A STUDY OF ANAEMIA IN DOGS

By

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Dissertation submitted for the Degree of Master of Veterinary Medicine
in the Faculty of Veterinary Medicine,
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Department of Veterinary Pathology,
University of Glasgow,
April 1997

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Ann Arbor, MI 48106 – 1346

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DEDICATION

This work is dedicated to my loving parents, Sudarshan and Hardesh

and Silloo

ACKNOWLEDGEMENTS

I would like to thank the Head of Department of Veterinary Pathology, Prof. D. Onions for allowing me to study here.

I would also like to thank my supervisor Prof. H. Pirie for accepting me in the Department of Pathology and for his advice and support throughout my course of study. I am grateful for his continuous efforts and encouragement which will be beneficial for the years to come for me.

I would also like to thank Dr. S. Toth for having showed me the world of haematology and cytology through the microscope. I sincerely appreciate her endless patience in teaching me, guiding me and her continued efforts in reading and correcting this work.

A special thanks to Mr. Ronnie Barron and Mr. Kenny Williamson for their efforts in teaching me the haematology laboratory procedures. A thanks to Mr. Ronnie Barron for helping me with the photographs and Kenny Williamson for his continuous efforts in teaching me the ins and outs of computing and database searches. A thanks also for tolerating me in the laboratory. Their awful puns and dreadful names kept me going.

I am also grateful to Prof. A. Nash and Dr. C. Little for having allowed me to see the cases in the Department of Small Animal Clinics.

I would like to express my appreciation to all the pathologists in helping me whenever required. I would also like to thank Mrs. Dania Anderson for helping me with the "Dataflex" database. Thanks also to Mr. Richard Irvine and Mr. John Ramsay at the post-mortem room.

A special thanks to Ms. Vicki Dale for scanning all the slides used in this thesis. I would also like to thank Mrs. Mary Findlay and Mrs. Maureen McGovern at the James Herriot Library for letting me use the library and for the support they gave me during my stay in Scotland.

Everyone in the Veterinary Pathology tea room who kept me feeling at home, it was always a nice break to stop for a cup of Scottish blend tea.

Lastly, I should not forget Douglas Hart who kept encouraging me with cups of tea and who also helped me in computing and made my stay in Scotland most memorable.

DECLARATION

I, Miss Reva Dheer, do hereby declare that the work in this dissertation is original, and was carried out by myself or with due acknowledgement, and has not been presented for the award of a degree at any other university.

Signed:

Dated: 8th July 1997

SUMMARY

Haematology is the study of one of the most vital systems, the blood, which is 'a window' to many other body systems often reflecting the abnormalities or pathological changes in diseased organs. The study of this system has been and still is of great interest to man. It is one of the most intensely studied systems of animals and man. Haematology is a vast subject which includes the study of all the cells of the blood, their formation and functions in normal and in pathological conditions. Haematological conditions are common and diverse, anaemia being one of the well recognised clinical conditions in animals and man. There is an increasing awareness amongst veterinarians regarding the implications of anaemia in animals, resulting in an increasing number of cases reported.

For a proper understanding of anaemia, it is necessary to know the details of the mechanisms of haemopoiesis which are reviewed in the first chapter. Pathological conditions affecting the erythropoietic system may be basically considered as hyperplasia, neoplasia or anaemia and these are discussed in the second chapter. For a systematic approach to diagnosing anaemia the clinical presentation of the patient is essential with a history of the subject. This information may provide a clue to the underlying mechanisms causing the anaemia and these matters are considered in the third chapter.

Once the diagnosis of anaemia has been established in a patient it is very important to classify the anaemia. There are various classifications of anaemia described for animals and man. A classification of anaemia in dogs has been proposed

on the basis of these classifications already described and this has been used throughout this study.

The retrospective study comprises of a survey of anaemic dogs presented at the University of Glasgow Veterinary School from 1st January to 31st December 1994. There were in all 245 dogs presented with Packed Cell Volume (PCV) less than 36.9% that were considered to be anaemic. From these dogs, 52 dogs (Group A) were examined post-mortem by the pathologists at the University of Glasgow Veterinary School. The haematological data of these 52 dogs was studied in more detail to co-relate the blood findings and the pathological observations for a better understanding of the underlying mechanisms causing the anaemia.

For the prospective part of this study, dogs presented as clinical cases to the veterinary school during the following year (1995), were selected. Knowing the pathological conditions observed in the retrospective study, dogs for the prospective study were chosen that were markedly anaemic. Only cases with significant anaemia as classified by the criteria adopted in the retrospective study and with some marked haematological findings were considered. These selected cases were grouped and studied in relation to the mechanism causing the anaemia.

This study has identified the commoner types of anaemia in the dogs investigated and classified the anaemias in the best possible way for veterinary use. Knowing the cause and the severity of an anaemia enables a better assessment for the prognosis of a case to be made. Thus, it was concluded that a complete haematological investigation should be an essential part of a diagnostic procedure for detecting the underlying causes of anaemia and for making a prognosis for the animal.

ABBREVIATIONS

ACD	anaemia of chronic disease
ADP	adenosine diphosphate
AIHA	auto-immune haemolytic anaemia
ALL	acute lymphoblastic leukaemia
Alk.Phos	alkaline phosphatase
ALT	alanine aminotransferase
ANC	all nucleated cells
AST	aspartate aminotransferase
ATP	adenosine triphosphate
Bas	basophils
BFU-E	burst forming units erythroid
BPA	burst promoting activity
BNeutro	band neutrophils
CBC	complete blood count
CFU	colony forming unit
CFU-E	colony forming unit-erythroid
CFU-GEMM	colony forming units-granulocytes, erythrocytes, megakaryocytes and monocytes
CLL	chronic lymphocytic leukaemia
CFU-Meg	colony forming unit megakaryocyte
CRF	chronic renal failure
CSFs	colony stimulating factors
cG-CSF	canine granulocyte colony stimulating factor

δ-ALA	delta-amino levulinic acid synthetase
DHAP	dihydroxyacetone-3-phosphate
2,3- DPG	2,3 diphosphoglycerate
DIC	disseminated intravascular coagulation
DNA	deoxyribonucleic acid
E-CSF	erythroid colony stimulating factor
EDTA	ethylene diaminetetraacetate
EM	Embden Meyerhof pathway
EMH	extramedullary haemopoiesis
Epo	erythropoietin
F-6-P	fructose-6-phosphate
FDP	fibrin degradation product
fl	femtolitre
g/dl	grams per decilitre
γIFN	gamma-interferon
G-6-P	glucose-6-phosphate
G-6-PD	glucose-6-phosphate dehydrogenase
GM-CSF	granulocyte macrophage colony stimulating factor
GSD	German Shepherd Dog
GSH	reduced glutathione
GSSG	oxidised glutathione
Hb	haemoglobin
HMP	hexose monophosphate pathway
IDA	iron deficiency anaemia
IgG	immunoglobulin G

IgM	immunoglobulin M
IL	interleukin
MAHA	microangiopathic haemolytic anaemia
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
MNPS	mononuclear phagocytic system
NAD	nicotinamide adenine dinucleotide (oxidised)
NADH	nicotinamide adenine dinucleotide (reduced)
NADPH	nicotinamide adenine dinucleotide phosphate (reduced)
NSAID	non-steroidal anti-inflammatory drug
PC	prospective case
PCV	packed cell volume
PFK	phosphofructokinase
pg	pico grams
Plt	platelet
RBC	red blood corpuscles
rHuEpo	recombinant human erythropoietin
RNA	ribonucleic acid
SCF	stem cell factor
SFMC	soluble fibrin monomer complex
TNFα	tumour necrosis factor alpha
vWD	von Willebrand's disease
WBC	white blood corpuscles
WHW	West Highland White terrier

CHAPTER 1

HAEMOPOIESIS

AND

ERYTHROPOIESIS

1.1 BLOOD

The blood has always been and still is an organ of great interest to man and his curiosity. The blood has always been regarded as a life-containing fluid, only rarely is the blood considered as an organ. The blood is the most vital organ contained as a fluid matrix, circulating through vascular channels into every part of the body. It is the life of every tissue and cell of the body, providing oxygen, hormones, nutrients and receiving the waste products of metabolism from all the various organs for excretion and maintaining a homeostatic environment for the cells of the body. It consists of a number of different types of cells which are suspended in a fluid matrix called plasma. This fluid matrix is a complex mixture of proteins controlling the pH and osmotic pressure. Many disease conditions can cause subtle changes in the blood most easily, making it the most important aid to a diagnosis of a disease.

Knowledge and progress in haematology has increased in the past few decades with increasing technology and have resulted in greater success in diagnosing and treatment of many disease conditions of the blood including anaemia. To understand the development of anaemias it is essential to know, the processes involved in the production of the blood cells and the factors involved in their regulation.

1.2 HAEMOPOIESIS

Haemopoiesis is derived from Greek language, where *haima* means blood and *poiesis* means making. It is a complex process by which the cells that make up the blood are produced. Haemopoiesis during intrauterine life begins in the yolk sac,

successively it takes place in the liver and spleen of the embryo, to a lesser extent the kidney and adrenals (Valli and Parry, 1993). In dogs, splenic haemopoiesis may be found at birth with a gradual diminution over the next six months. In mice, the spleen retains this function throughout life and hence has been used as a model in understanding certain mechanisms (Jain, 1979). After birth, the sustainment and production of haemopoietic cells takes place mainly in the shafts of long bones which contain the active red bone marrow the so-called myeloid tissue. After puberty, the flat bones of the sternum, pelvis, ribs, spine and proximal ends of long bones maintain haemopoiesis. The cavity of the long bones may be replaced by the fatty tissue called yellow marrow. Only in case of an increased demand for red blood cells will there be a haemopoietic conversion of the fat in the endosteal margins and in the cancellous bone areas (Valli and Parry, 1993). The red bone marrow is located centrally within the bone and is surrounded by the cortex of the bone. It has an arterial blood supply that delivers nutrients and also forms sinuses in the medullary bone. These sinuses are extravascular spaces within which haemopoietic cells grow and differentiate. The progenitor cells (discussed further), that grow and develop in these sinusoidal spaces are surrounded by the bone marrow stroma, which consists of endothelial cells, fibroblasts, adipocytes and macrophages. Cells from various cell lines grow in this bone marrow microenvironment until they are mature enough to be released into the venous circulation (Smith and Yee, 1992). The liver and spleen do have the potential to become active again and haemopoiesis may be re-established in case of increased demands. Formation of blood outside the marrow cavities is called extramedullary haemopoiesis (EMH). In dogs, this may be observed in cases of an increased demand which may be seen in blood loss or haemolysis.

Blood cells have different life spans and new cells have to be produced continuously to maintain a balance of these cells. The different functions of these cells makes their production very complex. Production, proliferation, maturation and release of the mature cells from the bone marrow is maintained in a steady state so that the production balances the loss. This is brought about by a number of circulating haemopoietic growth factors which are glycoproteins, and factors derived from the T-lymphocytes, monocytes, and from the specialised stromal elements of the bone marrow. These factors are the different colony stimulating factors (CSFs), interleukins (IL), interferons (IFN), thrombopoietin and erythropoietin (Epo) acting at different levels of the developmental process regulating the proliferation and differentiation of the haemopoietic stem cells, early and late progenitor cells (Metcalf, 1989; Smith and Yee, 1992). Metcalf (1989), observed that *in vitro* the CSFs, not only regulate proliferation, but also enhance the survival of the precursors, maintain the functional integrity of the cells, cause irreversible induction of commitment and differentiation, induce maturation of the cells and stimulate the activity of some mature cells. Development of the granulocytes, monocytes, platelets and lymphocytes are regulated by ILs and the CSFs, whereas erythropoiesis is mainly regulated by a hormone Epo produced by the kidneys.

Stem cells and early progenitor cells are morphologically unrecognisable and they were initially identified through cell culture colony forming assays. Hence, these cells are often referred to as colony forming units (CFU) or burst forming units (BFU). All the haemopoietic cells of the blood originate from a common pluripotent stem cell which is uncommitted and is able to differentiate into the various cell lineages, persisting throughout the life of the animal and capable of undergoing irreversible proliferation in a methodical process. The pluripotent stem cell is capable

of self-renewal and of differentiation into multipotential stem cells (these are cells producing progenitors of two or more cell lineages), which in turn differentiate into unipotential early precursor cells committed to a single lineage (Jain, 1993a; Metcalf, 1989). These early precursors undergo a number of cell divisions forming the late precursors which then reach a stage where they undergo maturation within the marrow. Only mature cells are released into the circulation. The presence of immature cells in the blood is associated with some haematological abnormality. Also, the production of the various cell types remains in proportionate numbers until this steady state is disturbed by some pathological condition.

The pluripotent stem cell gives rise to two types of multipotential stem cells which are the lymphopoietic and the haemopoietic (myeloid) stem cells and the subsequent development of these cell lineages in man is illustrated in Figure 1. These cells proliferate and form two pools, one for lymphopoiesis and one for myelopoiesis. They have a limited capacity for self renewal, and have the potential to become any progenitor cell termed multipotential cells (Smith and Yee, 1992). The myeloid progenitor cell (CFU-GEMM) derived from the myeloid stem cells is the cell from which the granulocytes, erythrocytes, monocytes and megakaryocytes are derived. The lymphopoietic stem cell is also derived from the same pluripotential stem cell but undergoes differentiation and maturation by different mechanisms than that of the myeloid series. The lymphopoietic stem cell, regulated by growth factors, forms the precursors for the B and T cells. These then mature to form the B lymphocytes and the T lymphocytes in the primary lymphoid organs that is in the bone marrow and the thymus respectively. The CFU-GEMM under the influence of CSFs and ILs form the precursors for the granulocytic (CFU-G, CFU-Eo, CFU-Bas), monocytic (CFU-M) and megakaryocytic cell lineages (CFU-Meg), and burst forming unit for the

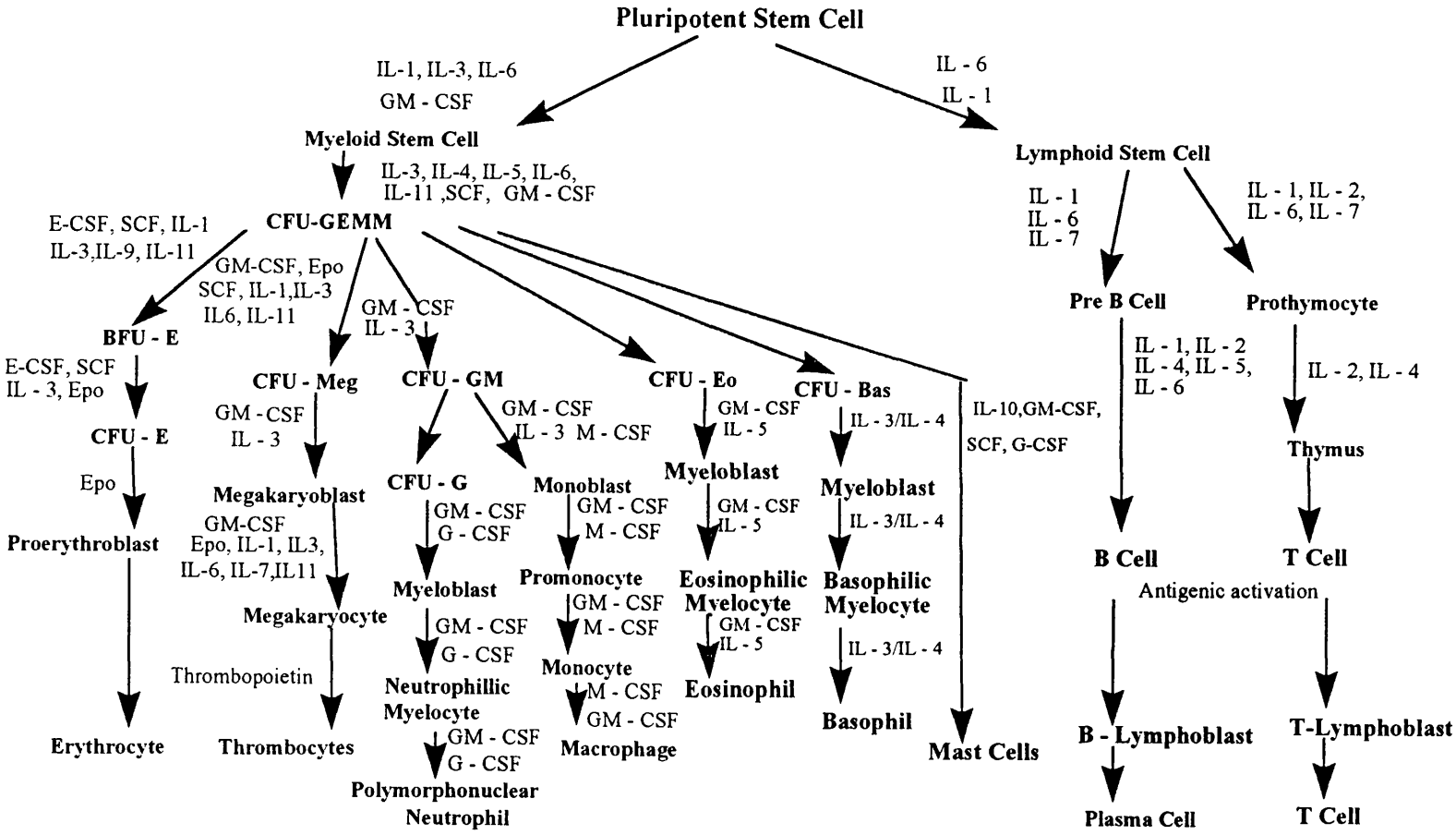

Figure 1: Schematic diagram of haemopoiesis and some of the regulatory factors.(Adapted from Beck, 1991 and Genzyme Diagnostics, 1995)

erythroid cell lineage (BFU-E). The erythroid burst forming unit, gives rise to the colony forming unit erythroid (CFU-E), which undergoes cell divisions to finally produce the erythrocytes (Metcalf, 1989).

The differentiation, production, maturation and regulation of erythrocytes takes place under the influence of the hormone erythropoietin and other factors (Krantz, 1991). It is essential to understand the mechanism involved in the formation of the erythrocytes and the action of erythropoietin since anaemia is an abnormality of the erythrocytes.

1.3 ERYTHROPOIETIN

This hormone is the main regulatory factor of erythropoiesis. Pathological conditions affecting the regulation of erythropoietin (Epo) production cause abnormalities ranging from secondary polycythaemia due to the excessive production of Epo to the anaemia of chronic renal failure due diminished Epo production (Cowgill, 1992a). Erythropoietin is a unique glycoprotein hormone amongst the haemopoietic growth factors since it is produced primarily by the adult kidneys, transported via the blood stream and acts specifically on the erythroid precursors, having little effect on other cell lineages (Krantz, 1991). It is present in the plasma, urine and other body fluids. Although it is produced primarily by the kidneys, in foetal life and in special circumstances in the adults, the liver has also been described as a site for extra renal production (Adamson, 1994). During the first weeks of pregnancy in human beings the foetal liver is the main source of Epo that disappears after 2 weeks and the kidney resumes the function (Cambi and David, 1994).

In a series of experiments by Fried (1972), bilaterally nephrectomised and eviscerated rats had low plasma Epo titers. However, these levels were greater than the levels observed when the rodents were subjected to total nephrectomy and partial hepatectomy of 80 percent. When these rats were exposed to hypoxia for 8 hours, the plasma Epo levels of the rats with nephrectomy and 80 percent hepatectomy was undetectable, when compared with the nephrectomised rats in which there was a low level of Epo. This suggested some extra renal site of Epo production and was assumed to be the liver (Fried, 1972).

Erythropoietin selectively stimulates the proliferation and differentiation of the early erythroid precursor cells (BFU-E and CFU-E) into the pronormoblast, (Figure 1), which is the first morphologically recognisable late precursor cell (Ikeda *et al.*, 1990). The BFU-E is resistant to the action of Epo. However, in the presence of a factor burst promoting activity (BPA), which is derived from T-lymphocytes and mononuclear cells, BFU-E becomes more sensitive and undergoes differentiation. In the first phase Epo stimulates mitosis and cellular growth, later its activity is directed towards the maintenance of cell survival. The haematological response of Epo depends on the number of circulating erythroid progenitors. At the cellular level, Epo increases RNA synthesis followed by haemoglobin synthesis and the action of Epo is increased at the CFU-E and pronormoblast stage (Cambi and David, 1994). At the CFU-E stage, the CFU-E is estimated to have 300 to 400 high affinity Epo receptors per cell and in healthy individuals is the cell with the highest number of receptors in the body (Adamson, 1994).

The normal levels of Epo have been measured in dogs. In a study of 25 dogs Ikeda *et al.*, (1990), using mouse spleen cells and serum from dogs, found the normal Epo levels to be 38.5-135 mU/ml. There was no difference in Epo levels between the

sexes. However, older dogs had lower levels compared to the high levels observed in puppies. The high levels in puppies was thought to be due to a physiological increase in erythropoiesis. In another study by Cook and Lothrop (1994), Epo was measured by radio immunodiffusion assay and in normal dogs levels were found to be 7-37 mU/ml.

Tissue oxygenation depends on a number of factors and hypoxia is sensed by the kidneys with the release of Epo (Cambi and David, 1994). Hypoxia induces the kidneys to produce renal erythropoietic factor or erythrogenein which interacts with an α-2-globulin (erythropoietinogen) synthesised by the liver to form Epo (Jain, 1979). As summarised by Woodman (1992), a variety of hormones may also be involved in the release of Epo such as growth hormones, lactogenic hormones and vasopressin. The action of these hormones and vasopressin seem to facilitate Epo release rather than affecting its action. Calcium levels may also increase when Epo production is stimulated (Woodman, 1992). Sudden tissue hypoxia as in case of severe haemorrhage, stimulates and increases the production of Epo which induces erythropoiesis to compensate for the developing anaemia. Whereas, hyperoxia reduces the amount of Epo and subsequently erythrocyte production (Krantz, 1991).

In dogs, advancing renal failure has been recognised to lead to a progressive non-regenerative anaemia due to the destruction of the renal parenchyma and associated reduced Epo secretory function (Cowgill, 1992a). In dogs with renal failure the measurements of serum Epo concentration, revealed low to normal levels indicating either an absolute or a relative decrease of Epo and this suggests that any impairment to the kidneys may lead to a non-regenerative anaemia (King *et al.*, 1992). In cases of certain haemolytic and blood loss anaemias there is an increase in the levels of Epo, stimulating erythropoietic activity which results in reduction in the maturation time and early release of immature cells leading to reticulocytosis (Jain,

1979). In a study by Cook (1994), it was observed by radioimmunoassay that in some dogs the levels of Epo increased in pure red cell aplasia. However, the bone marrow was unable to respond due to the aplastic conditions. Such animals probably would not benefit from Epo therapy.

