0.00	3.06	0.42	0.00	0.00	626.00

White blood cell and platelet variables

Dog ID	Treatment	Group	Day	WCC x10^9/l	AbNmat x10^9/l	AbNimm x10^9/l	AbLymph x10^9/l	AbMono x10^9/l	AbEos x10^9/l	AbBaso x10^9/l	Thr C x10^9/l
31	Dizene	nonblood	0	7.20	3.02	2.45	1.30	0.43	0.00	0.00	1.50
31	Dizene	nonblood	1	16.40	7.71	0.33	6.72	1.31	0.33	0.00	37.20
31	Dizene	nonblood	3	29.10	17.46	3.20	5.82	1.75	0.87	0.00	237.00
31	Dizene	nonblood	6	11.30	8.14	0.11	2.26	0.45	0.34	0.00	
33	Dizene	nonblood	0	8.70	2.26	3.57	1.48	1.31	0.09	0.00	11.10
33	Dizene	nonblood	1	9.60	6.53	1.63	0.48	0.96	0.00	0.00	2.00
33	Dizene	nonblood	3	22.60	13.70	4.52	2.24	1.67	0.50	0.00	31.10
33	Dizene	nonblood	6	28.80	19.87	4.90	3.46	0.29	0.29	0.00	252.00
34	Dizene	nonblood	0	4.10	1.85	0.41	0.57	1.03	0.25	0.00	1.50
34	Dizene	nonblood	1	11.00	6.20	0.55	2.00	1.94	0.31	0.00	8.60
34	Dizene	nonblood	4	22.30	16.01	2.68	2.41	0.36	0.85	0.00	251.00
34	Dizene	nonblood	7	7.40	4.44	0.44	1.18	0.67	0.67	0.00	246.00
35	Dizene	nonblood	0	4.20	2.43	0.50	0.56	0.51	0.20	0.00	1.80
35	Dizene	nonblood	1	11.50	5.75	0.46	2.76	1.84	0.35	0.00	5.70
35	Dizene	nonblood	4	12.50	6.65	0.13	3.94	0.71	0.85	0.23	191.00
35	Dizene	nonblood	7	10.00	6.18	0.00	2.48	0.34	1.00	0.00	283.00
36	Dizene	nonblood	0	14.50	8.70	2.61	0.73	2.47	0.00	0.00	1.80
36	Dizene	nonblood	1	26.20	20.44	2.10	0.52	3.14	0.00	0.00	1.20
36	Dizene	nonblood	3	43.10	26.29	7.33	4.31	2.59	2.59	0.00	66.50
36	Dizene	nonblood	6	38.30	29.49	3.45	1.53	1.92	1.92	0.00	451.00
37	Dizene	nonblood	0	5.80	2.61	0.23	2.61	0.35	0.00	0.00	
37	Dizene	nonblood	1	11.50	6.85	0.23	1.81	2.33	0.26	0.00	5.50
37	Dizene	nonblood	3	27.90	16.46	4.19	3.91	3.35	0.00	0.00	138.00
37	Dizene	nonblood	6	18.80	12.60	0.00	4.32	1.69	0.19	0.00	344.00
39	Fluids	nonblood	0	39.40	28.80	5.91	3.11	1.06	0.51	0.01	41.40
39	Fluids	nonblood	1	37.60	23.31	4.51	7.52	1.88	0.38	0.00	74.50
39	Fluids	nonblood	3	15.30	9.03	0.31	4.44	0.92	0.61	0.00	213.00
40	Dizene	nonblood	0	4.20	2.18	0.46	1.30	0.04	0.21	0.00	2.50
40	Dizene	nonblood	1	9.00	3.87	0.54	3.51	0.45	0.63	0.00	1.50
40	Dizene	nonblood	3	19.30	7.72	2.12	6.37	1.16	1.93	0.00	
40	Dizene	nonblood	6	12.60	5.80	0.00	4.54	1.13	1.13	0.00	305.00
42	Dizene	nonblood	0	6.90	4.49	0.07	1.79	0.48	0.07	0.00	2.60
42	Dizene	nonblood	1	12.70	4.45	0.64	4.95	2.29	0.38	0.00	4.00
42	Dizene	nonblood	3	19.70	11.43	1.97	4.14	1.18	0.99	0.00	
42	Dizene	nonblood	6	19.70	12.81	0.00	4.93	0.99	0.99	0.00	253.00
43	Fluids	nonblood	0	8.30	7.03	0.17	0.75	0.35	0.01	0.00	42.50
43	Fluids	nonblood	1	10.10	6.77	0.30	2.22	0.81	0.00	0.00	3.60
47	Dizene	nonblood	0	7.30	3.87	2.63	0.51	0.22	0.00	0.07	6.50
47	Dizene	nonblood	1	10.40	6.03	2.70	0.52	1.14	0.00	0.00	8.80
48	Fluids	nonblood	0	3.30	1.85	0.26	1.06	0.07	0.07	0.00	3.60
48	Fluids	nonblood	1	10.10	4.24	0.20	4.04	0.61	0.81	0.20	42.00
48	Fluids	nonblood	3	12.60	5.42	0.38	4.16	0.38	1.89	0.38	304.00
48	Fluids	nonblood	7	16.20	10.43	0.00	2.85	0.76	2.03	0.11	522.00
49	Fluids	nonblood	0	12.30	7.87	1.23	1.97	1.23	0.00	0.00	6.80
49	Fluids	nonblood	1	22.80	14.36	3.65	3.19	1.60	0.00	0.00	0.00
49	Fluids	nonblood	3	68.30	37.57	23.22	6.15	1.37	0.00	0.00	3.90