With increasing knowledge, many haemopoietic growth factors have been studied and produced through cloning and recombinant DNA technology. Their biological applications and therapeutic role has raised keen interest in human beings and in animals (Ogilvie, 1993). These growth factors have the ability to increase the number of erythrocytes, granulocytes and platelets in clinical situations of cytopenias when the stem cells are plentiful. Interleukins particularly, are likely to play an important role in treating cancer, infectious and immunodeficiency syndromes (Elmslie *et al.*, 1991). Groopman *et al.*, (1989), have well summarised their applications for human beings in certain clinical conditions. In veterinary medicine, there have been several reports and clinical trials in dogs and cats in studying the efficacy of these factors (Ogilvie *et al.*, 1992, Ogilvie, 1993, Cowgill, 1992a). The highly purified recombinant human erythropoietin (rHuEpo) is now clinically available and has been used in human patients.

Erythropoietin has been of great help to human patients in correcting the anaemia associated with chronic renal failure although its uses have also been observed in other conditions (Eschbach *et al.*, 1990). It has also been of significant value in case of short term treatment of chronic renal failure in dogs and cats (Cowgill *et al.*, 1990; Ogilvie, 1993; Bloomberg *et al.*, 1992). Subcutaneous injections improve the quality of life as well as improving the severity of anaemia in these animals. The current formulation of rHuEpo is in human serum albumin which could promote both local as well as systemic reactions in dogs and cats. Cellulitis, fever, arthralgia and

mucocutaneous ulcerations have been recognised in human beings, although an allergic basis could not be established, all these manifestations resolved when treatment was discontinued. It has also been observed that the use of rHuEpo has also resulted in the production of antibodies (Cowgill, 1992b). According to Bloomberg *et al.*, (1992), when rHuEpo (Epogen, Amgen Incorporated, Thousand Oaks,California, USA), was administered to a cat with chronic renal failure, there was a progressive improvement in the packed cell volume (PCV) and in the general status of the cat. Nevertheless, after 178 days the cat died of seizures which the author thought may have been similar to the seizures observed in human beings. However, with caution, it may still be useful for a short term therapy. With increasing interest and research, growth factors have been produced by biotechnology. Their use in human medicine is increasing while for dogs some of the factors have been cloned but are not yet commercially available for clinical use.

1.4 ERYTHROPOIESIS

Erythropoiesis is the systematic process in which the myeloid stem cells (CFU-GEMM) in the bone marrow differentiates to form the cells of the erythroid series, the erythrocytes (Figure 2). Differentiation of the myeloid stem cell (CFU-GEMM), to early committed erythroid progenitor cells called the burst forming unit erythroid (BFU-E), and then the development of the CFU-E results in the formation of morphologically recognisable erythroid precursors. The BFU-E is stimulated by Epo, various other cytokines (IL-3 and CSFs) and burst promoting activity (BPA) which is produced by various cells including macrophages and T-lymphocytes. Cytokines such as IL-3 and CSFs which are produced by T-lymphocytes, promote

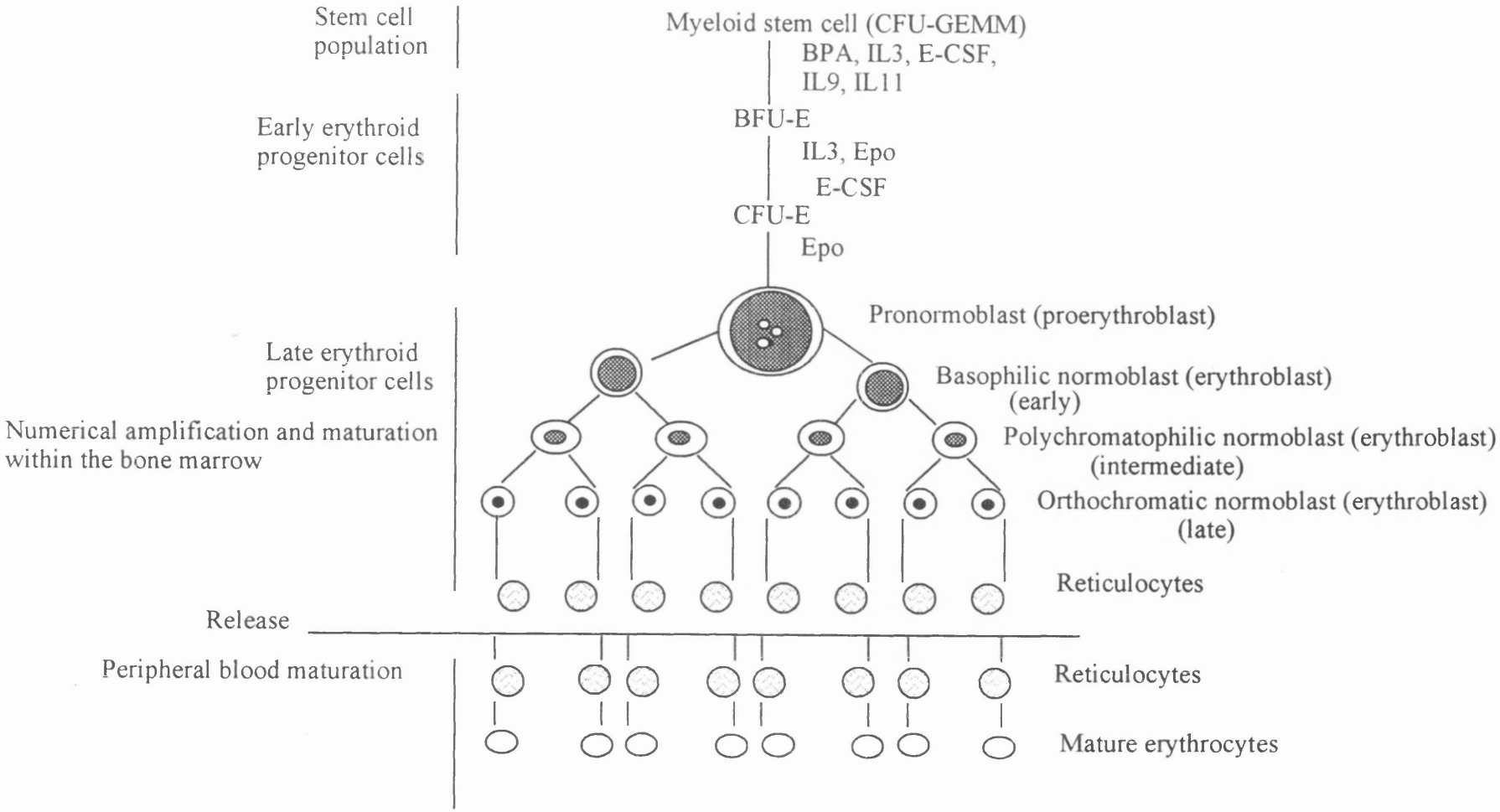

Figure 2: Erythropoiesis: Stages of erythrocyte development . (Adapted from Beck, 1991).

survival, proliferation and development of the multipotential stem cells and differentiation of erythroid progenitor cells (Erickson and Quesenberry, 1992). CFU-E is also sensitive to the actions of Epo which primarily stimulates proliferation and maturation of late erythroid precursors from the pronormoblasts onwards.

All the terminologies used to describe the late precursor cells are based on the appearance of the cell after staining. The process of blast transformation as seen with the Romanowsky stains takes place at the pronormoblast (proerythroblast) stage. It is the largest recognisable cell of the erythroid cell series, with a large central or eccentric nucleus, a large nuclear cytoplasmic ratio with one or more well-defined nucleoli. The cytoplasm stains deeply basophilic due to large amounts of RNA. In subsequent stages, there is a progressive condensation of nuclear chromatin and cytoplasmic structures such as mitochondria, with a loss of RNA activity and an increase in haem synthesis. Each pronormoblast undergoes three to four divisions and forms 8 to 16 cells which then undergo further progressive maturation.

The early basophilic normoblast is the next recognisable cell in the series where the nucleus lacks the nucleolus and has a coarser chromatin pattern with a dark blue staining cytoplasm. Each early basophilic normoblast undergoes further mitotic cell division resulting in the formation of the intermediate polychromatophilic normoblast, with the nucleus being a heavily clumped chromatin. At this stage the cells are smaller, with a blue to reddish tinge to the cytoplasm due to haemoglobinisation. Each cell undergoes maturation resulting in the late orthochromatophilic normoblast. The late orthochromatophilic normoblast has a pyknotic dense nucleus and a small amount of reddish cytoplasm. It is one of the most commonly observed cells of the erythroid series in the bone marrow

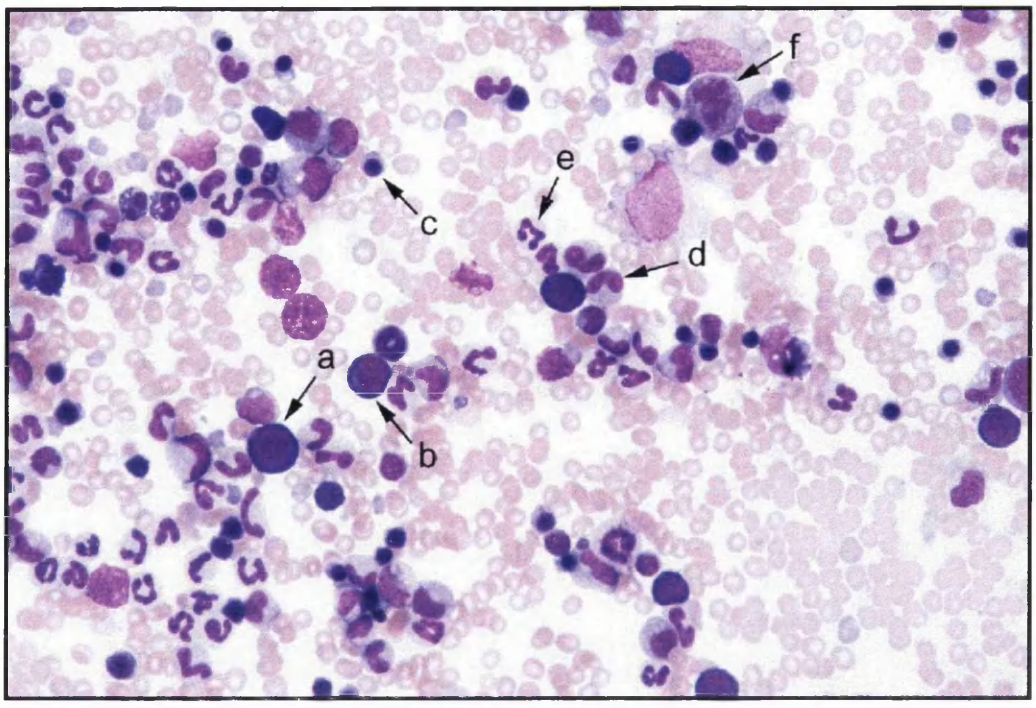

Figure 3A: Normal canine bone marrow cytology with different developmental stages of the myeloid and erythroid cell series and a background of mature erythrocytes. The cells are:

a: pronormoblast (proerythroblast)

b: basophilic normoblast (early)

c: orthochromatophilic normoblast (late)

d: metamyelocyte

e: mature neutrophil

f: myeloid cell in mitosis

Stained by May Grünwald Giemsa, (x 400).

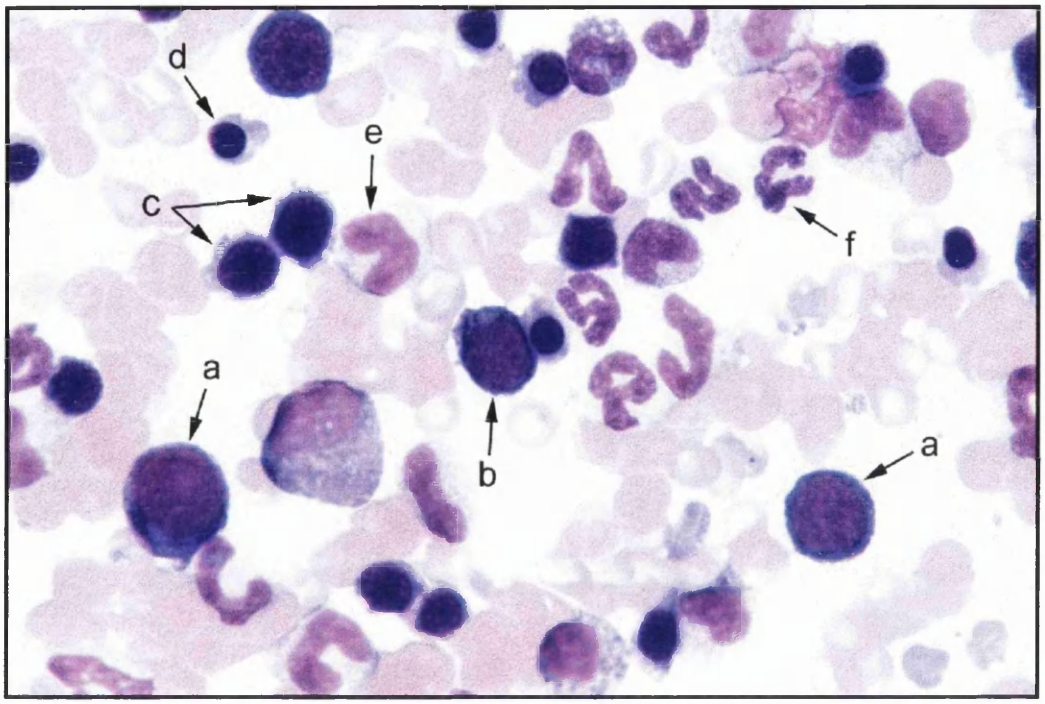

Figure 3B: Normal canine bone marrow cytology showing erythroid and myeloid precursors at various stages of development. The cells are :

a: pronormoblast

b: basophilic normoblast (early)

c: polychromatophilic normoblast (intermediate)

d: orthochromatophilic normoblast (late)

e: metamyelocyte

f: neutrophil

Stained by May Grünwald Giemsa, (x 1000).

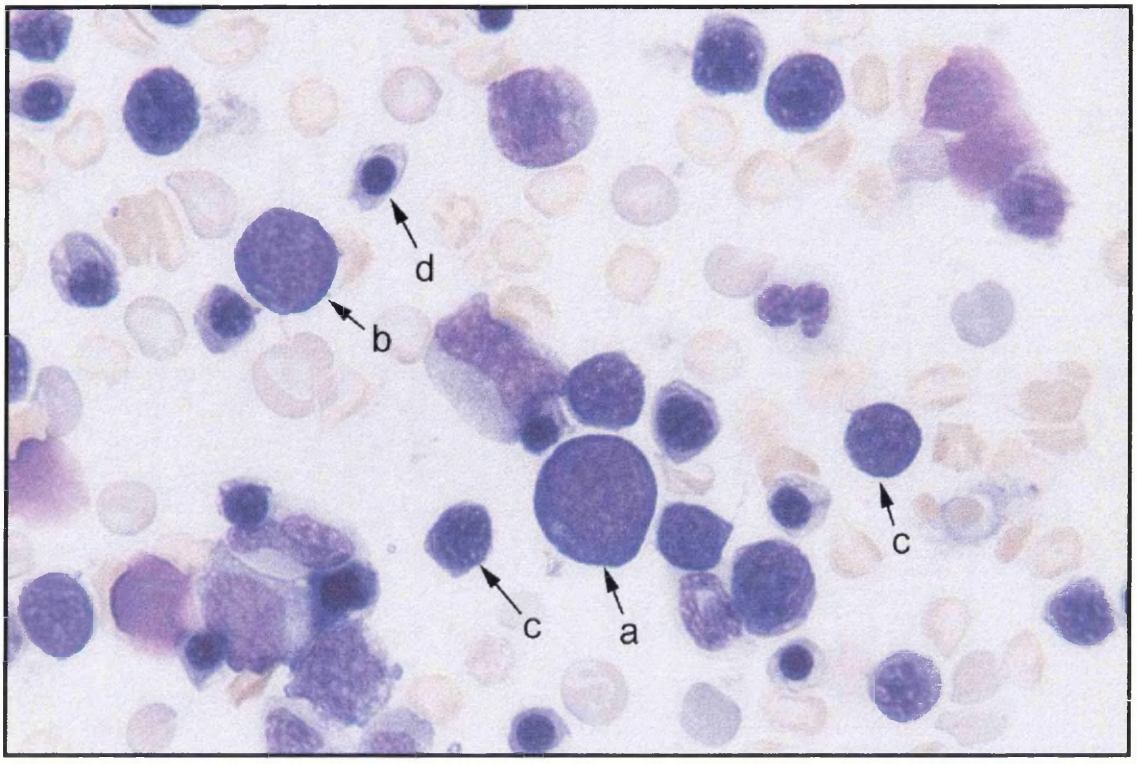

Figure 3C: Normal canine bone marrow cytology showing different development stages of the erythrocytes cell series. The cells are:

a: pronormoblast (proerythroblast)

b: basophilic normoblast (early)

c: polychromatophilic normoblast (intermediate)

d: orthochromatophilic normoblast (late)

Stained by May Grünwald Giemsa, (x 1000).

showing progressive accumulation of haemoglobin (Beck, 1991; Aufderheide, 1981). These cells are illustrated in Figure 3A, 3B and 3C.

Extrusion of the pyknotic nucleus leads to the formation of the immature erythrocyte, "the reticulocyte". This process requires four to seven days in a normal animal but may be accelerated to about two days under conditions causing erythroid stimulation (Weiser, 1995). Normally, nucleated erythrocyte precursors are not released into the circulation. However, when these cells are observed in excess they are indicative of increased bone marrow erythropoiesis or EMH.

1.5 THE RETICULOCYTE

According to the review by Houwen (1992), the history of the reticulocyte dates far back to the 19th century, when Erb described these cells which he observed were present in small amounts in healthy animals but increased in numbers when red cell regeneration took place. An overview of the history of the reticulocyte ever since its discovery has been given by Houwen (1992). Since then, there have been several investigations to elucidate their maturation and their role in regenerative anaemias. The reticulocyte is formed when the nucleus is lost from the orthochromatophilic normoblast during its passage through the marrow sinusoids. These cells are anuclear but still contain some ribosomes, Golgi bodies and mitochondria in their cytoplasm and undergo further maturation but no cell division. They are called reticulocytes because on a blood smear, after supravital staining with new methylene blue a method described by Schalm (1964), it produces a clumping of the above structures resulting in a granular blue stained reticulum (Figure 4A).

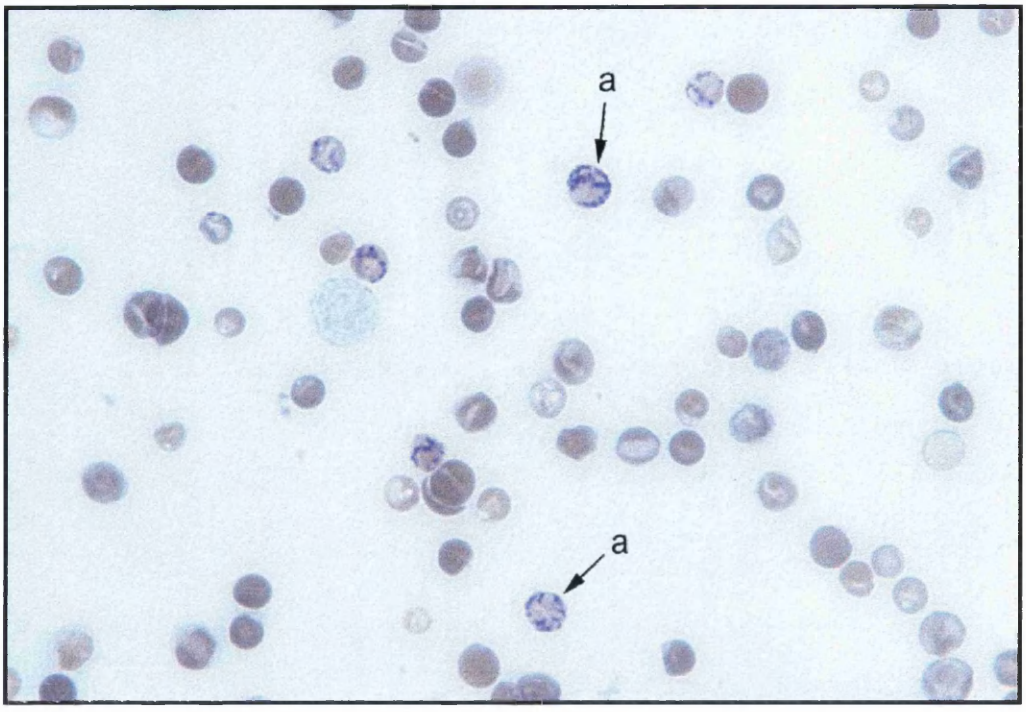

Figure 4A: Peripheral blood smear from a dog showing aggregation of RNA i.e. reticulum formation in immature erythrocytes (a). Reticulum is demonstrated by the supravital stain new methylene blue, (x 1000).

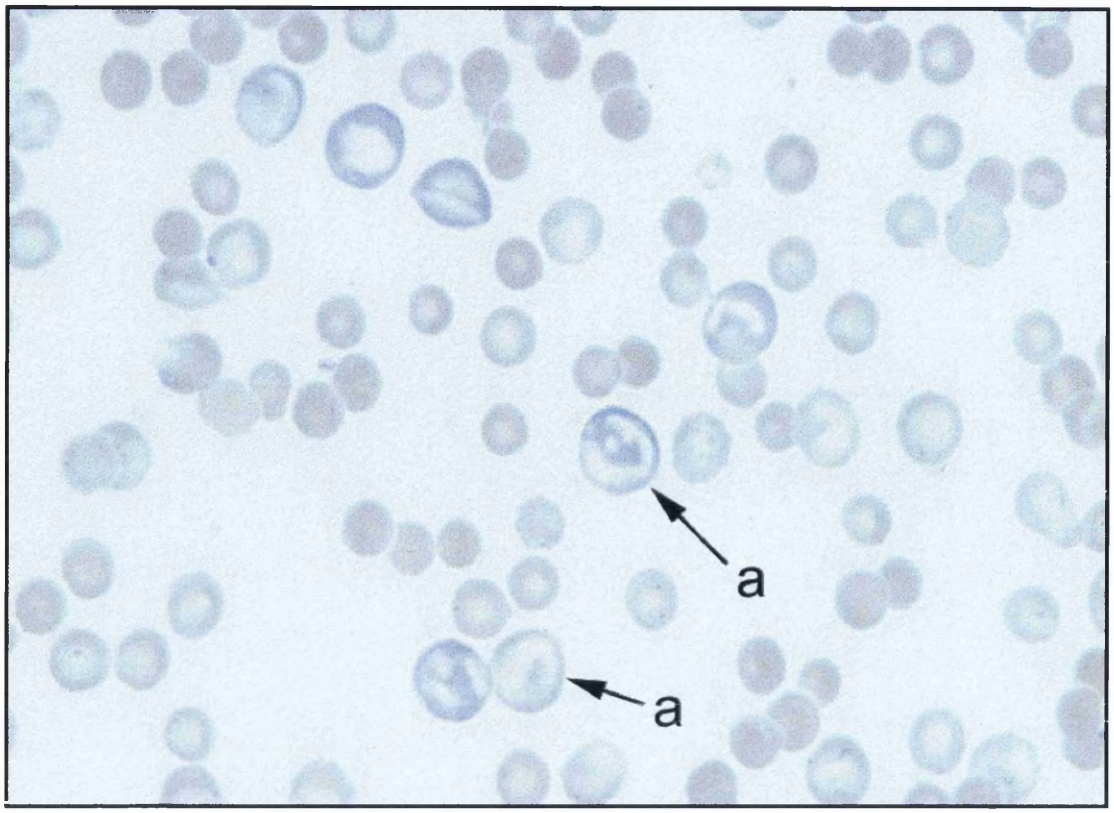

Figure 4B: Reticulocytes (a), have a bluish tint when the blood smear is stained with May-Grünwald Giemsa stain and are referred to as polychromatic macrocytes or polychromasia of red cells, (x 1000).

After staining with a standard Romanowsky stain such as Leishman or May - Grünwald Giemsa, they appear with a slight bluish tint which is called polychromasia and the mature erythrocytes appear eosinophilic due to their haemoglobin content (Figure 4B). In a blood smear the increased presence of polychromatic cells reflects increased erythropoiesis. The number of reticulocytes present in the blood can be counted manually and evaluation of reticulocytes has now become more accurate using flow cytometry methods by staining the cells with thiazole orange (Abbott and McGrath, 1991). Although there are drawbacks in this method, since Howell-Jolly bodies and small lymphocytes may be included in the counts, when compared to the manual method it has proven to be more precise.

Reticulocytes stay in the bone marrow for about two to three days where they undergo maturation before entering the circulation by diapedesis. Reticulocytes are sticky and lose their stickyness as they mature. As reticulocytes mature to form erythrocytes they undergo changes in addition to losing all its organelles (reticulum). They shrink in diameter and volume due to decrease in the water content, so that the haemoglobin is more concentrated along with a decrease in the total surface area. The amount of lipid present on the cell also decreases but the relative concentration of essential fatty acids and cholesterol increases resulting in the formation of a mature erythrocyte (Crosby, 1959). In the circulation or in the spleen they mature to erythrocytes within 24 to 48 hours but if the reticulocytes are produced under severe hypoxic conditions, they will have a greater amount of reticulum and tend to persist for a longer duration of three days (Laber *et al.*, 1974).

Normally, in the marrow reticulocytes contain more reticulum and are larger, less matured than those in the peripheral blood (Schalm *et al.*, 1975b). They can also be distinguished from erythrocytes easily as they have not yet achieved their

biconcave shape typical for canine erythrocytes. When observed in the circulation, they are larger in size than the mature cells. Hence, they are also referred to as "polychromatic macrocytes". Due to the fact that reticulocytes have larger volume (MCV) and or relatively low haemoglobin concentration (MCH) the morphology of a regenerative anaemia with marked reticulocytosis will either be macrocytic and normochromic or hypochromic.

Dogs and cats may normally have a reticulocyte count of 0.5-1 percent in the peripheral circulation (Schalm *et al.*, 1975b). Canine reticulocytes usually contain aggregates of RNA, while cats have aggregate as well as punctate forms of reticulocytes (Figure 4A). (Weiser, 1995).

Tissue anoxia due to anaemia after haemorrhage results in an immediate and premature release of reticulocytes into the circulation which is known as reticulocytosis. Accelerated release of reticulocytes is mediated by hypoxic conditions stimulating the release of Epo and consequently the stimulation of erythropoiesis in the bone marrow. Increased hypoxia may stress the marrow even further resulting in the release of some normoblasts. Thus, a reticulocyte count along with other parameters is an indicative tool in classifying and identifying the cause of anaemia. According to Schalm *et al.*, (1975b) and many other researchers, in the dog the magnitude of the reticulocyte response in the peripheral blood circulation is directly related to the degree of erythropoietic activity. Marked increase in erythropoiesis is associated with a regenerative blood picture which is characterised by anisocytosis due to an exaggerated variation in cell size, polychromasia due to polychromatic macrocytes, an increased number of Howell-Jolly bodies and often by the presence of nucleated red cell precursors.

"Howell-Jolly" bodies are nuclear remnants containing DNA (Figure 5).

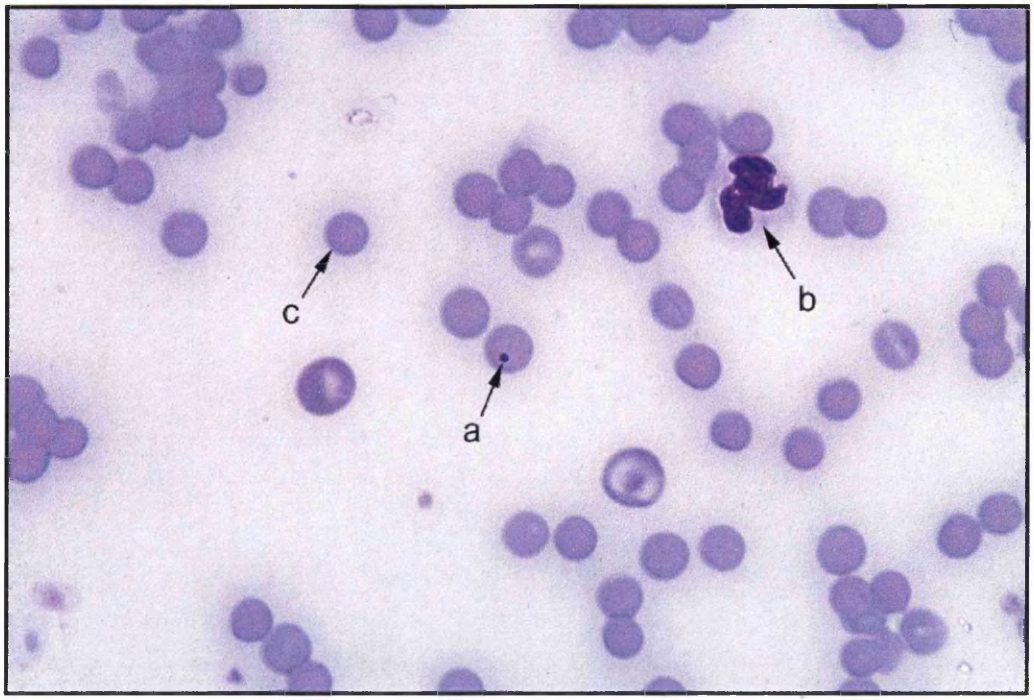

Figure 5: Howell -Jolly body in the erythrocyte (a), is nuclear remnants containing DNA and appear as densely stained structures.

b: neutrophil

c: erythrocyte

Stained with May-Grünwald Giemsa stain,(x1000).

They appear as dark staining structures in the reticulocytes and also in erythrocytes. These are frequently observed and may be present in high numbers when erythrocytes are being rapidly produced due to increased demand as in regenerative anaemia (Dacie and Lewis, 1991b). They may also be seen in the case of hyposplenism where there is an impaired splenic "pitting" function or due to corticosteroid therapy. This "pitting" function of the spleen is described later.

1.6 THE ERYTHROCYTE

The erythrocyte is formed after the reticulocyte loses all its organelles and undergoes certain changes. When blood is stained with a Romanowsky and examined under the microscope mature erythrocytes appear anuclear, reddish-brown in colour due to their haemoglobin content with a zone of central pallor. When a Romanowsky stained blood smear is examined, the normal feature is of a uniformity in all the cells (Figure 6A and 6B). A variation in this uniformity, which is the diameter of the cells, is called *anisocytosis*. At birth the erythrocytes of the neonate are large with a high mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) which decrease to the adult values by two to three months of age. By the first year the young dog attains all the values of an adult (Anderson and Gee, 1958).

Dogs have typical biconcave shaped cells and when observed with electron microscopy, the cells have a smooth surface. The life span of erythrocytes within the circulation is of 110-120 days and almost 0.9 to 1.3 percent are removed daily from the circulation (Weiser, 1995). The average size (diameter) of a canine erythrocyte is 7μm, with a of volume of 60-72 fl, which is expressed as the MCV and the normal range of haemoglobin content (MCH) of 19.5-24.5 pg. In dogs the normal range of

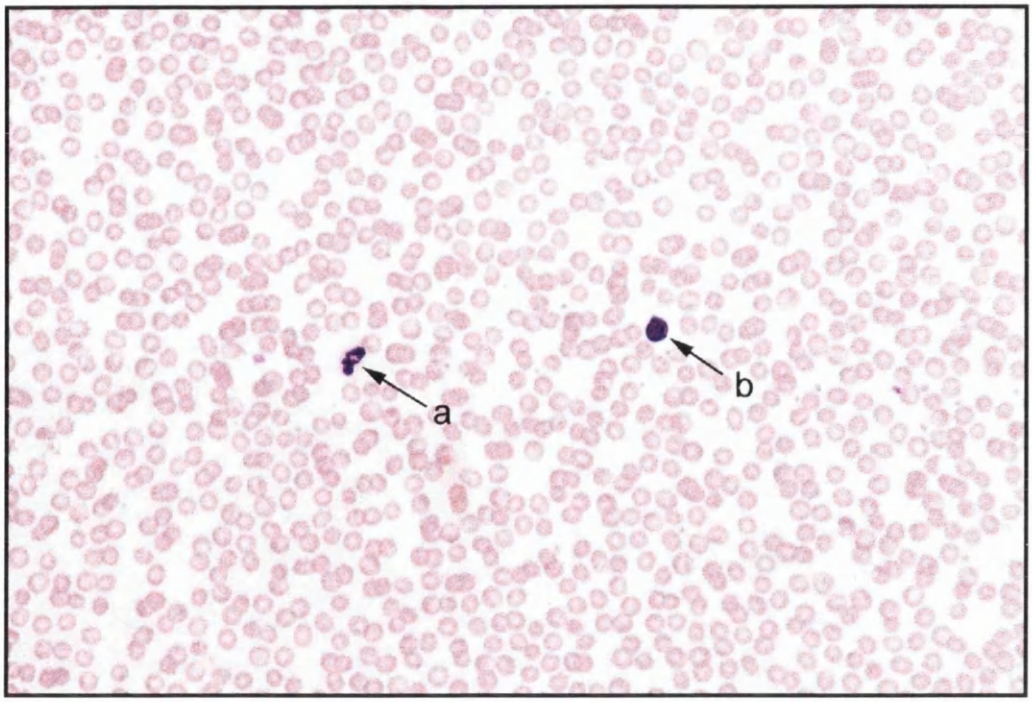

Figure 6A: Normal blood picture from a dog showing normocytic and normochromic red cells with uniform size and shape.

a: neutrophil

b: lymphocyte

Stained by May-Grünwald Giemsa, (x 400).

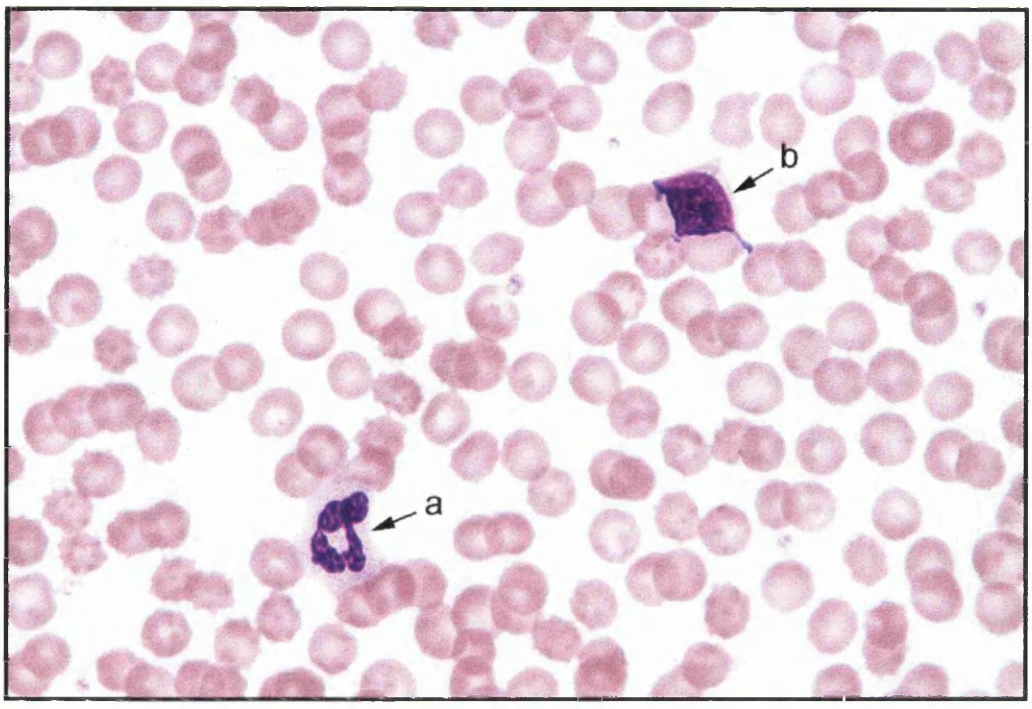

Figure 6B: Normal blood smear from a dog with normocytic and normochromic erythrocytes. Slightly crenated erythrocytes may be artifacts.

a: neutrophil

b: lymphocyte

Stained by May Grünwald Giemsa, (x 1000).

circulating erythrocytes is 5.5-8.5 x 10 ^{12}/l (Appendix A, Table A1). The cellular composition of the bone marrow is another important aspect which is a ratio of the myeloid and erythroid cells (M:E). The normal ratio in dogs is 0.75:2.5:1.0 which varies in different disease conditions (Schalm *et al.*, 1975a).

Certain breeds have atypical red cell values which are not associated with anaemia but are normal features for that particular breed. When any of these breeds are encountered in practice these features should be considered in order to correctly interpret the haematological results. Red cells of Japanese Akita dogs have a low MCV of 55 to 65fl, whereas miniature and toy Poodles, both have high MCV ranging from 85 to 95fl. The cause of these features in Poodles according to Schalm *et al.*, (1975a) and Schalm (1976), was unknown and may be physiological and not associated with any disease. Canfield and Watson (1989), investigated macrocytosis in detail in a Poodle and concluded that it may be similar to a congenital dyserythropoietic and megaloblastic disorder. Adult Greyhounds, German Shepherd Dogs and Poodles have higher than normal red cell counts, haemoglobin and pack cell volumes (PCV) (Squires, 1993, Schalm *et al.*, 1975a; Doxey, 1966).

Anderson and Gee (1958), studied the normal red cell values of 500 Beagle dogs and observed male dogs to have higher erythrocyte counts than females and the PCV and haemoglobin values were found to be higher in males. There are physiological adjustments too, in which the haemoglobin concentrations may fluctuate for example during oestrus, pregnancy and lactation in females. During gestation the PCV first decreases, then gradually starts to increase in the next few months. Later stages of pregnancy are associated with a pronounced decrease in erythrocytes called as "spurious anaemia" which is due to an increase in the plasma volume or iron deficiency, with a corresponding increase in leucocyte numbers (Wintrobe *et al.*,

1974). During lactation the recovery is apparent but not complete till the litter is weaned (Anderson and Gee, 1958).

Erythrocytes are unique cells since they contain no cell organelles or nucleus and still survive. The primary function of erythrocytes is to transport oxygen via haemoglobin to various tissues of the body and to return carbon dioxide to the lungs. During their life span erythrocytes have to travel through a number of vessels and come across different environments within the body. To achieve this special function they are made up of a membrane consisting of a lipid bilayer and a cytoskeleton. The lipid bilayer is composed of phospholipids and cholesterol. The cytoskeleton is made up of proteins spectrin alpha and β (α and β), ankyrin, actin and protein 4.1 which form a filamentous network in maintaining the cellular flexibility, biconcave shape and integrity of the cell (Smith, 1987).

For survival erythrocytes depend entirely on glycolysis by obtaining their energy mainly from glucose which is metabolised to lactate. This is by the anaerobic glycolytic Embden-Meyerhof (EM) pathway, the hexose monophosphate pathway (HMP) and the Rapaport-Luebering shunt (Figure 7). Through these pathways energy is generated in the form of reduced glutathione (GSH), reduced pyridine nucleotides (NADH, NADPH), 2,3-diphosphoglycerate (2,3-DPG) and adenosine triphosphate (ATP). Approximately 95 percent of the glucose is metabolised by the EM pathway, of which 30 percent is shunted via 2,3-diphosphoglycerate (2,3-DPG) and only 5 percent goes by the HMP shunt (Kaneko, 1987). The ATP generated is essential for energy, maintaining the cell shape by controlling the movements of sodium into and potassium outside the cell. The reduced nicotinamide adenine dinucleotide phosphate (NADH) also formed in the EM pathway is utilised for the enzymatic reduction of methaemoglobin (iron in ferric form) to functional

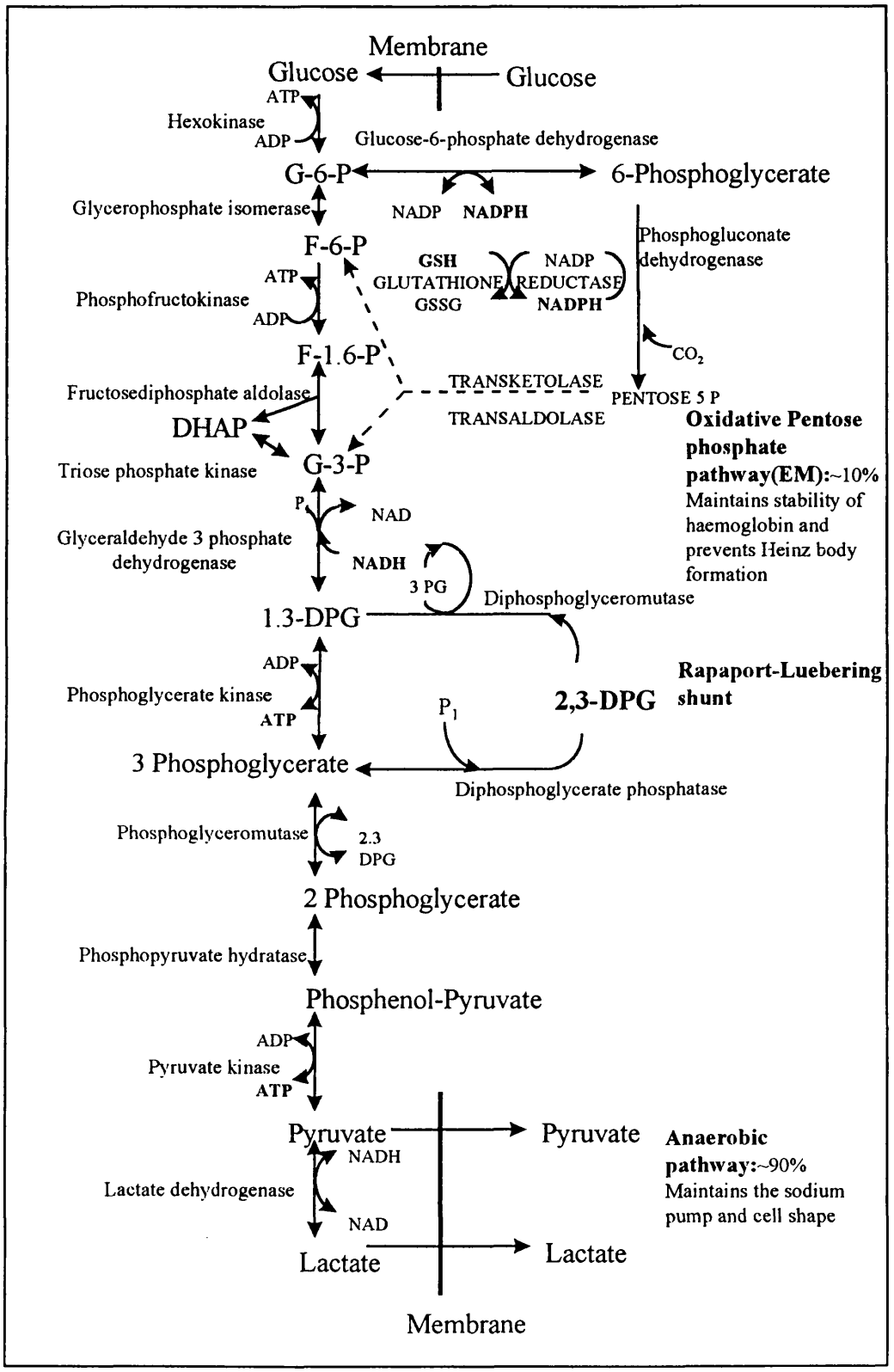

Figure 7: The anaerobic and pentose phosphate pathways of erythrocyte glucose catabolism. (Adapted from Haut and Litchman, 1980).

haemoglobin (iron in ferrous form) for oxygen transport. The reduced nicotinamide adenine dinucleotide phosphate (NADPH), formed in the pentose cycle is utilised for the conversion of oxidised glutathione (GSSG) to reduced glutathione (GSH) which is essential for the conversion of methaemoglobin to haemoglobin and for the binding of haemoglobin molecules to oxygen within the erythrocytes. It is also essential for the prevention of oxidative damage to the erythrocyte membrane. Any deficiency may result in Heinz body formation and methaemoglobinaemias (Schalm *et al.*, 1975b). A deficiency of any enzyme in the EM pathway leads to a decrease in ATP concentrations. The erythrocytes assume abnormal shape and or size and may be associated with haemolytic anaemias.

In dogs and cats, the sodium content within the cell is higher than the potassium because of a loss of membrane sodium-potassium ATP during the late stages of erythrocyte maturation, unlike in human beings where a reverse condition is observed (Jain, 1993b). The Shiba is the smallest breed of dog, indigenous to Japan. It has been reported that in some of these Japanese Shiba dogs the potassium content is higher than the sodium content. These dogs also have high reduced glutathione levels (GSH), increase in the osmotic fragility and an increase in the MCV. This characteristic finding is described to be an autosomal mode of inheritance (Maede *et al.*, 1990). Some Akitas too, have high erythrocyte potassium which results in spontaneous in vitro haemolysis producing pseudohyperkalaemia (Degen, 1987).