White blood cell and platelet variables

Dog ID	Treatment	Group	Day	WCC x10^9/l	AbNmat x10^9/l	AbNimm x10^9/l	AbLymph x10^9/l	AbMono x10^9/l	AbEos x10^9/l	AbBaso x10^9/l	Thr C x10^9/l
49	Fluids	nonblood	6	22.70	17.03	1.14	2.50	1.36	0.68	0.00	272.00
50	Dizene	nonblood	0	2.40	1.51	0.50	0.17	0.19	0.02	0.00	3.80
50	Dizene	nonblood	1	6.30	3.53	0.25	1.39	1.13	0.00	0.00	2.30
50	Dizene	nonblood	3	15.60	7.96	3.12	1.87	2.34	0.31	0.00	68.90
50	Dizene	nonblood	7	8.60	5.77	0.52	1.63	0.50	0.18	0.00	272.00
51	Dizene	nonblood	0	8.50	5.70	0.60	1.53	0.68	0.00	0.00	9.30
51	Dizene	nonblood	1	11.70	6.44	0.35	3.39	1.40	0.12	0.00	50.30
51	Dizene	nonblood	3	12.40	7.19	0.50	3.60	0.74	0.37	0.00	253.00
52	Fluids	nonblood	0	4.90	2.11	0.78	0.83	1.18	0.00	0.00	14.40
52	Fluids	nonblood	1	12.60	6.93	0.76	2.14	2.65	0.13	0.00	19.70
52	Fluids	nonblood	3	31.00	18.91	1.55	2.79	6.20	1.55	0.00	124.00
52	Fluids	nonblood	7	30.00	23.40	2.10	3.60	0.30	0.60	0.00	335.00
54	Dizene	nonblood	0	5.00	1.45	0.65	2.05	0.75	0.10	0.00	21.80
54	Dizene	nonblood	1	9.02	3.17	0.72	3.55	1.26	0.32	0.00	9.00
54	Dizene	nonblood	3	16.50	4.95	1.65	8.42	0.83	0.66	0.00	165.00
54	Dizene	nonblood	6	10.00	4.10	0.30	3.90	0.90	0.70	0.10	327.00
56	Fluids	nonblood	0	6.10	4.47	0.44	0.58	0.48	0.15	0.00	5.80
56	Fluids	nonblood	1	9.00	6.48	0.54	0.72	1.26	0.00	0.00	3.60
56	Fluids	nonblood	3	14.40	9.50	0.58	1.01	2.45	0.72	0.14	80.00
56	Fluids	nonblood	6	24.50	14.95	2.70	3.19	2.94	0.74	0.00	152.00
58	Dizene	nonblood	0	5.30	3.23	1.22	0.58	0.27	0.00	0.00	5.30
58	Dizene	nonblood	1	14.30	9.58	1.86	1.29	1.43	0.14	0.00	
58	Dizene	nonblood	3	22.10	14.14	3.09	1.99	2.65	0.22	0.00	288.00
58	Dizene	nonblood	6	21.80	18.53	0.87	0.87	1.31	0.22	0.00	966.00
59	Dizene	nonblood	0	4.60	1.79	0.18	2.21	0.37	0.05	0.00	9.90
59	Dizene	nonblood	1	13.00	4.42	0.52	6.24	1.82	0.00	0.00	3.20
59	Dizene	nonblood	3	14.50	4.79	1.02	6.53	1.74	0.44	0.00	102.00
59	Dizene	nonblood	6	12.80	5.25	0.13	4.99	2.18	0.26	0.00	291.00
63	Fluids	nonblood	0	9.30	5.95	1.30	0.93	1.12	0.00	0.00	8.10
64	Dizene	nonblood	0	5.10	3.77	0.20	0.61	0.31	0.20	0.00	0.80
64	Dizene	nonblood	1	10.50	6.09	0.21	2.94	0.84	0.42	0.00	0.10
64	Dizene	nonblood	3	21.00	10.92	3.15	3.78	1.68	1.26	0.21	44.70
64	Dizene	nonblood	6	13.70	9.32	0.00	3.01	0.96	0.31	0.00	228.00
65	Dizene	nonblood	0	6.60	2.57	0.66	2.38	0.92	0.07	0.00	0.20
65	Dizene	nonblood	1	11.20	5.61	1.01	3.00	1.37	0.21	0.00	3.60
67	Dizene	nonblood	0	8.70	5.14	1.83	0.90	0.84	0.00	0.00	0.00
67	Dizene	nonblood	1	19.20	11.52	2.69	2.69	1.54	0.77	0.00	3.00
67	Dizene	nonblood	3	62.80	43.21	15.70	2.01	1.63	0.31	0.00	43.10
67	Dizene	nonblood	6	16.40	12.14	1.31	1.97	0.98	0.00	0.00	
68	Dizene	nonblood	0	13.50	7.70	0.81	4.32	0.27	0.41	0.00	7.50
70	Dizene	nonblood	0	4.00	2.57	0.48	0.43	0.51	0.01	0.00	4.60
70	Dizene	nonblood	1	10.00	4.31	0.90	2.60	1.98	0.21	0.00	0.90
70	Dizene	nonblood	3	25.30	12.40	5.82	1.77	5.06	0.25	0.00	137.00
70	Dizene	nonblood	7	10.00	7.30	0.80	0.90	0.60	0.40	0.00	61.50
72	Dizene	nonblood	0	4.40	2.68	0.09	0.92	0.70	0.00	0.00	0.40
72	Dizene	nonblood	1	9.00	4.05	0.09	3.24	1.53	0.00	0.09	4.90