1.7 HAEMOGLOBIN SYNTHESIS

The oxygen carrying capacity of erythrocytes is associated with their haemoglobin content. With the help of its iron component, one haemoglobin molecule can combine with four molecules of oxygen. The compound 2,3 diphosphoglycerate which is formed in the Rapaport-Luebering shunt of anaerobic glycolysis is the potential regulator of energy and for the release of oxygen from this haemoglobin molecule. An increase in the levels of 2,3-diphosphoglycerate which may be observed in anaemia, may be caused by a change in the intracellular pH of erythrocytes, that results in a decrease in the oxygen affinity (Erslev, 1990a). The oxygen affinity of haemoglobin is expressed as P_{50} which is, the oxygen tension at which haemoglobin is half saturated. Haemoglobin is a conjugated protein made up of two pairs of globin chains and each chain associated with an identical iron containing porphyrin, or haem group, with amino acid residues arranged in the form of an α-helix. Each globin chain has eight helical areas numbered A to H and haem occupies the position between the E and F helices of the chain (Jain, 1993a). There are three types of haemoglobin, embryonic, foetal and adult. The concentrations of foetal haemoglobin is highest at birth and gradually diminishes within a few months after birth and is replaced by adult haemoglobin. In dogs, these prenatal haemoglobins cannot be differentiated from the adult haemoglobin (Schalm *et al.*, 1975b).

The synthesis of haemoglobin is under genetic control and occurs in the nucleated erythroid precursors and some amount takes place in the reticulocytes (Valli and Parry, 1993). Haem is synthesised in the mitochondria, by the combination of glycine and succinyl coenzyme A, leading to the formation delta amino levulinic acid (δ-ALA) which is dependent on the presence of pyridoxal phosphate of

pyridoxine (vitamin B-6) origin. The rate limiting enzyme is delta-amino levulinic acid (δ-ALA) synthetase. Subsequently, this results in the formation of a protoporphyrin III ring with an ferrous iron atom in the centre which combines with a globin chain to form the haemoglobin molecule. Globin synthesis occurs in the cytoplasmic ribosomes of nucleated erythrocytes. It consists of two alpha and two non alpha (beta, delta or gamma) protein chains. Differences in the amino acid sequences in these globin chains account for species variance as well as differences within each species in the haemoglobin molecule (Jain, 1993b).

Certain haemoglobinopathies such as thalassaemias and sickle cell disorders are observed in human patients and have not been observed in dogs (Weiser, 1995; Squires,1993). The thalassaemias are a heterogenous group of disorders which are characterised by various genetically determined defects of globin chain synthesis and are classified into α and β types in whichever chain the defect is observed. Sickle cell disease is associated with the presence of sickle cell S haemoglobin which is genetically determined. In anoxia erythrocytes assume a bizarre shape of a sickle hence the name (Cotran *et al.*, 1994a).

The incorporation of iron is a key component of erythropoiesis for haemoglobin synthesis. Iron is absorbed by the gut mucosa from the diet. The intestinal mucosal cells absorb ferrous iron more easily than the ferric iron. It is transported by the protein transferrin, to the receptors on the erythroid precursors (Adamson, 1994). Iron metabolism is discussed in the following section.

1.8 ERYTHROCYTE DESTRUCTION

Erythrocytes have a limited life span and are removed from the circulation after 110 or 120 days as a physiological process balanced by a continuous renewal from the bone marrow (Schalm, 1975a). Senescence of erythrocytes is brought about by the exhausation of their enzyme systems resulting in spherocyte formation and consequently removal from the circulation by the splenic macrophages. Shortening of the life span of erythrocytes may be due to abnormalities in the cell shape, defects and oxidation of the membranes, trauma, attachment of immunoglobulins as in auto-immune haemolytic anaemia (AIHA), or excess loss in haemorrhagic conditions (Cotran *et al.*, 1994a). In haemolytic anaemias, erythrocytes are destroyed either intravascularly or extravascularly. Intravascularly, they lose their cell membranes and enzyme systems whereas extravascular destruction involves phagocytosis by the macrophages of the mononuclear phagocytic system (MNPS) mainly in the spleen, liver and bone marrow. Intravascular lysis involves the release of free haemoglobin into the plasma imparting a pink colour to it. The haem component of haemoglobin becomes bound to haptoglobin, a plasma protein, and it is then phagocytosed by cells of the MNPS. Excessive destruction of erythrocytes may result in surplus degradation of haemoglobin to dimers or monomers leading to saturation of haptoglobins and haemopexin leading in turn to the excretion of haemoglobin through kidneys, resulting in haemoglobinuria and in chronic conditions haemosiderinuria. Some part of haemoglobin may be oxidised to methaemoglobin, which dissociates and liberates haematin (Weiser, 1995; Jain, 1993b).

Extravascular haemolysis involves the process where the macrophages engulf the erythrocytes resulting in the release of free iron, as haemoglobin is degraded into its essential constituents. Free iron released from haem is carried as a ferric form in

the plasma to the early erythroid precursors in the bone marrow. This process is achieved by a highly specialised beta globulin protein called transferrin, which is produced by the liver (Valli and Parry, 1993). Iron must exist in the ferric state to bind to transferrin. Ceruloplasmin a copper-containing globulin, facilitates iron transport and converts ferrous to ferric iron.

Iron is present in a variety of compounds and compartments within the body and is referred as iron pools. In the adult animal 75 percent of the iron is in haemoglobin and there are no sufficient reserves if the loss exceeds more than 30 percent. The reticuloendothelial system stores some amount of iron as ferritin or haemosiderin. Ferritin is an iron-protein complex occuring in most cells and plasma, consisting of apoferritin protein subunits and iron. The serum ferritin levels are an accurate measure of body iron stores (Smith, 1992). Haemosiderin the other form of stored iron is most stable but least available. (Valli and Parry, 1993).

Bilirubin is produced in the phagocytic cells of the MNPS by degradation of haem pigment. The biliverdin that is first formed is converted to bilirubin which combines with albumin to be transported to the hepatic cells. Here a portion of this bilirubin is conjugated with glucoronide and excreted to the bile. Most of it undergoes enterohepatic circulation while the remaining is stored as bile. Changes in the amount of bilirubin are proportional to the degree of haem degradation (Cotran *et al.*, 1994a). In severe haemolytic conditions bilirubin production may exceed the conjugating capacity of the liver. This may lead to the development of jaundice. Haemolytic jaundice is associated with high levels of unconjugated (indirect) bilirubin in the plasma as opposed to the high levels of conjugated (direct) bilirubin found in posthepatic jaundice.

1.9 THE FUNCTIONS OF THE SPLEEN AND ITS ROLE IN HAEMOPOIESIS

The spleen is the largest unit of the lymphoreticular mononuclear phagocytic system and is closely related to the haemopoietic system since it undergoes changes in a number blood diseases. It is a dumbbell-shaped flat organ, covered by a fibromuscular capsule, situated in the left anterior quadrant of the abdomen in a dorsoventral position in canines (Couto and Hammer, 1995). The architecture and position of the spleen makes it a multi-functional organ. It is positioned in the main circulation and performs a major role of a filter, entrapping pathogens present in the blood stream.

The spleen can also be viewed as a peripheral lymphoid organ because it has the presence of a white pulp, which consists of periarterial lymphatic sheaths contributing to lymphocyte production particularly after antigenic stimulation. The red pulp of the canine spleen has well developed venous sinusoids and has an effective "pitting" function as opposed to the spleen of cats which has poorly developed sinusoids and poor pitting functions. Thus, EMH is observed more often in dogs than in cats (Couto and Hammer, 1995).

The red pulp functions as a cleansing department of the erythrocytes by its "pitting" function. This pitting function is the ability of the splenic macrophages to remove a solid particle from the cytoplasm of the erythrocyte without destroying the cell itself (Crosby, 1959). Erythrocytes are highly deformable and during their passage through the spleen erythrocytes traverse a network of sinusoids present in canines and which are absent in felines. Erythrocytes lose inclusion bodies during their passage from the splenic chords, into the splenic sinuses by squeezing through the spaces between the cytoplasmic processes of the endothelial cells that form the sinus wall

(Crosby, 1977). The splenic macrophages remove abnormal structures such as the intraerythrocytic inclusions, the Howell-Jolly bodies and Heinz bodies, parasites and antibodies attached to the cell surface without damaging the morphology of rest of the erythrocyte (Crosby, 1959).

From a haematological point of view, the red pulp of the spleen is most significant. It is the site where maturation of the reticulocytes occurs and the effect of the spleen on the red cell surface may influence the time when reticulocytes enter the circulation (Crosby, 1959). In pathological states, remodelling of the erythrocytes coated with immunoglobulins takes place by the splenic macrophages which either phagocytose the erythrocytes or remodel the erythrocyte membrane surface eventually resulting in the releasing of erythrocytes as spherocytes into the circulation (Couto and Hammer 1995; Crosby, 1959). In addition, the spleen retains its ability to initiate EMH in most adult animals especially in dogs (Couto and Hammer, 1995).

Platelets are also stored in the spleen and approximately 30 percent of the total platelet mass is in the spleen at any given time, where they circulate slowly. As a result of this feature, splenectomy in pathological conditions may result in an elevation of the platelet counts (Couto and Hammer, 1995). The spleen also regulates the kinetics of platelets and contributes to the development of anaemias caused by extravascular lysis. It is also involved in iron metabolism and immunological functions. The sinusoids are lined by splenic macrophages which have among others, receptors for both the Fc portion of immunoglobulins and C3b.

The erythropoietic bone marrow is most efficient in producing erythrocytes which are all similar in shape and size. However, sometimes it may produce mishapen cells which are removed by the "culling" function of the spleen. This function of the spleen enables it to scrutinise every cell before it enters the general circulation and the

abnormal cells are removed (Crosby, 1959). The red pulp of the spleen is the "graveyard" of senescent erythrocytes which due to the exhaustion of their enzyme systems lose their biconcave shape and may become spheroid. This is more pronounced in human beings with hereditary spherocytosis. The red pulp also functions as a filter for not only the spherocytes but also every abnormally shaped erythrocyte such as acanthocytes which get entrapped by the sinusoids (Couto and Hammer, 1995).

In animals the spleen has a great capacity to store blood which can be released when required by splenic capsular contractions. The spleen of a sleeping dog can sequester a third of the red cells and when awake the spleen injects them all back into the circulation. The human spleen however, does not perform this function (Crosby, 1977). Sometimes larger volumes of blood may suddenly be expelled into the circulation causing polycythaemia. Alternatively, splenomegaly which may be due to inflammatory, congestive and infiltrative causes, results in hypersplenism due to increase in the splenic red cell volume concentrating in the splenic red pulp which is usually associated with cytopenias. This is observed to a greater extent in animals than in human patients (Couto and Hammer, 1995; Crosby, 1959). When splenectomy is considered for patients with cytopenias the bone marrow should be always evaluated since the spleen may take on the function of haemopoiesis as EMH.

CHAPTER 2

PATHOLOGICAL CONDITIONS

OF

THE ERYTHROPOIETIC SYSTEM

AND

THE CLASSIFICATION OF ANAEMIA

2.1 INTRODUCTION

The function of the whole erythropoietic system is regulated and maintained in a steady physiological state. A number of pathological changes may disturb this steady state accompanied by physiological adjustments that eventually result in affecting the whole erythropoietic system at different rates and stages of development. Erythrocytes may be affected at their site of production which may result in either hyperplasia, neoplasia, hypoplasia or aplasia.

The number of erythrocytes can be affected by a variety of factors such as increased or decreased amounts or lack of Epo. Inherited defects in haemoglobin synthesis result in the haemoglobinopathies of human beings such as thalassaemias (α and ß) and sickle cell anaemia. Enzymes are essential for the metabolism of erythrocytes and defects in these enzyme systems such as pyruvate kinase, glucose-6-phosphate dehydrogenase and phosphofructokinase deficiencies may occur, causing shortening of the life span of erythrocytes. There are also hereditary membrane defects of erythrocytes, such as hereditary elliptocytosis or spherocytosis or hereditary stomatocytosis which shorten their life span. Erythrocytes also require essential nutrients for their metabolism such as vitamin B-12 and folate, and deficiencies of both causing ineffective erythropoiesis have been recognised more in human patients.

The number of conditions affecting the erythrocytes are vast but eventually all result in either hyperplasia or neoplasia or anaemia. Anaemia is the consequence of a large number of conditions causing a reduction in the number of erythrocytes and it is discussed further in detail (Section 2.5 of this chapter).

2.2 HYPERPLASIA OF THE ERYTHROPOIETIC SYSTEM

An increase in the red cell mass occurs as a result of a primary increase in the production of the red cells or as a compensation for impaired delivery of oxygen to the tissues. This group of disorders is called "polycythaemia"; it is described as an increase in the red cell count, haemoglobin concentration and PCV (Chanarin, 1992). Polycythaemia has been classified as relative or absolute polycythaemia.

Relative polycythaemia develops secondary to disturbances which cause a decrease in the plasma volume, resulting in increase in the PCV and haemoglobin concentration whilst, the RBC mass (i.e. within the bone marrow) remains normal. This has been observed in animals usually as a result of dehydration associated with vomiting, diarrhoea high fever or excessive burns (Peterson and Randolf, 1983). Absolute polycythaemia can be either primary or secondary. Primary polycythaemia or polycythaemia rubra vera is a chronic myeloproliferative disorder which has been reported in man, it is rare in dogs and cats (Chanarin, 1992; Peterson and Randolf, 1983; Foster and Lothrop, 1988).

Secondary polycythaemia is the result of excessive production of Epo leading to increased erythropoiesis. This polycythaemia may be appropriate due to the Epo response to tissue hypoxia at high altitudes, cardiac disease, pulmonary disease or haemoglobinopathies. The inappropriate secondary polycythaemia is caused by excessive production of Epo without tissue hypoxia. In dogs there have been reports of this condition which exclusively is caused by renal neoplasms and renal cysts (Waters and Prueter, 1988).

The accurate diagnosis and differentiation of primary and secondary polycythaemia depends on the measurement of the levels of Epo in the serum and

urine. The level of Epo is elevated in secondary polycythaemia unlike primary polycythaemia where the levels are undetectable (Peterson and Randolph, 1983). High oxygen affinity haemoglobins, familial polycythaemia and increased carboxyhaemoglobin levels are other causes of absolute secondary polycythaemias which have been described in human patients (Chanarin, 1992).

2.3 NEOPLASIA OF THE ERYTHROPOIETIC SYSTEM

Haemopoietic neoplasms are classified as myeloproliferative or lymphoproliferative disorders depending on the cell types involved. Leukaemia is defined as a neoplastic proliferation of haemopoietic cells affecting the bone marrow and the blood. The two main large groups of leukaemias are the myeloid and lymphoid leukaemias. Leukaemias can be further classified and identified on the basis of the maturity of cells and on the course of the disease as acute or chronic leukaemias. A variety of diagnostic tools such as light and electron microscopy, cytochemical staining, immunophenotyping or molecular analysis are used in human medicine. However, standard methods of identifying cell morphology and cytochemical staining are used mainly in animals (Raskin, 1996; Cotran *et al,* 1994b).

In acute leukaemias the predominant cells are blasts or other immature cell forms, while in chronic leukaemias the cell types involved are mainly well-differentiated mature cells. Acute leukaemias usually have a short course of disease and cause severe cytopenias. Chronic leukaemias have a more protracted course of disease and may or may not cause cytopenias. Myeloproliferative disorders originate from neoplastic haemopoietic (myeloid) stem cell clones which are capable of differentiating into erythroid, granulocytic, monocytic and megakaryocytic cell

lineages. Myeloproliferative disorders result from the abnormal proliferation of one, several or all of the above cell lineages and include the acute myeloid leukaemia, the chronic myeloproliferative and dysplastic conditions (Raskin, 1996; Cotran *et al.*, 1994b; Moulton and Harvey, 1990).

According to the FAB (French, American, British) classification in human patients acute myeloid leukaemia comprises of eight classes. This scheme of classification considers the degree of maturation (M0 to M3) and the predominant line of differentiation of the leukaemic stem cells (M4 to M7) which are illustrated in Table 1 (Raskin, 1996; Cotran *et al.*, 1994b). In the dog the causes of these leukaemias still remain obscure. There has been a report by Jain (1993f), in which 24 beagles were experimentally exposed to ^{60}Co-γ-irradiation and 5 of these dogs developed erythroleukaemia. In cats, FeLV seems to be the cause of all haemopoietic neoplasms depending on the subgroup and the point of integration of the viral genome (Jain, 1993f; Evans and Gorman, 1987; Grindem *et al.*, 1985). In man, the causes of myeloproliferative disorders may range from chemicals to radiation. It has also been established that chromosomal translocations are involved (Cotran *et al.*, 1994b).

The erythropoietic system may undergo neoplastic changes resulting in erythroleukaemia which is according to the FAB classification (M6) a rare subgroup of the acute myeloid leukaemias. The FAB classification has been used in animals with certain modifications. A classification for acute myeloid leukaemias in dogs and cats, was proposed by Jain *et al.*, (1991) using certain criteria's from the FAB classification. In human patients, erythroleukaemia was first described by, and was named after DiGuglielmo in 1917 (Moulton and Harvey, 1990). Erythroleukaemia is rare in dogs, although there have been reports of affected cats (Jain, 1993f; Grindem *et al.*, 1985).

Table 1: Classification of Acute Myeloproliferative Disorders

Class	Acute Myeloid Leukaemia (AML)	Predominant Cell Type in the Bone Marrow
M0	Minimally or poorly differentiated AML	Blasts cells non-reactive for cytochemical markers
M1	AML without maturation	Blast cells >90% of NEC and PO or SB positive blast cells >3%
M2	AML with maturation	Myeloblasts >30% to <90% of NEC, differentiated granulocytes >10%
M3	Promyelocytic leukaemia	Blasts cells >30% of NEC, predominantly hypergranular, promyelocytes
M4	Myelomonocytic leukaemia	Myeloblasts and differentiated granulocytes >20%, monocytic cells >20% of NEC
M5	Monocytic leukaemia	Monocytic cells >80% of all NEC
M6	Erythroleukaemia	Erythroid cells >50% of ANC, myeloblasts and monoblasts <30% of NEC
M6-Er	Erythraemic myelosis	Erythroid cells >50% of ANC, myeloblasts and monoblasts and proerythroblasts >30% of ANC
M7	Megakaryocytic leukaemia	Megakaryoblasts >30% of NEC

(Adapted from Cotran *et al.*, 1994b and Jain *et al.*, 1991)

Abbreviations:

NEC=non-erythroid component

ANC=all nucleated cells

SB=Sudan Black stain

PO=Peroxidase stain

Erythraemic myelosis (M6-Er), has been described by Jain *et al.*, (1991), and in this condition erythroid precursors are more than 50 percent of all nucleated cells (ANC), myeloblasts are rare, and the total blast cell counts including proerythroblasts, is more than 30 percent of ANC. As this condition progresses, the red cell precursors become bizarre and multinucleated with increase in the number of dysplastic blast cells and eventually it develops into erythroleukaemia which may terminate as myeloblastic leukaemia.

Erythroleukaemia (M6), is the abnormal proliferation of both the erythroid and granulocytic cell lineages. In erythroleukaemia, the erythroid component of the bone marrow is more than 50 percent and the blast cells (myeloblasts and monoblasts) are less than 30 percent of all the nucleated cells (Table 1). It can also be characterised by the presence proerythroblasts of more than 30 percent of ANC. A designation of "M6-Er" may be used for the latter condition with predominance of proerythroblasts (Jain *et al.*, 1991).

A myelodysplastic syndrome involving the erythroid component (MDS-Er) has also been described by Jain *et al.*, (1991), characterised by more than 50 percent erythroid cells of ANC and less than 30 percent of blast cells including proerythroblasts of ANC.

Polycythaemia rubra vera is a subgroup of the chronic myeloproliferative disorder (Table 2). It is due to excessive proliferation at the stem cell levels of the cells of the myeloid series, with the predominance of the erythroid precursors resulting in excessive red cell production (Chanarin, 1992; Cotran *et al.*, 1994b). There is marked increase in the blood volume and viscosity in affected patients due to the high red cell numbers (Chanarin, 1992). On haematological examination, there is an increase in the haemoglobin and PCV values. The number of granulocytes and

Table 2: Classification of Chronic Myeloproliferative Disorders

Chronic Myeloproliferative Disorders	**Predominant Cell Type in the Blood**
Chronic granulocytic leukaemia	Mature neutrophils
Chronic eosinophilic leukaemia	Mature eosinophils
Chronic basophilic leukaemia	Mature basophils
Polycythaemia rubra vera	Mature erythrocytes
Essential thrombocythaemia	Platelets
Myelofibrosis	Cells of extra medullary haemopoiesis
Myelodysplastic syndrome (MDS)	Cytopenias
Myelodysplastic syndrome erythroid (MDS-Er)	Cytopenias

(Adapted from Cotran *et al.*, 1994b, and Jain *et al.*, 1991).

platelets may also be increased reflecting the fact that this is a stem cell disorder. Bone marrow examination reveals a markedly cellular marrow showing erythroid hyperplasia and replacement of the fatty marrow. As the disease progresses myeloid metaplasia and myelofibrosis may develop in man (Cotran *et al.*, 1994b).

2.4 ANAEMIA

Anaemia is defined as a reduction in the concentration of the erythrocyte number or the haemoglobin concentration or both (Wintrobe *et al.*, 1974). It is an important and frequently encountered haematological abnormality in clinical practice. Anaemia is rarely a primary disease and is usually a result of a generalised disease process (Schalm *et al.*, 1975c; Weiser, 1981; Squires, 1993). All anaemias are caused either by abnormal loss or destruction of erythrocytes and failure of normal erythropoiesis to compensate for it or by a decreased erythropoiesis which fails to replace the normal daily one percent loss of red blood cells.

On the basis of red cell mass, anaemia and polycythaemia can be described as relative or absolute (Erslev, 1990a). Relative anaemia is observed when the red cell mass is normal and it is usually not a haematological disorder but a disturbance in the regulation of the plasma volume or a physiological expansion of the plasma volume in neonates and in pregnancy (Erslev, 1990a; Jain, 1993c). Whereas an absolute anaemia is mainly a decrease in the amount of circulating red cell mass which is observed more often and is clinically more significant (Jain, 1993c). This type of anaemia has been studied in great detail by haematologists and there have been different approaches in identifying and classifying it. Human and veterinary literature seem to have similar

ways of classifying anaemia, although there are some variations in the types of anaemia observed in animals and in man.

The classification of anaemia is based either on the morphology of the red cells or on the activity of the bone marrow or on the aetiology involved. However, an overall consideration of all these classifications is essential in the evaluation of a particular case of anaemia.

2.5 CLASSIFICATION OF ANAEMIA

2.5.1 CLASSIFICATION BASED ON ERYTHROCYTE MORPHOLOGY

The mean cell volume (MCV), the mean cell haemoglobin (MCH) and the mean cell haemoglobin concentration (MCHC) have been referred to as "absolute" values. These values, calculated from the results of the red cell count, haemoglobin content and the packed cell volume (PCV), have been widely used in the classification of anaemia (Dacie and Lewis, 1991a).

In the dog, the normal range of the mean cell volume (MCV) is 60-77 fl, the mean cell haemoglobin concentration (MCHC) is 32-36 g/dl and the mean cell haemoglobin is 19.5-24.5 pg (Schalm *et al.*, 1975a). In certain disease conditions there may be a disproportionate variation in these values resulting in a variety of morphological changes. The MCV may be within, above or below the normal range and accordingly an anaemia can be described as normocytic, macrocytic or microcytic. The haemoglobin content of the erythrocytes can be expressed as the mean cell haemoglobin concentration (MCHC), which is the average concentration of

haemoglobin in red cells and is independent of cell size. This value measures the ratio of weight of the haemoglobin to the volume of red cells in grams per decilitre. It can also be expressed as the mean cell haemoglobin (MCH) which reflects the weight of haemoglobin in picograms (pg) in a red cell and this value is dependent on the cell size (Dacie and Lewis, 1991a).

If the MCH and the MCHC are within the normal range the anaemia is normochromic, if they are below the normal range the anaemia is described as hypochromic. However, a low MCH may also indicate microcytosis. A high MCH is always associated with a high MCV reflecting the presence of macrocytosis (Dacie and Lewis, 1991a). A high MCHC is seen only if prominent spherocytosis is present which reflects the increased haemoglobin volume associated with decreased cell membrane and size (Feldman, 1983).

With the use of electronic cell counters a graphical representation of the volumes of erythrocytes can be obtained. The red cell distribution width (RDW) which is a histogram of the distribution of the volumes of erythrocytes is a coefficient of the variation of erythrocytes and quantifies the degree of anisocytosis. High values would indicate a heterogeneous population and low values indicate a homogenous population. It also helps in quantifying the mixed population of cells that is the microcytes as well as the macrocytes. Therefore, it is essential to consider these features because if only the MCV is considered these mixed population of cells may be overlooked (Stone and Freden, 1990). In a study by Weiser and O'Grady (1983), RDW was very useful in accurately diagnosing microcytic-hypochromic anaemias.

In 1934, Wintrobe classified anaemias morphologically based on the changes in the absolute values. These changes were described as associated with different pathological disturbances in human beings, which were found to be useful in

identifying the cause and deciding the type of therapy. Also, according to Erslev (1990a), morphological classification enables the clinician to consider certain types of anaemias such as iron deficiency, vitamin B12 and folic acid deficiency anaemias in human patients. However, Jain (1993c), has described this classification as non-specific and is useful only when other ways of classifications are also considered because morphological changes are slow and often do not indicate an early bone marrow response to the anaemia. Feldman (1983), too describes this classification as inadequate particularly when a mixed population of red cells with poikilocytosis or a moderate degree of anisocytosis is present.

Morphology of erythrocytes

On the basis of the absolute normal values (Appendix A: Table A1), anaemias can be morphologically classified as follows and as summarised in Table 3.

Normocytic-normochromic anaemia

This is the commonest type of anaemia found in man and animals. This morphology is observed immediately after an acute blood loss. It is also observed when erythropoiesis is suppressed which may be either due to chronic diseases such as malignancies, infections and CRF causing mild anaemias or due to bone marrow hypoplasia or aplasia causing more severe anaemias (King *et al.*, 1992; Dacie and Lewis, 1991b; Madewell and Feldman, 1980, Cartwright, 1966)

Normocytic-hypochromic anaemia

This is an occasional finding in chronic diseases such as chronic infections, cancers and arthritis. Fractures or soft tissue injuries too may cause such a morphology (Cartwright, 1966). Also, chronic blood loss by a bleeding tumour may result in this type of anaemia. Depending on the severity and duration of the blood loss, these conditions may progress to a microcytic-hypochromic anaemia (Madewell and Feldman, 1980).

Macrocytic-normochromic anaemia

This morphology is often seen in man associated with megaloblastic anaemias caused by Vitamin B12 or folic acid deficiency (Cotran *et al.*, 1994a). It is also observed in regenerative anaemias.

Macrocytic-hypochromic anaemia

This type of anaemia is seen in remission from an acute blood loss and particularly in acute haemolytic anaemia with marked reticulocytosis which increases the MCV and decreases the MCHC. The degree of reticulocytosis depends on the severity of anaemia and the bone marrow response (Schalm *et al.*, 1975c).

Microcytic-hypochromic anaemia

This type of morphology is seen in iron deficiency anaemia which may be nutritional or associated with chronic blood loss. Microcytic-hypochromic anaemia is frequently seen in dogs due to chronic blood loss caused by intestinal neoplasms, ulcerative colitis, intestinal parasitism, severe flea and lice infestations (Madewell and Feldman, 1980; Schalm *et al.*, 1975c). Nutritional deficiency of vitamin B6 or copper

Table 3: Classification of Anaemia Based on Erythrocyte Morphology.

Erythrocyte Morphology	MCV (fl)	MCHC (g/dl)
Normocytic normochromic	60 - 70 fl	32 - 36 g/dl
Normocytic hypochromic	60 - 70 fl	less than 32 g/dl
Macrocytic normochromic	more than 60 fl	32 - 36 g/dl
Macrocytic hypochromic	more than 60 fl	less than 36 g/dl
Microcytic hypochromic	less than 60 fl	less than 32 g/dl

Table 4 Some Commonly Observed Morphological Variations in Canine Erythrocytes and Associated Disease (Adapted from Jain, 1993b; Wintrobe *et al.*, 1974)

Terminology	Associated Disease Conditions
Acanthocytes (Spur cells)	Increase in cholesterol phospholipid ratio of the cell membrane, diffuse liver disease, haemangiosarcoma, portocaval shunts and high cholesterol diets
Codocyte (Target cells)	Liver disease with cholestasis, iron deficiency anaemia, postsplenectomy and portocaval shunts
Dacrocytes	Myeloproliferative disorders, hypersplenism
Echinocytes (Burr cells or crenated cells)	Artifactual, uraemia, glomerulonephritis and neoplasia
Macrocytes	Increased erythropoiesis and megaloblastic anaemia
Microcytes	Iron deficiency anaemia, anaemia of chronic disease and portosystemic shunts
Schistocytes (Helmet or fragmented cells)	Microangiopathic haemolytic anaemia, DIC, haemangiosarcoma, neoplasms, hypersplenism and myelofibrosis
Spherocytes (Microspherocytes)	Immune-mediated haemolytic anaemia, post-transfusion and Heinz body anaemia

may also produce this type of anaemia but this is not often seen in dogs (Cotran *et al.*, 1994a; Aufderheide, 1981). Because iron is mainly absorbed from the upper parts of small intestine, malabsorption of iron may occur in some intestinal disease; this is a rare cause in animals but it may require parenteral iron therapy.

In addition to the above changes in the absolute values affecting the erythrocyte morphology, certain disease conditions also affect the erythrocyte causing some typical morphological changes which are listed in Table 4.

2.5.2 CLASSIFICATION BASED ON BONE MARROW RESPONSE

Morphological classification is helpful but limited especially in the case of a normocytic-normochromic anaemia, since the latter has been observed to be associated with a number of disease conditions. Furthermore, morphological classification is also limited when the degree of anaemia is only slight; alterations in the size and haemoglobin content may not be pronounced making it difficult to classify the anaemia (Wintrobe, 1934). Hence, the study of the pathophysiological and the bone marrow response to anaemia are better ways of classifying anaemia.

The erythroid marrow responds to changes in the levels of erythropoietin by altering the rate of formation of erythrocytes. The production and loss of erythrocytes in the circulation is maintained in an equilibrium and anaemia may result when there is an alteration in any of these factors. Wintrobe *et al.*, (1974), have classified anaemias on the basis of erythrokinetics which can result in effective or insufficient erythropoiesis. Effective erythropoiesis is associated with reticulocytosis whereas, if the reticulocyte count is low, erythropoiesis is presumed to be impaired either due to

insufficient or ineffective erythropoiesis. Insufficient erythropoiesis as a result of lack of sufficient erythroid precursors can be caused either by infiltration by tumour cells or aplasia of the bone marrow. Ineffective erythropoiesis refers to the production of defective erythrocytes that are destroyed before leaving the marrow or shortly thereafter. Anaemias of this type are the thalassaemias, sideroblastic and megaloblastic anaemias that occur mainly in man.

Based on the bone marrow response anaemia has been classified as regenerative (responsive) or non-regenerative (non-responsive) by Jain (1993c), Squires (1993), and many other haematologists. The reticulocyte count is a simple and practical way to assess the marrow response to anaemia. In regenerative anaemias erythropoiesis is stimulated by an increase in Epo production. The greater the stimulation the greater is the reticulocytosis. Erythropoietin reduces the intermediate time for the developing normoblasts, increases the rate of haemoglobin synthesis per cell and produces an early release of reticulocytes from the bone marrow. The number of Howell-Jolly bodies may also increase. In non-regenerative (non-responsive) anaemias associated with renal diseases, there is a diminished Epo production resulting in an inadequate stimulus for erythroid precursors leading to anaemia. In other non-regenerative anaemias the pathogenesis is not Epo dependent.

2.5.3 CLASSIFICATION BASED ON AETIOLOGY

Classification of anaemias based on the aetiology involved seems to be the most satisfactory approach. According to Cotran *et al.*, (1994a), Jain (1993c), and Wintrobe *et al.*, (1974), this type of classification involves an understanding of the aetiology and pathogenesis of the disease process causing the anaemia. Human

literature has a broad approach considering all the possible erythrocyte disorders (Erslev, 1990a). Megaloblastic anaemias due to Vitamin B-12 and folate deficiency, the haemoglobinopathies such as haemoglobin structural deformities (sickle cell disease) and globin chain synthesis (thalassaemias) and other extrinsic and intrinsic factors affecting the red cell survival are discussed in great details many of which are not applicable to domestic animals especially in dogs (Kaneko, 1987).

In this study, anaemia in dogs was classified on the basis of the aetiology. Accordingly, anaemia was considered to be caused either by abnormal loss of erythrocytes due to haemorrhage and haemolysis or by decreased production of erythrocytes. The other important factor, the bone marrow response, was also considered in order to classify an anaemia as regenerative or non-regenerative. For this purpose the presence or absence of reticulocytes is established either by the reticulocyte count or by the microscopic examination of the blood smear. An illustration of the aetiological classification of anaemia is presented in Figure 8.

2.5.3.1 ANAEMIA OF ABNORMAL RED CELL LOSS

Haemorrhagic anaemia

Haemorrhage can occur from a number of diverse conditions depending on the cause, amount of blood loss and the tissues involved. Blood loss may be external in which blood is lost from the body or it may be internal in which the essential components of haemoglobin are available for re-utilisation (Valli and Parry, 1993). Haemorrhagic anaemia may occur either due to an acute or a chronic blood loss.

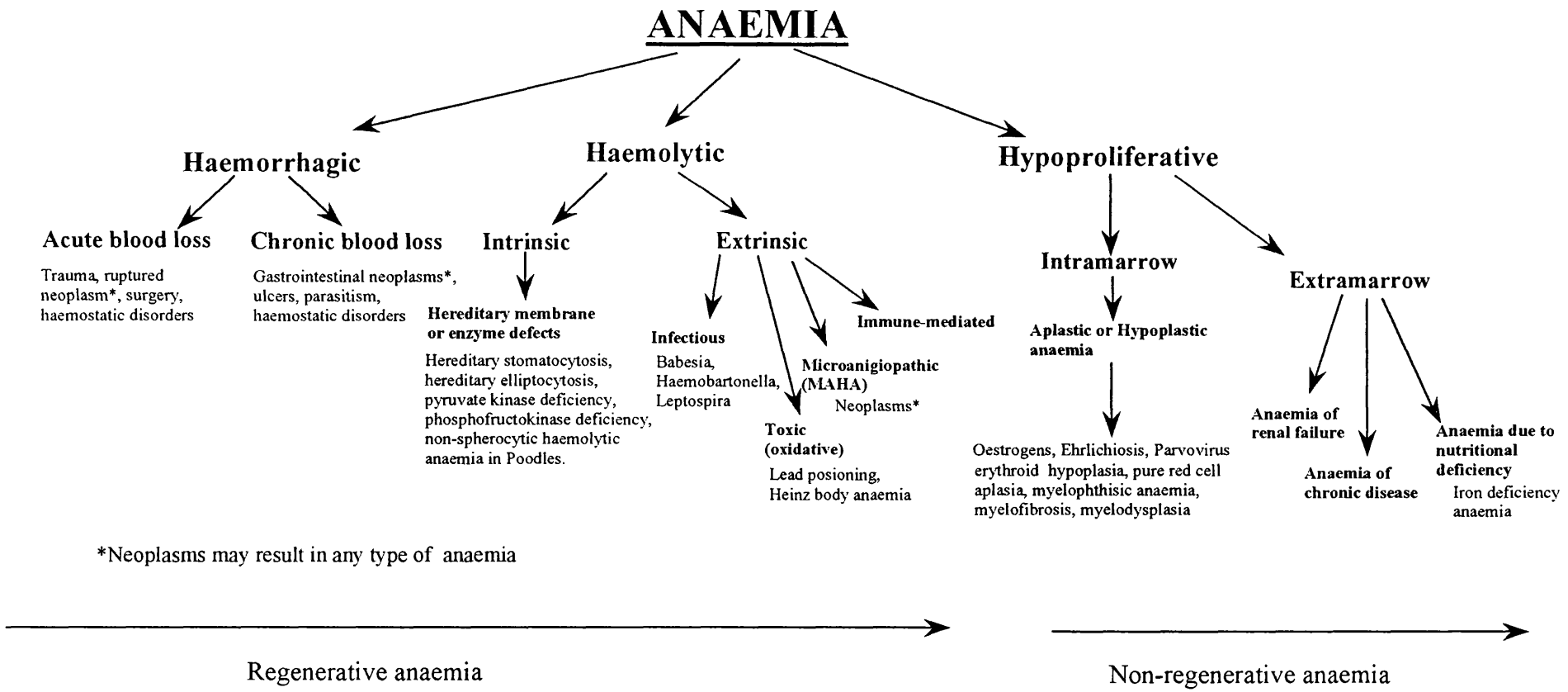

Figure 8: Classification of anaemia on the basis of aetiology.

(A) Acute blood loss anaemia

A balance of the total blood volume is maintained by the plasma volume and the circulating red cell mass. Severe blood loss can acutely decrease the blood volume to such an extent that it can result in cardiovascular collapse, irreversible shock and death. In this situation the loss of blood cells is not as important as the loss of total blood volume causing a sudden hypovolemic shock (Hillman, 1990). To prevent this shock, there is rapid replacement of plasma fluid from the interstitial compartments and intracellular compartments to the intravascular compartments. The mechanisms involved for restoring plasma volume operate slowly. The initial evaluation of the PCV does not reflect the magnitude of blood lost until three days, although anaemia will be evident within 24 hours (Feldman, 1981).

Acute blood loss is known to be observed in conditions that may be either focal such as acute trauma which may be surgical, gastrointestinal, genitourinary, wounds, ruptured spleen, ruptured neoplasm or systemic involving a significant amount of blood loss (Crystal and Cotter, 1992). Certain acquired bleeding tendencies of dogs may be related to thrombocytopenia or thrombocytopathy caused by immune-mediated thrombocytopenia or *Ehrlichia canis*, all which may lead to acute blood loss anaemia by epistaxis, malaena or haematuria (Carr and Johnson, 1994; Kuehn and Gaunt, 1985). Hereditary coagulopathies such as Factor VIII deficiency, von Willibrand's disease (vWD) may also result in blood loss. Certain poisons such as hydroxycoumarin (warfarin), indandione (pindone), and diphacinone can also cause severe bleeding (Feldman and Mount 1983; Crystal and Cotter 1992). Diphacinone is more potent than warfarin and poisoned dogs die of severe internal

haemorrhages due to increased capillary permeability and decreased plasma coagulability in addition to tissue hypoxia.

Disseminated intravascular coagulation (DIC), also called consumptive coagulopathy or defibrination syndrome, is due to excess coagulation and fibrinolysis; it may result in a haemorrhagic diathesis (Bell, 1994).

Dogs with neoplasms, especially with haemangiosarcomas of the spleen, usually die suddenly either due to the intra-abdominal rupture of the primary tumour resulting in acute blood loss anaemia or by metastasis of the tumour to vital organs which may cause bleeding (Brown *et al.*, 1985).

Haematological observations

There are no reserve pools of matured erythrocytes to replace the cells lost immediately after haemorrhage. The oxygen supply to the tissues is maintained by cardiovascular adjustments by arteriolar constriction in organs such as the skin and a decrease in vascular resistance to the vital organs such as the kidneys. Plasma levels of Epo increase within 6 hours after anaemia appears in a bled patient (Hillman, 1990). But the magnitude of increase in Epo levels depends on the severity of anaemia. The red cell precursors must first proliferate and mature over a period of two to five days prior to release into the circulation. Therefore, there is a considerable time lag in the response to an acute anaemia (Hillman, 1990).

Acute blood loss is not immediately associated with a reticulocytosis, because of the time that is required for Epo production and stimulation for an increase in the marrow proliferation. There is initially, a marked increase in the platelet number to shorten the coagulation time and hasten clot retraction. However, as fluid adjustments also take place, the PCV and haemoglobin levels start to fall within 12 to 24 hours, depending on the amount of blood lost. Erythrocytes are normocytic and

normochromic, with normal MCV, MCH and MCHC. The next response to an acute blood loss is Epo-related shift of marrow reticulocytes into the circulation which is seen by the appearance of polychromatophilic, macrocytic erythrocytes (reticulocytes) and by the next few days marked erythroid hyperplasia of the marrow is apparent (Hillman, 1990). Grossly, the marrow hyperplasia is evident by an expansion of the haemopoietic areas along the endosteal surfaces of the long bones and microscopically, by an increase in the proportion of haemopoietic cells to fat cells in the red marrow areas from the normal 50 percent to 100 percent (Valli and Parry, 1993).

Although the reticulocyte count may appear to increase soon after anaemia, the reticulocyte production does not increase for three to five days (Hillman, 1990). Some leucocytosis may also occur within a few hours of haemorrhage, due to redistribution of the leucocytes from the spleen, lungs and splanchnic vessels (marginal pool) and from the marrow reserves (Valli and Parry, 1993; Hillman, 1990). Thrombocytosis may also be present with larger platelets due to the mobilisation from the marginal pools (Hillman, 1990).

Subacute blood loss, that is after three days, may be associated with a slight polychromasia due to the presence of reticulocytes. After an increase in the number of reticulocytes, which indicates a release of young red cells from the bone marrow to compensate for the ongoing anaemia, a characteristic macrocytosis is observed (Cotran *et al.*, 1994a; Valli and Parry, 1993). This results in a high MCV, the MCHC is normal, therefore the anaemia is macrocytic and normochromic.

If there is still a severe anaemia, then EMH may be established which is characterised by the presence of normoblasts and erythroblasts in the peripheral circulation. A reticulocytosis which persists for longer than one or two weeks is

usually indicative of some ongoing blood loss which may progress into an anaemia of chronic blood loss (Valli and Parry, 1993).

(B) Chronic blood loss anaemia

Chronic haemorrhage is observed when the rate of blood loss exceeds the regenerative capacity of the bone marrow. This may occur either by an external blood loss due to parasitism such as hookworms, heavy flea and lice infestations or internally due to endoparasites, gastrointestinal ulcerations or gastrointestinal adenocarcinomas and other neoplasms (Harvey *et al.*, 1982; Cosenza, 1984; Madewell and Feldman, 1980; Curtis, 1977). In a study by Harvey *et al.*, (1982), from 25 dogs studied, there were 19 dogs with chronic blood loss anaemia, mainly due to external or internal parasites and hookworms were found most frequently in these dogs. Curtis (1977), had reported a dog with seizures and severe anaemia due to chronic blood loss, from *Ancylostomum caninum* and a flea infestation.

Hypoproteinaemia may occur in addition to chronic blood loss (Weiser and O'Grady 1983; Harvey *et al.*, 1982). In a study by Weiser and O'Grady (1983), there were four dogs of the 12 studied that developed hypoproteinaemia, as most other dogs were able to replace the lost plasma proteins. However, hypoproteinaemia may also occur due to secondary infections and may not be proportional to the amount of blood lost.

Haematological observations

Non-haem iron is stored primarily as haemosiderin and ferritin in the cells of the mononuclear phagocytic system particularly in the bone marrow. When an external blood loss occurs for a very long duration, then this iron store may be depleted and iron is needed for supplementation (Stone and Freden, 1990). In chronic

blood loss continuous loss of both blood and iron takes place, which at first is associated with polychromasia and anisocytosis of the red cells due to reticulocytosis (Adamson, 1980). The morphology is normocytic and normochromic at this stage.

If the blood loss persists for a longer time, then a few cells may become hypochromic as a result of greater external loss than uptake of iron, and if it still continues, then the red cells become microcytic and hypochromic and this morphology is typical of a chronic haemorrhagic anaemia (Meyer and Harvey, 1994; Stone and Freden, 1990). These features are illustrated in Figure 9. According to Griffiths *et al.*, (1981), dogs with portosystemic shunts may be presented with microcytic erythrocytes based on the MCV alone and hypochromasia may or may not be present indicating that iron deficiency is relative in these cases.

Although this occurrence was poorly understood, it was confirmed by Meyer and Harvey (1994). In their study, there were 60 percent dogs with microcytosis when compared with 72 percent of the previous study and the serum iron levels were within the low normal. These findings suggested that a microcytic-hypochromic anaemia may be seen in portosystemic shunts. Although the mechanisms causing hypochromasia was still unclear it appeared to be different from the mechanisms described in chronic disease.