White blood cell and platelet variables

Dog ID	Treatment	Group	Day	WCC x10^9/l	AbNmat x10^9/l	AbNimm x10^9/l	AbLymph x10^9/l	AbMono x10^9/l	AbEos x10^9/l	AbBaso x10^9/l	Thr C x10^9/l
72	Dizene	nonblood	3	15.40	5.88	1.23	5.44	2.20	0.65	0.00	133.00
73	Dizene	nonblood	0	5.60	1.51	0.50	3.36	0.17	0.06	0.00	3.90
73	Dizene	nonblood	1	7.90	1.19	0.16	5.21	1.19	0.16	0.00	105.00
73	Dizene	nonblood	3	9.40	1.69	0.66	6.39	0.38	0.00	0.00	397.00
76	Fluids	nonblood	0	12.80	7.81	1.28	2.69	1.02	0.00	0.00	4.30
78	Fluids	nonblood	0	4.80	3.55	0.34	0.58	0.34	0.00	0.00	1.00
78	Fluids	nonblood	1	17.80	14.30	0.18	2.46	1.14	0.00	0.00	1.80
81	Fluids	nonblood	0	7.90	6.10	0.24	1.05	0.51	0.01	0.00	2.60
81	Fluids	nonblood	1	18.70	10.85	0.94	4.86	1.87	0.19	0.00	78.80
81	Fluids	nonblood	3	20.80	12.27	1.25	5.62	1.46	0.21	0.00	204.00
82	Fluids	nonblood	0	3.70	2.37	0.33	0.74	0.26	0.00	0.00	2.80
82	Fluids	nonblood	1	6.90	4.17	0.55	1.47	0.65	0.06	0.00	0.70
82	Fluids	nonblood	3	16.60	7.80	1.16	6.14	1.49	0.00	0.00	35.10
82	Fluids	nonblood	7	11.30	7.33	0.23	3.01	0.41	0.33	0.00	637.00
83	Dizene	nonblood	0	10.00	5.00	0.30	4.00	0.70	0.00	0.00	62.30
83	Dizene	nonblood	1	10.30	4.49	0.21	4.90	0.57	0.14	0.00	
83	Dizene	nonblood	3	7.20	2.45	0.07	3.96	0.36	0.36	0.00	285.00
83	Dizene	nonblood	7	11.90	8.73	0.12	2.24	0.42	0.38	0.00	481.00
87	Dizene	nonblood	0	7.40	3.11	0.59	2.89	0.81	0.00	0.00	1.00
87	Dizene	nonblood	1	9.90	3.76	0.79	4.36	0.89	0.10	0.00	2.40
87	Dizene	nonblood	3	8.20	2.13	0.90	3.85	1.07	0.25	0.00	
87	Dizene	nonblood	6	11.80	3.89	0.47	6.14	0.59	0.71	0.00	448.00
90	Fluids	nonblood	0	3.30	2.18	0.17	0.60	0.33	0.02	0.00	0.40
90	Fluids	nonblood	1	4.80	2.54	0.05	1.68	0.43	0.10	0.00	7.90
90	Fluids	nonblood	3	12.70	5.33	0.00	4.32	2.54	0.51	0.00	85.10
90	Fluids	nonblood	6	11.50	5.87	1.50	3.34	0.46	0.35	0.00	296.00
93	Dizene	nonblood	0	12.50	8.38	0.50	2.23	1.13	0.13	0.00	37.00
93	Dizene	nonblood	1	16.10	7.02	1.61	5.30	2.00	0.18	0.00	64.40
93	Dizene	nonblood	3	26.10	17.85	1.31	2.92	2.98	1.04	0.00	224.00
94	Dizene	nonblood	0	6.70	3.69	0.34	2.14	0.54	0.00	0.00	0.60