Because of an extra cell division and poor haemoglobinisation, microcytic and hypochromic cells are produced and these poorly formed erythrocytes may break easily resulting in the formation of poikilocytes giving an overall picture of anisocytosis (Rogers, 1995; Schalm *et al.*, 1975c). In a study by Weiser and O'Grady (1983), schistocytes were present in most of the dogs with severe microcytosis which may have been due to the severity of the anaemia. Another feature seen in iron

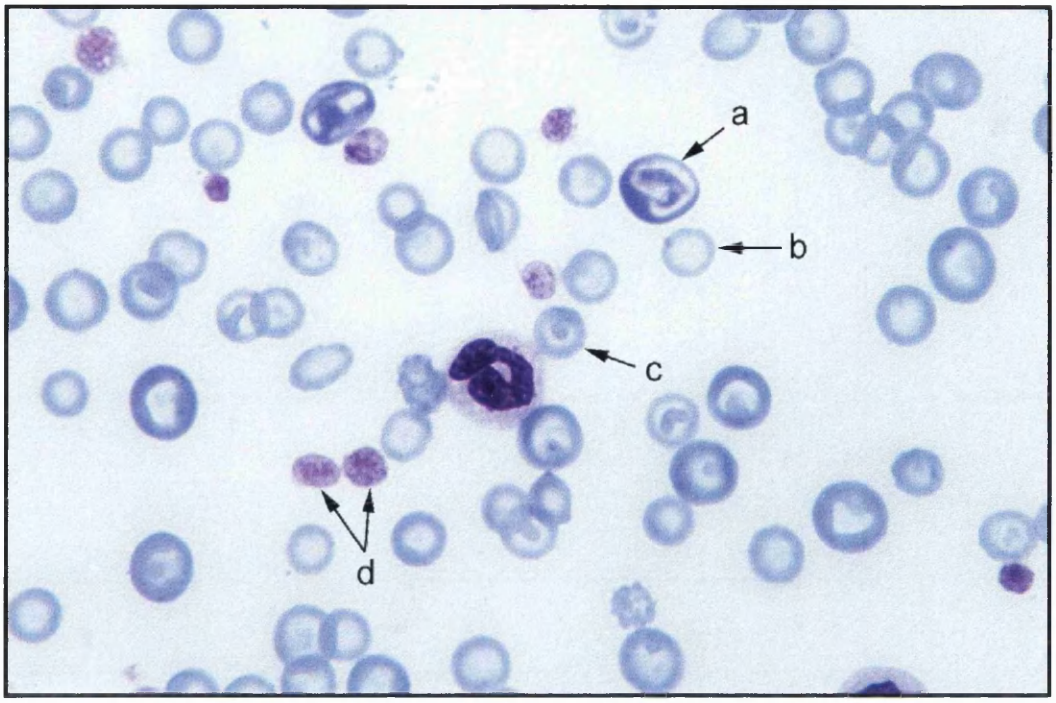

Figure 9: Blood smear of a dog with blood loss anaemia showing reticulocytes.

a: reticulocyte (polychromatic macrocyte)

b: microcytic hypochromic red cell

c: target cell

d: large platelets

Stained by May-Grünwald Giemsa (x 1000).

deficiency anaemia is the size of the reticulocytes. In their study, Weiser and O'Grady (1983), also observed that the size of the reticulocytes was smaller which may be a significant finding associated with iron deficiency anaemia.

In severe iron deficiency anaemia, as long as sufficient iron is not available, haemoglobin synthesis lags behind and the hypochromasia becomes more evident than the microcytosis and a non-regenerative anaemia may be seen in addition to a hypoproteinemia. In such cases, there may also be some amount of EMH present as the spleen is enlarged and has an increase in the sinus stroma (Valli and Parry, 1993).

2.5.3.2 ANAEMIAS OF INCREASED RED CELL DESTRUCTION

Haemolytic anaemia

In haemolytic anaemia, the red cells are destroyed prematurely and are not able to survive the normal 100-120 days. There are two main types of haemolytic anaemias which are distinct. In one, lysis of erythrocytes is the consequence of inherited intrinsic factors such as abnormalities of the cell membrane, deficiencies of the enzyme systems, abnormalities of the haemoglobin structure and globin chain synthesis. In the other, haemolysis is due to various acquired extrinsic factors such as infections, fragmentation, exposure to toxic-oxidative substances and to anti-red cell antibodies.

The main features of all haemolytic anaemias are the reduction in red blood cell survival time and the re-utilisation of iron which is not lost as in haemorrhagic anaemias. The shortened red cell survival time can be determined by radioactive sodium chromate labelling techniques (Dacie and Lewis, 1991c).

There are also a number of clinical and haematological findings which reflect, on one hand the increased rate of destruction and on the other, increased rate of production of red cells. Destruction of erythrocytes may be either intravascular which may lead to haemoglobinaemia and haemoglobinuria or extravascular where the red cells are phagocytosed mainly by the splenic macrophages, which may lead to splenomegaly (Cotran *et al.*, 1994a). Destruction of erythrocytes may result in the development of jaundice, reduced haptoglobin levels and occasionally haemoglobinuria. Hyperplasia is exhibited by an elevated reticulocyte count and normoblastic hyperplasia of the bone marrow. It is only in exceptionally rare cases that a low reticulocyte count is seen in uncomplicated haemolytic disease but this has, in general a poor prognosis (Jones and Gruffydd-Jones, 1991).

Haemolytic anaemias have an increased Epo stimulation of the bone marrow resulting in a shortened transit time of red cell precursors and the number of mitotic divisions is reduced, resulting in the release of rather large normoblasts and reticulocytes. The latter raises the MCV and marked anisocytosis and polychromasia is observed. Depending on the severity of the disease, the anaemia may be normochromic and normocytic or macrocytic and in some cases, hypochromic and macrocytic (Valli and Parry, 1993; Cotter, 1992).

(A) Intrinsic factors causing lysis of erythrocytes

Membrane and enzyme defects of erythrocytes

Since erythrocytes mainly depend on the anaerobic glycolysis for ATP, any deficiency of the enzymes involved in glycolysis may have significant effects on the function and survival of these cells.

Table 5: Intrinsic Conditions Associated with Haemolytic Anaemia in Dogs

Conditions	Breed Affected	Reference
Hereditary stomatocytosis	Alaskan Malamute, Miniature Schnauzer	Brown *et al.*, (1994); Pinkerton *et al.*, (1974)
Hereditary elliptocytosis	Cross-Bred dog	Smith *et al.*, (1983)
Pyruvate kinase deficiency	Basenji, Beagle, West Highland White Terrier, Cairn Terrier	Searcy *et al.*, (1971), Prasse *et al.*, (1975), Chapman and Giger (1990), Schaer *et al.*, (1992).
Phosphofructokinase deficiency	English Springer Spaniel	Giger and Harvey (1987), Harvey and Smith (1994).
Familial non-spherocytic anaemia	Standard Poodle	Randolph *et al.*, (1986)

The membrane and enzyme defects of erythrocytes are not commonly seen conditions in dogs and have been identified only in certain breed. These however, should be considered in the differential diagnosis of haemolytic anaemias. These conditions are similar to the ones seen in human beings. However, in animals due to the reduction in the breeding from the affected animals this type of anaemia is less frequently seen. Only some of these conditions are discussed as a part of this study. A summary of some of these conditions is shown in Table 5.

Hereditary stomatocytosis

Stomatocytes are erythrocytes with rectangular or elongated pale areas across their centre. Conditions similar to human hereditary stomatocytosis have been reported in Miniature Schnauzer and Alaskan Malamute breeds of dogs (Brown *et al.*, 1994; Fletch and Pinkerton, 1972). An autosomal-recessive mode of inheritance has also been reported in Miniature Schnauzers without clinical signs of the disease but with macrocytosis, high range of the PCV and slightly shortened erythrocyte osmotic fragility (Brown *et al.*, 1994). The condition recognised with haemolytic anaemia has been observed in chondro-dysplastic dwarf pure bred young Alaskan Malamute dogs and is thought to be caused by membrane changes of erythrocytes due to a pleiotropic effect of the dwarfism gene called *dan* (Fletch and Pinkerton, 1972).

A newly recognised disease has been reported by Slappendel *et al.*, (1994), called as familial stomatocytosis-hypertrophic gastritis (FS-HG). It is associated with a haemolytic anaemia, hereditary stomatocytosis and hypertrophic gastritis which seems to resemble Menetrier's disease in man. The erythrocytes also have an increased osmotic fragility and the condition may be as a result of an abnormal lipid metabolism.

Haematological observations

Dogs usually have a mild anaemia with stomatocytes, macrocytosis, low MCHC and increased MCV. The haematological findings are typical of a haemolytic anaemia. Mechanical and osmotic fragility is increased indicating an intrinsic red cell membrane abnormality. There is also a shortened erythrocyte life span with reticulocytosis and marrow hyperplasia with an increase in the iron turnover and reduced gluthatione levels in these dogs (Pinkerton *et al.*, 1974). However, according to Brown *et al.*, (1994), in the Miniature Schnauzer studied there was macrocytosis and a high PCV value with the absence of marked erythrocyte regeneration. These erythrocyte abnormalities were suggestive of defects in volume regulation rather than membrane defects which increases as the cells get older.

Hereditary elliptocytosis

This condition is an inherited autosomal recessive trait of an erythrocyte membrane abnormality due to a protein band 4.1 deficiency resulting in altered erythrocyte morphology, elliptocytosis, membrane fragmentation and poikilocytosis (Smith *et al.*, 1983). A cross bred dog was studied with this condition, although anaemia was not present, there was marked reticulocytosis and an increase in the osmotic fragility of red cells, indicating that some haemolytic process was taking place including a good bone marrow response (Smith *et al.*, 1983).

Pyruvate kinase deficiency

Pyruvate kinase enzyme is involved in the maintenance of the sodium pump and hence the cell size. This enzyme normally catalysis the conversion of phosphoenolpyruvate to pyruvate. When this reaction becomes rate limiting the

conversion of glucose to lactate in the EM pathway is impaired resulting in a deficiency of ATP and an increase in the glycolytic intermediates in the anaerobic pathway (Searcy *et al.*, 1979; Searcy *et al.*, 1971). This enzyme deficiency was first described in a young Basenji dog by Searcy *et al.*, (1971). Subsequently, it seemed to be a well recognised autosomal recessive inherited trait in very young Basenji, Beagle, West Highland White Terriers and quite recently, in Cairn Terriers (Searcy *et al.*, 1971; Prasse *et al.*, 1975; Chapman and Giger, 1990; Schaer *et al.*, 1992).

Deficiency of pyruvate kinase enzyme leads to an impaired metabolism of erythrocytes resulting in an decrease in the ATP formation and glucose utilisation leading to the formation of macrocytes. The deficiency of pyruvate kinase can be demonstrated by an increase in the M_2 type isoenzyme and a decrease or absence of the R-type isoenzyme normally found in mature erythrocytes with a simultaneous increase in the 2,3-DPG and phosphoenolpyruvate (Chapman and Giger, 1990).

Haematological observations

Affected dogs may or may not be anaemic but always have marked reticulocytosis. Reticulocytosis, anisocytosis, thrombocytopenia and erythroid hyperplasia were seen in affected dogs (Searcy *et al.*, 1971; Schaer *et al.*, 1992). Secondary myelofibrosis and osteosclerosis were also commonly seen in these dogs causing death before they were three years old. Much of the red cell destruction also takes place in the spleen and liver; splenomegaly may therefore be present in affected dogs (Chapman and Giger, 1990; Schaer *et al.*, 1992). Splenomegaly was seen due to EMH, which resolved after splenctomy and the thrombocytopenia improved. According to Schaer *et al.*, (1992), in the Cairn Terrier breed of dog, the haematological parameters remained stable after a splenectomy and the longevity of the dog with this enzyme deficiency may have been due to the absence of

myelofibrosis (Schaer *et al.*, 1992). However, the pathogenesis of myelofibrosis and osteosclerosis in all affected dogs still remains to be elucidated. Many of the features of PK deficiency in dogs are similar to man. However, the fibrosis and sclerosis within the marrow cavities, a constant feature in dogs, has not been seen in man and the pathogenesis seems uncertain (Searcy *et al.*, 1979)

Phosphofructokinase deficiency (PFK)

Erythrocytes lack mitochondria and obtain their energy mainly from anaerobic glycolysis. In the oxidative EM pathway, there is an alternative route via the Rapaport-Luebering shunt whereby, 2,3-DPG is formed which influences the haemoglobin oxygen affinity. In PFK deficiency there is a decrease in the 2,3-DPG concentrations resulting in an increase in the oxygen affinity to haemoglobin.

The deficiency of this enzyme has been reported in middle-aged English Springer Spaniels and was suggested to be due to an autosomal recessive mode of inheritance caused by compensated haemolytic disorder characterised by bilirubinuria and reticulocytosis despite normal PCV or mild anaemia. Sporadic intravascular haemolytic crisis may occur due to hyperventilation caused by stressful situations (Giger and Harvey, 1987). In such crisis, severe anaemia, haemoglobinuria and hyperkalaemia were also present. It has been shown that PFK deficient erythrocytes were more alkaline fragile than the normal cells which indicated a pH-dependent lysis. (Harvey and Smith, 1994).

Thus, phosphofructokinase deficiency should be suspected in English Springer Spaniels and Cocker Spaniels when presented with the above findings. Despite the severe haemolytic crisis, PFK deficient dogs have much better life expectancy than the

dogs with pyruvate kinase deficiency because no myelofibrosis occurs (Giger and Harvey, 1987).

Non-spherocytic haemolytic anaemia in Poodles

In standard Poodles a familial non-spherocytic haemolytic anaemia has been observed by Randolph *et al.*, (1986). It is usually seen in very young dogs and may be fatal as they attain three years of age. This disorder is characterised by a severe anaemia , with marked regeneration and hepatosplenomegaly. The dogs may be Coombs' positive and the red cell survival time may be reduced. In the dogs studied by Randolph *et al.*, (1986), all the dogs had pathological changes of haemosiderosis, myelofibrosis and osteosclerosis. According to the author, all the above changes in Poodles may be related to an intrinsic erythrocyte abnormality.

(B) Extrinsic factors causing lysis of erythrocytes

Infectious haemolytic anaemia

In dogs infectious causes of haemolytic anaemia occur mainly due to the blood parasites Babesia and Haemobartonella. Haemolysis may also be seen in canine Leptospirosis particularly, in *Leptospira icterrohaemorrhagicae* infections with haemoglobinuria, icterus, and a moderate to marked anaemia (Jain, 1993d; Valli and Parry, 1993).

Babesia

This is a tick borne, protozoal parasite that can affect vertebrate erythrocytes and cause severe intravascular haemolysis. All domestic species are susceptible to it

and young dogs are reported to be affected especially by *Babesia canis*. However, *Babesia gibsoni* infection in dogs is restricted more to the Asian countries and the parasitised red cells are removed by the spleen with no evidence of intravascular haemolysis (Valli and Parry, 1993). This intracellular erythrocytic piroplasm, once on the surface of the erythrocyte membrane, enters the cell by the disintegration of red cell membrane at the attached site eventually rupturing the erythrocytes and leading to haemoglobinuria.

Canine babesiosis can be subclinical, peracute, acute or chronic. Anorexia, depression, anaemia, pyrexia, splenomegaly and jaundice are some of the commonly observed clinical signs (Harvey *et al.*, 1988; Farwell *et al.*, 1982). Mildly affected animals develop anaemia and fever but they fail to show jaundice or haemoglobinuria and recover within a few days. Petechial or ecchymotic haemorrhages are observed in severely affected dogs with DIC or thrombocytopenia (Breitschwerdt, 1984).

Haematological observations

Haematological features may vary depending on the course of the disease. However, the anaemia is usually regenerative with anisocytosis, poikilocytes and spherocytes (Breitschwerdt, 1984). Haematological complications of vascular stasis is a prominent finding in severe disease, which causes hypoxia and subsequent tissue damage. Sequestration of the erythrocytes is also contributory factor to the decrease in the PCV levels (Taboada and Merchant, 1991). Severe coagulation disturbances and DIC are also caused by virulent strains of *Babesia canis* which may further complicate the disease (Valli and Parry, 1993).

Presence of the organisms can be demonstrated by examination of a peripheral blood smear stained by May-Grünwald Giemsa and by serology. The anaemia is usually, regenerative, macrocytic-normochromic. Blood findings include anisocytosis,

polychromasia, poikilocytosis, reticulocytosis, normoblastosis and lymphocytosis. Spherocytes may also be seen (Farwell *et al.*, 1982). Thrombocytopenia may also be present and may or may not be associated with a DIC (Harvey *et al.*, 1988). Dogs may also be Coombs' positive due to the anti-erythrocyte antibodies formed as a result of alterations induced in erythrocyte surface antigens by the parasite (Jain, 1993d)

This parasitic infection is fairly uncommon in the United Kingdom and there have been reports of it being more prevalent in the tropical countries and the United States.

Haemobartonella

This is a rickettsial organism found in many domestic animals, which is highly pleomorphic and occurs in coccoid or rod forms arranged as chains or rings or small specks on the erythrocyte surface. Although it is observed world-wide, there have been very few reports of its occurance in dogs when compared to the number of cases reported in cats, especially in the United Kingdom (West, 1979).

Haemobartonella canis is non-pathogenic and affected dogs are asymptomatic. The infection may become more pronounced in dogs which have undergone splenectomy resulting in severe haemolytic anaemia (West, 1979; Kuehn and Gaunt, 1986). Affected dogs have similar clinical signs to the ones seen in *Babesia canis* infections although, haemoglobinuria is not seen since no intravascular haemolysis takes place.

Haematological observations

On examination of a blood smear stained by May-Grünwald Giemsa, these organisms can be identified. In anaemic dogs, the blood smear shows all the features

of regeneration with normoblastosis, Howell-Jolly bodies and leucocytosis. The anaemia is caused by removal of the parasite infected cells by the spleen. In addition, an immune-mediated mechanism may also be involved (Middleton *et al.*, 1982).

Toxic haemolytic anaemia

Lead poisoning

Lead poisoning has been reported in young dogs and cats resulting in neurological and severe gastrointestinal disorders and Poodles and German Shepherds seem to be more frequently affected (Morgan *et al.*, 1991). Chronic lead poisoning causes increased red cell fragility but it also interferes with haem synthesis resulting in anaemia which may be normocytic or microcytic and even hypochromic. Lead also affects other tissues and the major presenting signs are referable to the nervous system. The presence of nucleated red cells without low PCV and basophilic stippling of erythrocytes are suggestive of lead poisoning. Basophilic stippling of the erythrocytes is caused by an unusual clumping of the ribosomes due to lead induced deficiency of pyrimidine-5'-nucleotidase (P5N) enzyme, thus inhibiting several other enzyme systems most importantly the δ-aminolevulinic acid dehydrase (Jain, 1993b). This condition must be differentiated from other causes such as an intense bone marrow response to anaemia which may also cause basophilic stippling to occur.

Oxidative or Heinz-body anaemia

Inherited enzyme deficiencies, e.g. G-6-PD, can lead to oxidative damage of the red cells. Powerful oxidative agents in drugs, chemicals and certain vegetables may also reduce the normal glutathione levels on the red cell surface. This may cause oxidation of haemoglobin to methaemoglobin. The oxidised and precipitated

haemoglobin appears as Heinz inclusion body in the red cell. Heinz bodies attach to the cell membrane and their release into the circulation may lead to methaemoglobinaemia as seen in copper poisoning of sheep. Normally, sulphydryl (-SH) groups maintain the protein tertiary structure and when it gets irreversibly oxidised haemoglobin loses this tertiary structure and becomes denatured and precipitates to form morphologically recognisable Heinz bodies (Lees, 1980). Heinz-bodies are also called "erythrocyte refractile bodies" and appear as highly refractile small dense bodies protruding from the erythrocyte surface (Figure 10A and 10B).

In cats, haemoglobin is more easily oxidised than in the dog or man as more sulphydryl groups are present in feline haemoglobin. Heinz bodies may be normally seen in cats without anaemia or they may be associated with anaemia (Weiser, 1995; Christopher, 1989). Presence of numerous Heinz-bodies in the red cells may lead to erroneously high haemoglobin estimation in blood samples. Heinz bodies may be pitted out by the splenic macrophages and so called "bite" cells may be seen in the blood. The severity of haemolysis depends on many factors including the dose and type of drug or vegetable such as onions responsible.

The mechanisms that cause lysis of red cells is quite complex. Haemolysis is due to the following event, oxidation of the sulphydryl groups of haemoglobin in the red cells, formation of Heinz-bodies and attachment of the Heinz bodies to the cell membrane. Next is the removal (pitting out) of Heinz-bodies and Heinz body-containing cells by the spleen, loss of membrane, spherocyte formation, increased membrane permeability, the end result is intravascular and extravascular lysis of affected cells.

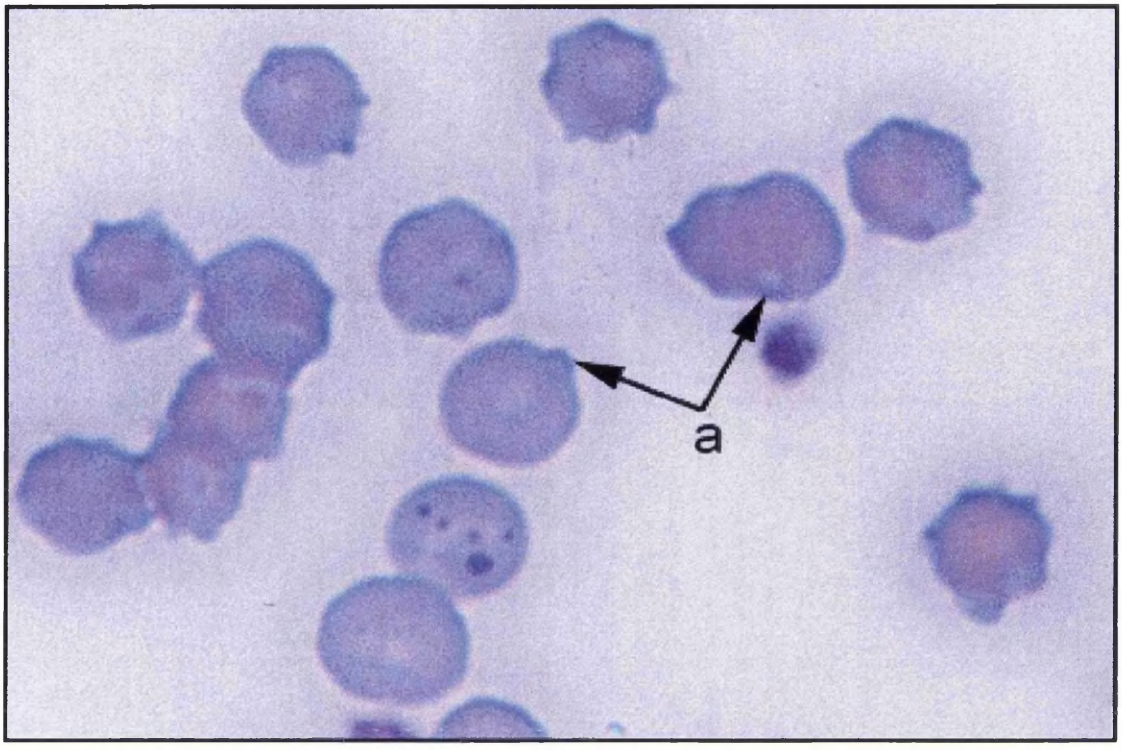

Figure 10A: Heinz bodies in the erythrocytes (a), appear as refractile protruding bodies when stained with May Grünwald Giemsa stain. These are feline red cells but Heinz bodies look similar in every species, (x1000)

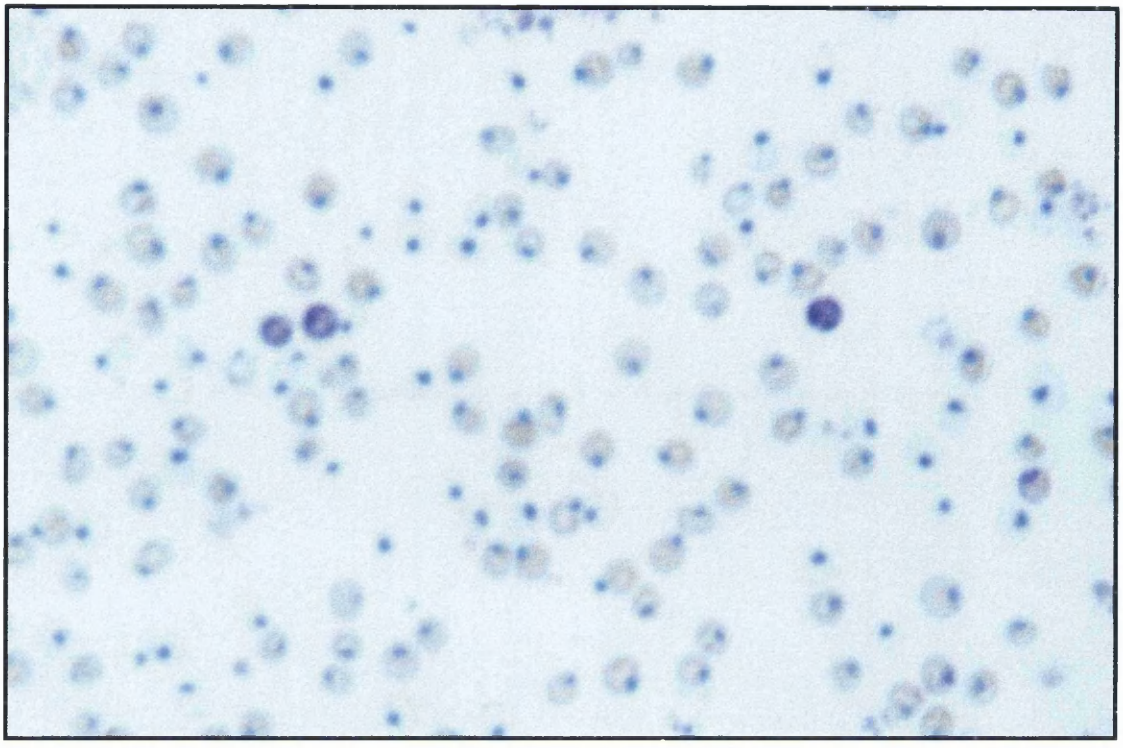

Figure 10B: Heinz bodies in the erythrocytes when stained with new methylene blue, appear blue in colour. These are feline red cells but Heinz bodies look similar in every species, (x 400).

There are reports of Heinz body-induced anaemia in dogs due to a number of causes such as zinc toxicity, onion and drug-induced causes. Methylene blue is also known to cause severe Heinz body haemolytic anaemia (Fingeroth *et al.*, 1988).

A case of a dog eating onions which resulted in a marked haemolytic anaemia with Heinz bodies present has even been reported (Spice, 1976). This dog also had haemoglobinuria, bilirubin and urobilinogen in the urine. For the first 96-120 hours after ingestion of onions, no bone marrow regeneration was seen. Later, increasing regenerative anaemia was noticed which resolved within a few days on supportive treatment. (Spice, 1976). Zinc toxicity has been also reported in a dog which resulted in a severe haemolytic anaemia with Heinz body formation (Luttgen *et al.*, 1990). Another report of zinc toxicosis was ingestion of zinc nuts which resulted in acute non-spherocytic haemolytic anaemia (Torrance and Fulton, 1987). The above factors should be considered in the differential diagnosis of dogs presented in a severe haemolytic crisis.

Microangiopathic haemolytic anaemia (MAHA)

This condition includes a group of disorders characterised by fragmentation of the erythrocytes within the circulation mainly due to shearing forces, leading to intravascular haemolysis (Martinez, 1990). In microangiopathic haemolytic anaemia, also called fragmentation anaemia, fragmentation may occur due to the passage of erythrocytes through damaged arteriolar endothelium which may be associated with a number of causes. This condition has been less studied in animals than in man (Rebar *et al.*, 1980). This type of anaemia may be due to primary causes such as thrombotic thrombocytopenic purpura and uraemic haemolytic syndrome seen mainly in man. Other secondary causes may be include malignancy, drugs, DIC, immune-mediated

conditions and many others. According to Rebar *et al.*, (1980), for MAHA to occur with neoplasms, the total neoplastic mass should be large enough and the blood flow through the neoplasm should be significant.

Hypertension, narrowing and hardening of the arterioles and endothelial cell swelling, probably are contributory factors to fragmentation of the erythrocytes (Martinez, 1990). However, fibrin deposition in the blood vessels is a known major factor causing MAHA. In dogs there are few reports but MAHA has been seen with congestive heart failure, glomerulonephritis, myelofibrosis and neoplasia, especially haemangiosarcoma of the spleen, heart and liver (Rebar *et al.*, 1980). One of the other important cause is DIC which may be due to infections or neoplasia (Bell, 1994).

In heart worm disease caused by *Dirofilaria immitis*, there is obstruction of the normal blood flow and circulating erythrocyte may fragment easily due to mechanical forces resulting in intravascular haemolysis. In a study by Kitagawa *et al.*, (1992), the half-life of erythrocytes in dogs affected with pulmonary heartworm disease was investigated. The erythrocytes of dogs that were mild to severely affected had shorter life span than the less severely affected dogs.

Erythrocytes are teared by strands of fibrin within the vessels and by the force of the blood flow are released as fragments. Some cells flow in the circulation as schistocytes which can be seen on the blood smear whereas, some fold and acquire different shapes (Martinez, 1990; Madewell and Feldman, 1980).

Haematological observations

Fragmented and irregularly distorted red cells which may assume a helmet shape are the most prominent findings in addition to reticulocytosis. Schistocytes are

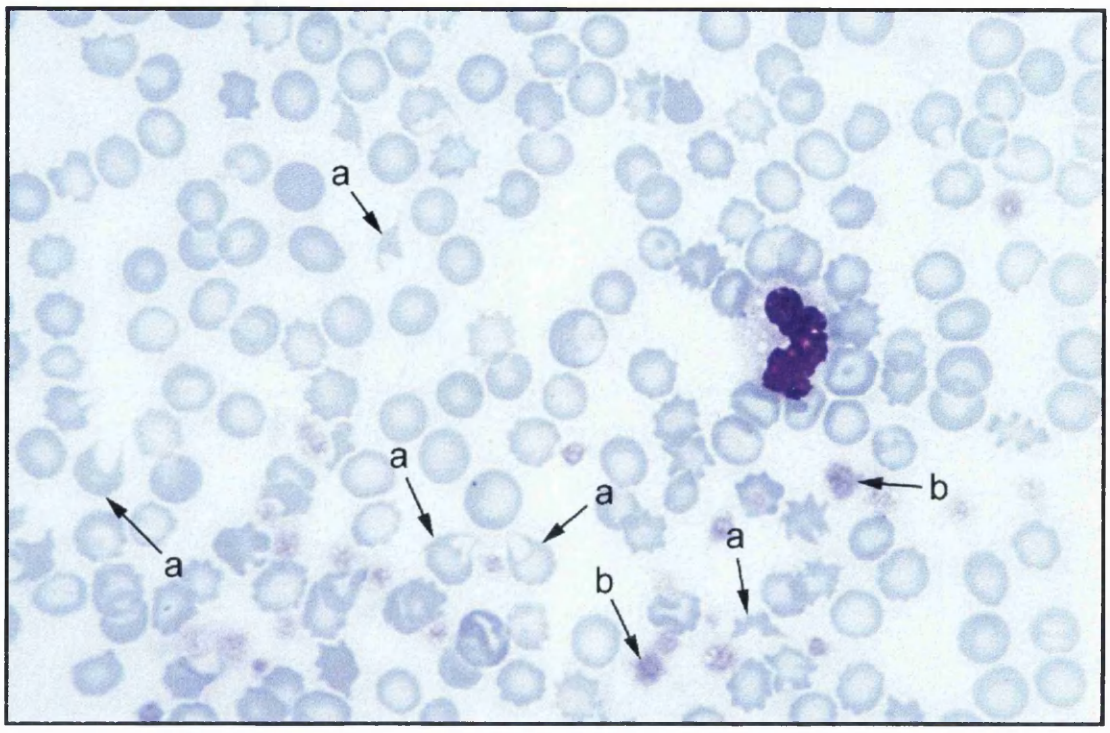

Figure 11A: Blood smear from a dog with microangiopathic haemolytic anaemia showing anisocytosis and poikilocytosis caused by increased number of schistocytes (a). Large platelets (b). Stained by May Grünwald Giemsa, (x 1000).

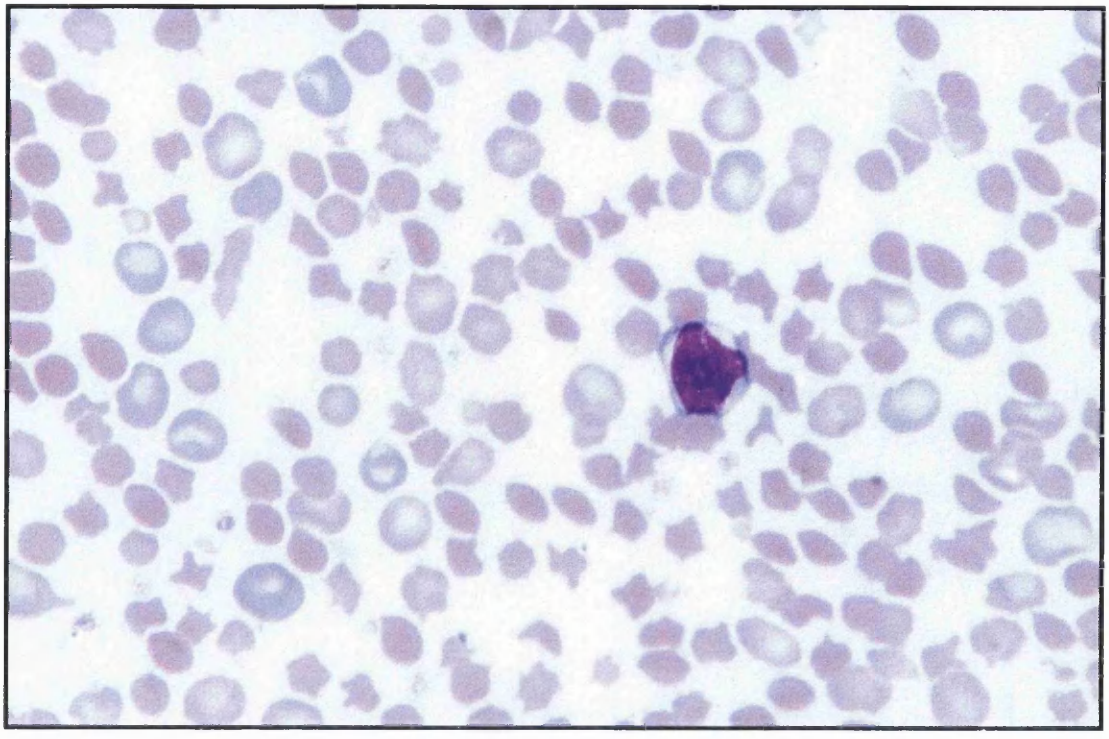

Figure 11B: Another blood smear from a dog with microangiopathic haemolytic anaemia. There is marked anisocytosis and poikilocytosis due to the many small schistocytes, normal red cells and large polychromatic cells. Stained by May Grünwald Giemsa, (x 1000).

illustrated in Figure 11A and 11B. Neutrophilia and thrombocytopenia may also be present depending on the extent and cause of the lysis. There may be a marked erythroid and megakaryocytic hyperplasia in the bone marrow (Martinez, 1990). According to Rebar *et al.*, (1980), elongated erythrocytes were a common finding in addition to other shapes in cases which had more than 50 percent of schistocytes. Features of haemolytic anaemia such as haemoglobinuria and haemosiderinuria may also be present. The presence of schistocytes may reflect DIC which can be confirmed by the presence of raised FDP's, low fibrinogen and low platelet count.

Immune-mediated haemolytic disorders

The immune-mediated haemolytic disorders are of two types. The first group consists of conditions induced by allo-antibodies (iso-antibodies) which may occur naturally or develop after exposure to allo-antigens (iso-antigens), haemolytic disease of the new born and incompatible transfusions are examples of this type (Giger *et al.*, 1995; Jain, 1993e). However, these are rarely observed conditions in dogs.

The other group consists of diseases caused by auto-antibodies produced against self antigens at some time during the post-natal life of the animal. This group includes auto-immune haemolytic anaemias (AIHA), auto-immune thrombocytopenia or thrombocytopenic purpura (AITP), auto-immune neutropenia, certain drug-induced haemolytic anaemias and leucopenias (Jain, 1993e; Slappendel, 1986; Dodds, 1977).

Immune-mediated haematological disorders are observed more commonly in dogs than any other species and has been well documented (Jones and Gruffydd-Jones, 1991; Dodds, 1983; Bennett, 1984; Halliwell, 1978). The number of dogs

affected with these conditions has increased two to three fold since 1979, but according to Dodds (1983), whether this increase is due to an increased awareness about the disorder or due to the true increase in the number of cases is still unclear. Approximately 60-70 percent of the cases have been classified as idiopathic the remaining 30-40 percent being secondary (Dodds, 1983). According to Bennett (1984), there are several theories regarding the cause involving several mechanisms. Well known predisposing factors that can induce an immune-mediated disease are viruses, bacteria, pregnancy and specific drugs. Less specific causes can be hormonal irregularities, genetic influences and stress (Dodds, 1983).

According to Dodds (1983), a modified live virus vaccine may also be an important cause for immune-mediated diseases. The viral antigen could trigger a hapten-type immune-mediated reaction in a susceptible host. In about 25 percent of the 223 cases presented at her laboratory, there had been a definite association with a recent exposure to vaccination. Although this does not indicate that all vaccines may cause haemolytic anaemia the possibility should be considered.

Auto-immune haemolytic anaemia (AIHA)

Auto-immune haemolytic anaemia is caused by auto-antibodies which are produced against the patient's own erythrocytes resulting in erythrocytes with a shortened life span *in vivo*. It is associated with an increase in the erythropoietic activity of the bone marrow. The presence of auto-antibodies and complement on the surface of the affected red cells can be diagnosed by the direct anti-globulin test (DAT) or direct Coombs test (DCT) using polyspecific antisera (Jain, 1993e; Halliwell, 1978; Dodds, 1977).

In the DAT, the patient's erythrocytes are suspended in saline to which a polyspecific antisera containing antibodies raised against canine IgG, IgM, and complement (C) and C3b are added. If red cells contain any of the above components singly or in combination they will be agglutinated and this can be visualised under the microscope.

This is the most commonly observed auto-immune disorder in dogs. The aetiology is either primary or idiopathic, or secondary. The latter may be associated with lymphoproliferative disorders, systemic lupus erythrematosus, viral, bacterial or parasitic infections or may be drug-induced (Dodds, 1983; Halliwell, 1978). Genetic influences play a significant role in the genesis of AIHA and in auto-immune disorders in general.

According to Jain (1993e), Jackson and Kruth (1985), there is no particular breed predilection. But Dodds (1983), suggested that Old English Sheepdogs and Miniature Dachshunds were commonly affected. There has been a higher incidence in Cocker Spaniels, Poodles, Collies, Whippets and Musterlanders (Bennett, 1984). Several studies showed that the disease occurs more frequently in females with an average age of 6.4 years (Jackson and Kruth, 1985; Halliwell, 1978) and according to Dodds (1983), females whether intact or spayed are affected more than males in a ratio of 2:1 with an average age of 4-5 years affecting young adult dogs.

There are theories regarding the factors triggering this event. The first theory states that a change on the erythrocyte membrane forms a new antigen which stimulates a normal immune response and the antigen is now altered or foreign to the host. The second theory is that the immune system fails to recognise itself. These theories are mere speculations but adequate for explaining the mechanisms (Dodds 1977).

The auto-antibodies involved are mainly IgG and IgM. These auto-antibodies are classified as warm, optimally active at 37°C, and cold antibodies active between 2°C to 10°C but with thermal activity upto 37°C (Table 6). Antibodies may be agglutinins and lysins. The differences between the ability of individual antibodies to cause erythrocytes to undergo agglutination or lysis by complement and phagocytosis by macrophages are related to the structure of the antibody. In particular, to the size of the molecule (for example, IgG or IgM), its valency, (that is, the number of Fab sites), and chemical differences (within its constant and hinge regions). Complement is activated by the classical pathway. To activate complement the antibody should be an IgM molecule or two IgG molecules closely adjacent on the red cell membrane. About 200-or more antibodies are required per cell for detection by splenic macrophages (Dacie, 1992b).

The auto-antibodies most commonly occurring in dogs, are IgG, IgM or IgA, the latter two being observed less often (Stockham *et al.*, 1980; Dodds, 1977). These have been summarised by Slappendel (1986), as follows:

- Type I in which immunoglobulin alone, usually IgG, is present.
- Type II in which both immunoglobulins IgG and or IgM with complement are present.
- Type III in which only complement are present and
- Type IV in which neither complement nor immunoglobulins can be detected.

A combination of the above may be observed causing various reactions. Type I and Type II reactions, in which IgG antibodies are detected by the DAT, indicate overt or latent AIHA. Haemolysis is noticed to be severe or acute only in the Type II reactions. Type III reactions have a strongly positive anti-C type DAT, due to activation by IgM, this type is associated with severe intravascular haemolysis. IgM

Table 6: A Summary of the Autoantibodies Causing AIHA

Thermal Range	Type	Pathogenesis
Warm (37°C)	IgM	In vivo agglutination (rarely observed)
	IgM	In vivo haemolysis (intravascular lysis)
	IgG-high concentration	May activate complement in vivo haemolysis
	IgG-incomplete antibodies	Coat erythrocytes, extravascular lysis (phagocytosis) and spherocyte formation. Commonest type in dogs.
	C3b	Coat erythrocytes (extravascular lysis)
Cold (below 37°C)	IgM	In vivo haemolysis due to complement activation (rare)
	IgM	In vivo agglutination, cold haemagglutinin disease affecting extremities

type warm and cold antibodies either fix complement causing lysis or agglutinate red cells. Type IV reaction, that is a negative DAT usually represents a false negative result which may be due a number of factors.

There are however true negative cases too. The mechanisms of Coombs' negative AIHA has been studied in detail and it has been shown that the patient's erythrocytes are coated with too few antibodies for detection by antiglobulin sera.

The mechanism of AIHA involves intravascular or extravascular lysis of the red cells. In extravascular lysis, the erythrocytes are coated by IgG and/or C3b and are phagocytosed by the MNPS, especially by the spleen which is the primary organ involved. The splenic macrophages have surface receptors for the Fc portion of the IgG and the C3b portions of complement. Partial erythrophagocytosis causes reduction in the erythrocyte membrane and the formation of spherocytes which are released into the circulation. The presence of spherocytes is diagnostic in identifying this type of AIHA, in addition to a moderate to severe splenomegaly the latter being due to the fact that spherocytes are sequestered and removed by the spleen (Cotran *et al.*, 1994a; Dacie, 1992a; Halliwell and Gorman, 1989).

In case of intravascular haemolysis, which may be severe, there is haemoglobinaemia and haemoglobinuria which is due to vigorous complement fixation by IgG or more frequently by IgM antibodies, leading to the formation of a membrane attack complex which involves the attachment of C8 to C5,6,7 and of C9 to C8.

Cold auto-antibodies can activate complement and cause intravascular lysis or agglutinate red cells. If agglutination is caused by IgM type auto-antibodies the condition is called "cold haemagglutinin disease". Cold agglutinin disease may be evident by auto-agglutination of erythrocytes at room temperature or by exaggerating

it at 4°C. Auto-agglutination may also occur in association with monoclonal gammopathies which will not disappear at 37°C (Greene *et al.,* 1977; Cotran *et al.,* 1994a).

Rouleaux formation of red cells occurs when there is an increase in the plasma proteins causing the red cells to stick together. However, the head of a blood smear with normal protein levels may also contain rouleaux, but the tail of the same smear would show more evenly spread cells (Figure 12A and 12B). Rouleau can be distinguished from true auto-agglutination by the addition of isotonic phosphate buffered saline to the blood. Aggregated erythrocytes with rouleaux will be dispersed, whereas true auto-agglutination will remain unaffected (Halliwell, 1978; Slappendel, 1986). A blood smear from a dog with IgM type AIHA would appear as shown in Figure 13, wherein the red cells are aggregated towards the tail of the smear.

The clinical symptoms result either from in vivo agglutination of red cells or from fixation of complement when temperatures drop below 30°C especially at distal ends of the body. Affected dogs may show necrosis of their extremities, for example, the nose, tail and ears due to microcapillary stasis due to exposure to a cooler environment and continuous exposure may cause gangrene to develop (Greene *et al.,* 1977). When complement is activated, intravascular haemolysis may be macroscopically seen which is a rare occurrence in dogs.

The onset of these disorders can be acute (sudden) or chronic (gradual). Clinical signs will depend on the mechanism involved. In the rare non-agglutinating cold antibody disease there is a sudden onset of anaemia, with haemoglobinuria, icterus, increased serum bilirubin levels and weakness (Dodds 1983).

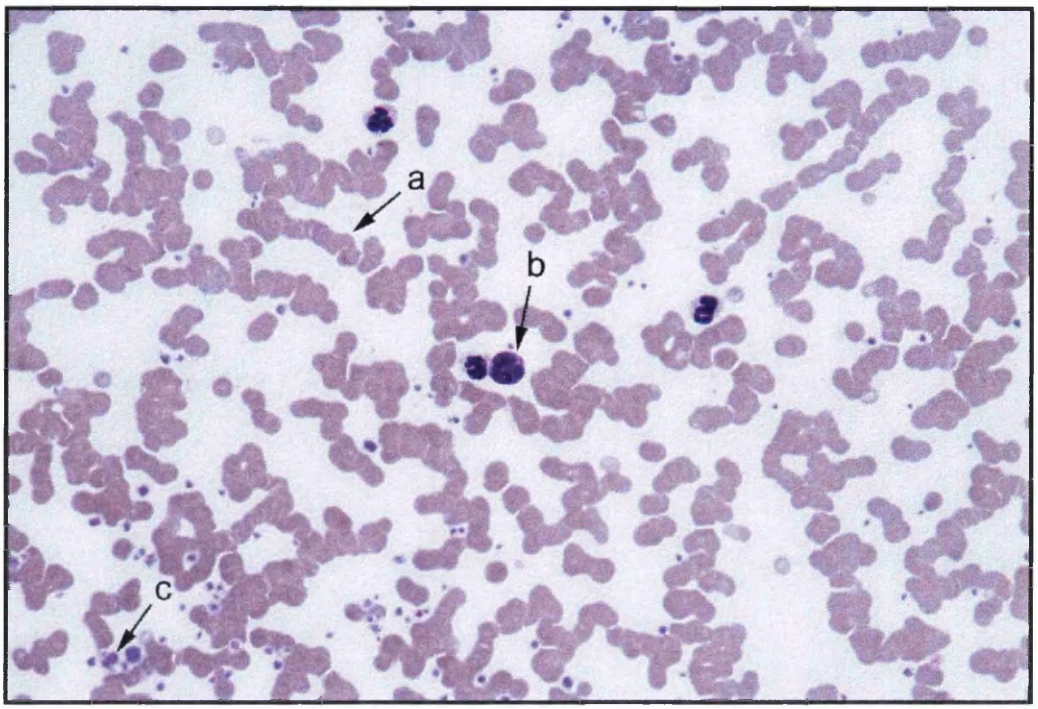

Figure 12A: Numerous rouleaux are present at the head of a smear made from a sample with normal plasma protein levels.

a: Rouleau i.e. a roll of red cells resembling a pile of coins.

b: neutrophil

c: platelet aggregation

Stained by May Grünwald Giemsa, (x 400).

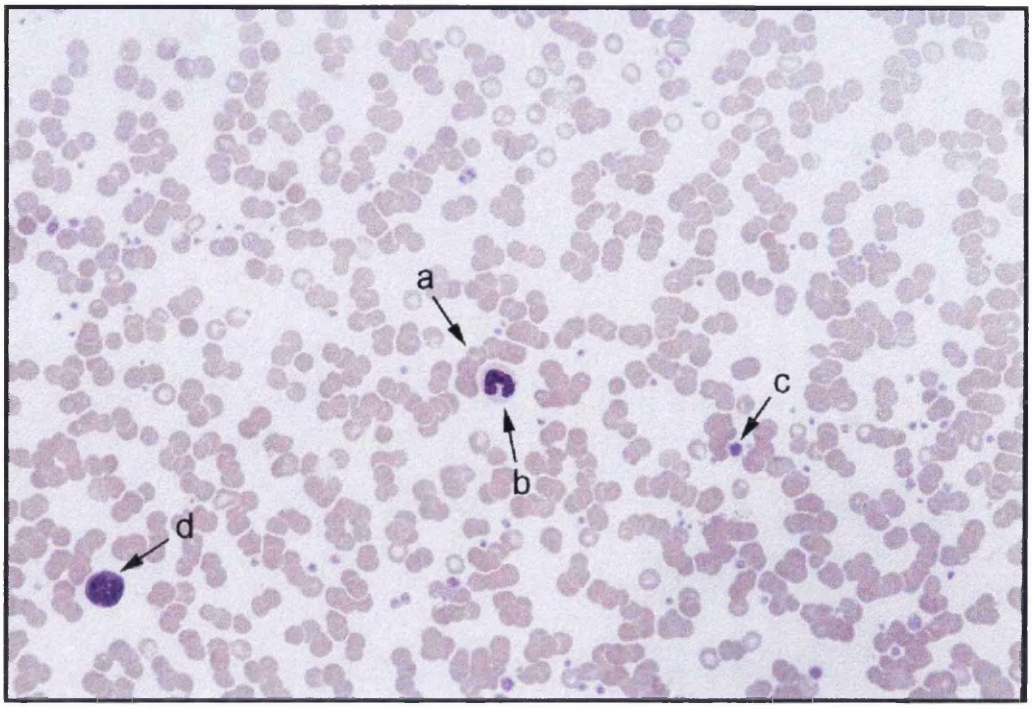

Figure 12B: Rouleaux and dispersion of erythrocytes at the tail of a smear made from a sample with normal protein levels.

a: Rouleau

b: neutrophil

c: platelet

d: lymphocyte

Stained by May-Grünwald Giemsa, (x 400).

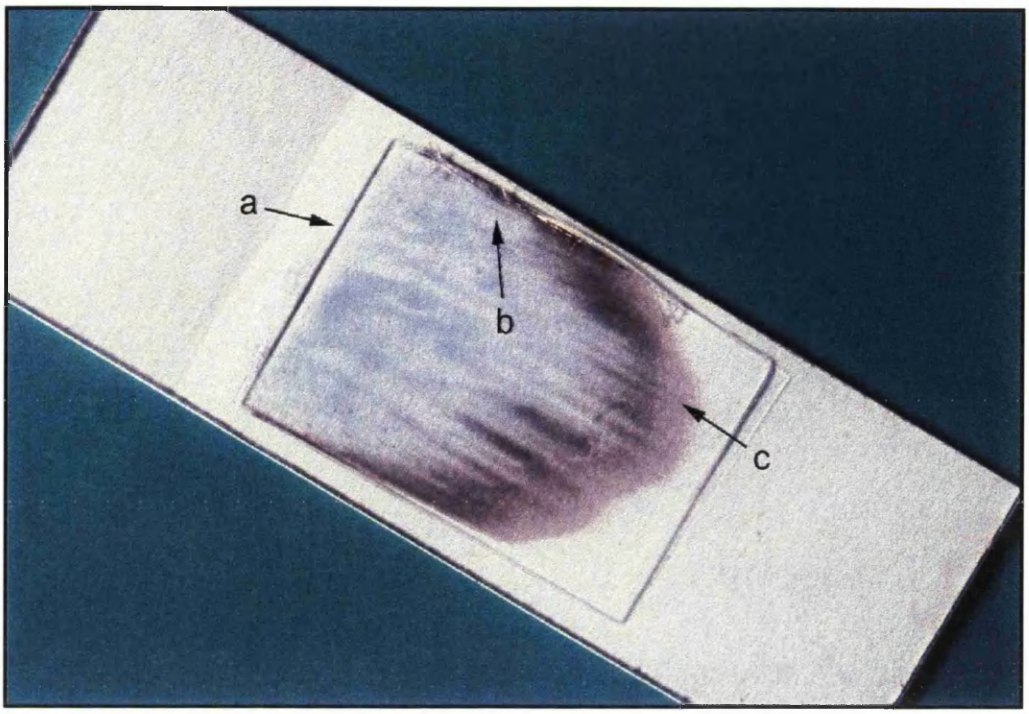

Figure 13: The gross appearance of a blood smear from a dog with IgM type auto-immune haemolytic anaemia demonstrating uneven distribution of cells.

a: head of the smear (starting point of the smear)

b: centre of the smear with sparsely distributed cells

c: tail of the smear showing agglutination of red cells (end of the smear)

The blue tinge of the background is caused by the increased globulin content **(b).** The smear is stained by May-Grünwald Giemsa.

Haematological observations

There is a markedly increased bone marrow response to the anaemia reflected in the blood as marked anisocytosis, polychromasia, many Howell-Jolly bodies leptocytosis and normoblastosis. Spherocytosis and auto-agglutination of red cells are characteristic findings associated with AIHA. If the auto-agglutination is massive it is most likely to be caused by high thermal amplitude cold antibodies. Warm antibodies, however, can also produce auto-agglutination which may be IgG or IgM or both. There is a marked increase in the MCV due to the increased reticulocytosis. The MCH and MCHC are within normal ranges and may be increased in patients with increased spherocytosis (Dacie, 1992a). Normoblastosis is often present in patients with IgG type AIHA and may exceed the white cell count (Dacie, 1992a). Some of the features as seen in dogs are illustrated in Figures 14A, 14B, 14C.

As erythropoiesis is increased in AIHA, there is hypertrophy due to an increase in the erythropoiesis and the M:E ratio may exceed the normal (Dacie, 1992a).

A leuco-erythroblastic reaction is often noticed in AIHA; that is a high WBC count or erythroblast count (Mandell *et al.*, 1989). There is leucocytosis with neutrophilia showing a left shift and monocytosis. Very high WBC counts (leukaemoid reaction) are also often seen (Halliwell and Gorman, 1989). Erythrophagocytosis by the circulating monocytes can be observed. It is an important feature by which the erythrocytes coated with auto-antibodies and or complement can be eliminated from the circulation (Dacie, 1992a). Thrombocytopenia may also be present which may be caused either by a true immune-mediated thrombocytopenia with AIHA (Evan's Syndrome) or it may be due to haemorrhages or DIC.(Cotter, 1995; Valli and Parry 1993; Jackson and Kruth, 1985). In cases of IgM type AIHA,

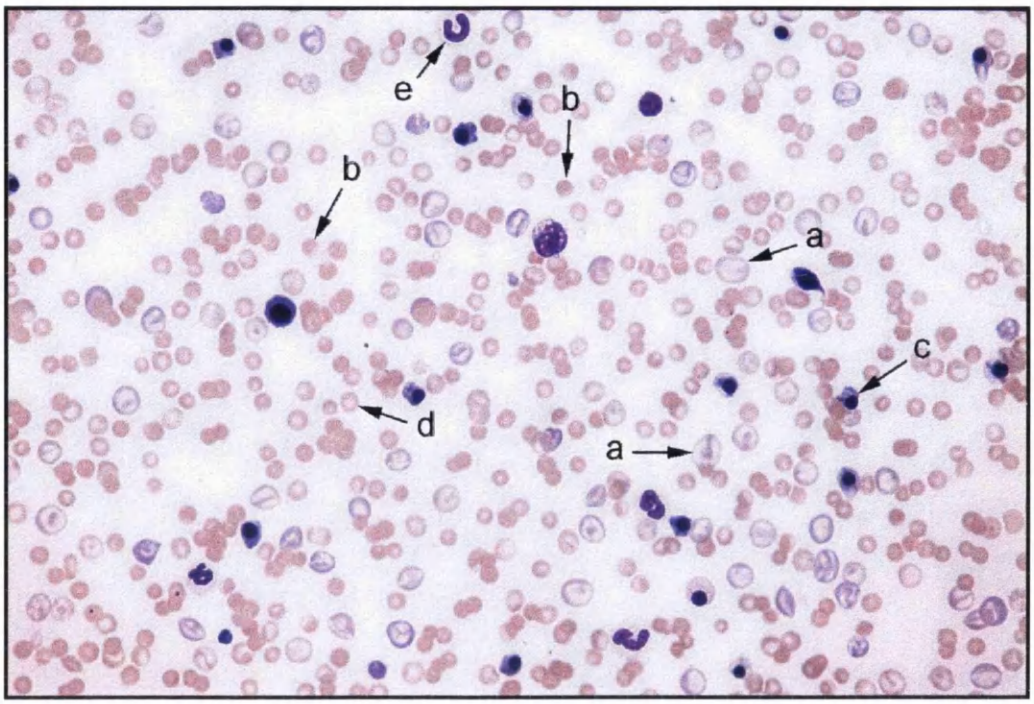

Figure 14A: A blood smear from a dog with IgG type auto-immune haemolytic anaemia showing features of marked regeneration with normoblastosis and spherocytosis

a: reticulocytes (polychromatic macrocyte)

b: spherocytes

c: normoblast

d: erythrocyte

e: band neutrophil

Stained by May-Grünwald Giemsa, (x 400).

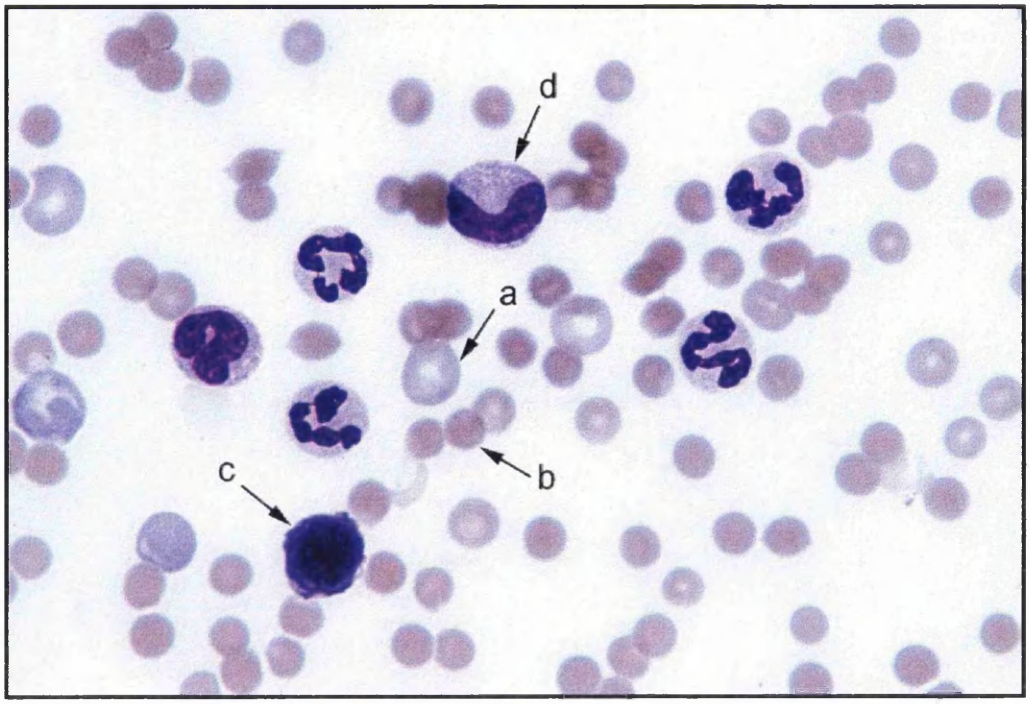

Figure 14B: A blood smear from a dog with IgG type auto-immune haemolytic anaemia showing regenerative features and spherocytosis.

a: reticulocytes (polychromatic macrocyte)

b: spherocytes

c: basophilic normoblast (early)

d: late metamyelocyte

Stained by May-Grünwald Giemsa, (x 1000).

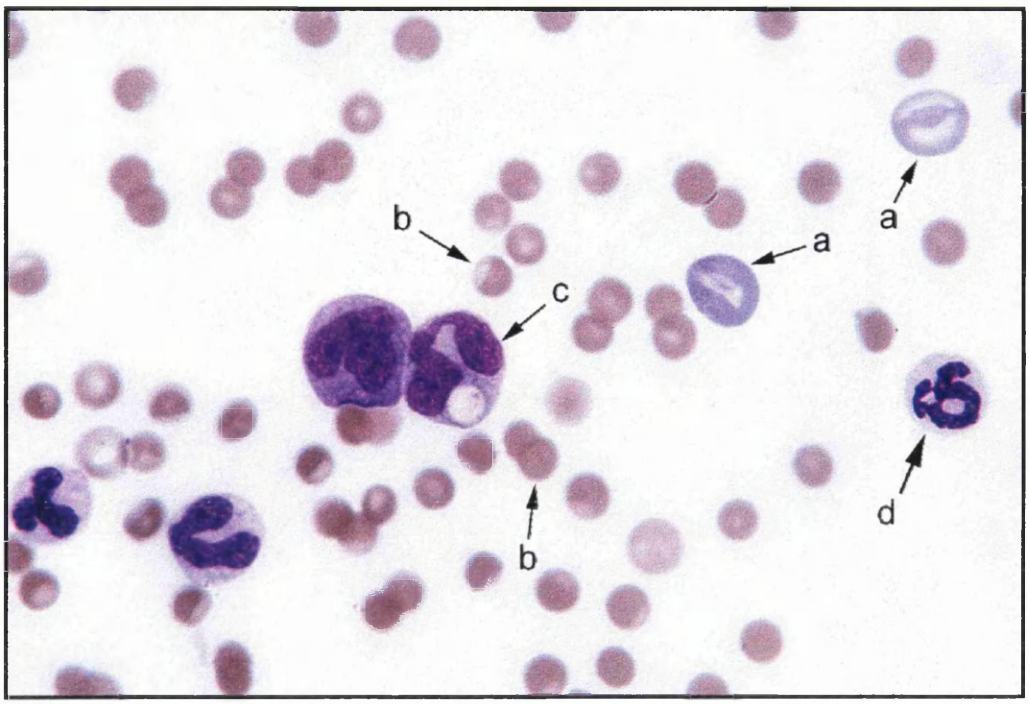

Figure 14C: A blood smear from a dog with IgG type auto-immune haemolytic anaemia, showing features of regeneration and spherocytosis

a: reticulocytes (polychromatophilic macrocytes/leptocytes)

b: spherocytes

c: erythrophagocytosis by a typical monocyte

d: neutrophil

Stained by May-Grünwald Giemsa, (x 1000).

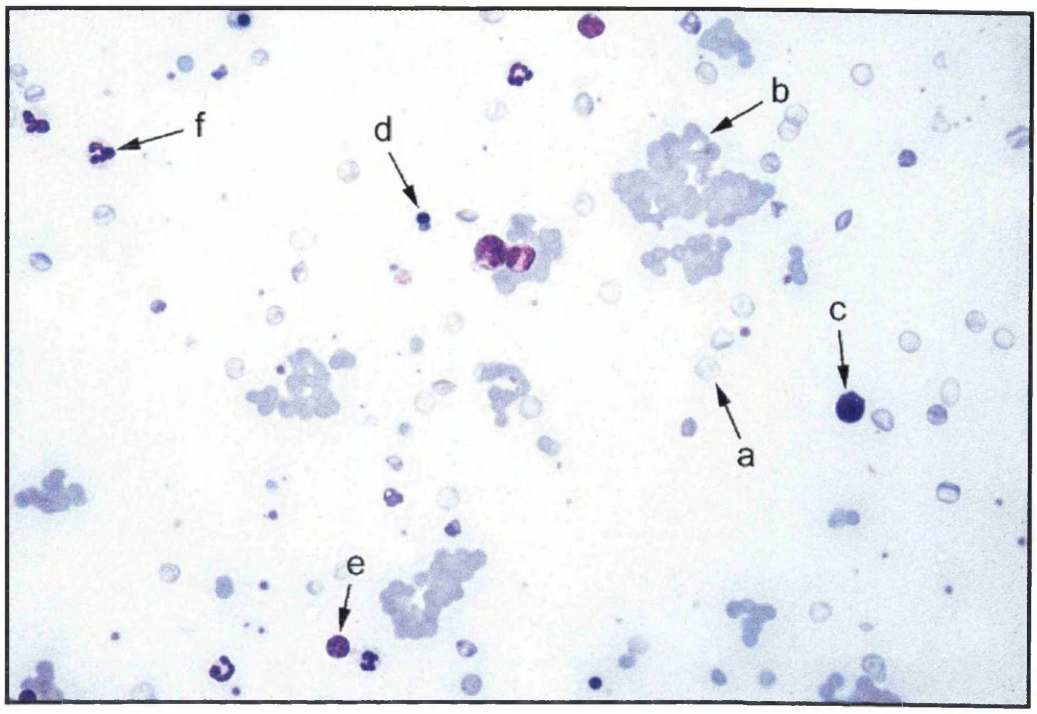

Figure 15A: A blood picture from a dog with IgM type auto-immune haemolytic anaemia showing auto-agglutination of red cells and regenerative features.

a: reticulocyte (polychromatic macrocyte)

b: auto-agglutinated red cells

c: intermediate normoblast

d: late normoblast

e: lymphocyte

f: neutrophil

Stained by May-Grünwald Giemsa, (x 400).

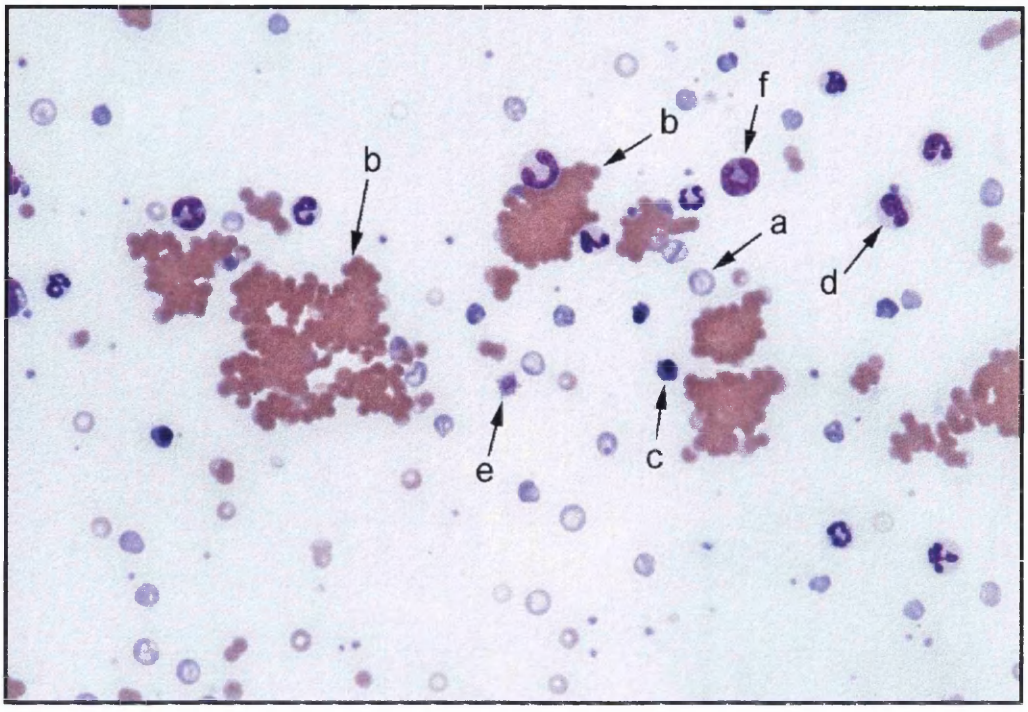

Figure 15B: A blood smear from a dog with IgM type auto-immune haemolytic anaemia showing various characteristic features.

a: reticulocytes (polychromatic macrocytes)

b: auto-agglutination of erythrocytes

c: normoblast

d: metamyelocyte

e: large platelet

f: monocyte

Stained by May Grünwald Giemsa, (x 400).

similar features may be observed but auto-agglutination is more pronounced and is a diagnostic feature as illustrated in Figure 15A and 15B.

Reticulocytopenia has also been recognised as a complication reflecting an immune-mediated hypoplastic or aplastic condition (Jones and Gruffydd-Jones 1991; Holloway *et al.*, 1990; Jonas *et al.*, 1987). Suppression of various haemopoietic precursors have been observed implicating that an immune-mediated mechanism may be involved (Weiss, 1986). Affected dogs may require a blood transfusion or stronger use of immunosuppressive therapy and there have been reports of recovery in some cases (Cotter, 1992; Holloway *et al.*, 1990).

2.5.3.3 ANAEMIAS OF DECREASED RED CELL FORMATION

Hypoproliferative anaemias are seen in a large number of diseases which affect erythropoiesis resulting a reduction in the number of red cells. These may broadly be classified as intramarrow and extramarrow conditions depending on the mechanisms involved. The extramarrow conditions include the decreased stimulation by erythropoietin due to renal damage, chronic metabolic diseases, chronic inflammatory diseases and lack of essential nutrients such as iron. The intramarrow conditions include the direct damage to the bone marrow microenvironment, especially to the haemopoietic cells due to irradiation and toxic effects of drugs and chemicals, infiltrative diseases such as leukaemias leading to myelophthisis and myelofibrosis.

(A) Intramarrow conditions causing anaemia

Aplastic or hypoplastic anaemia

The terms aplastic and hypoplastic imply that the anaemia is due to acellular or hypocellular marrow. Aplastic anaemia or bone marrow aplasia is defined as multifactorial disease involving a primary failure of all haemic cell lines. It is associated with cytopenias in the blood and with a hypocellular or acellular bone marrow replaced by adipose tissue (Nissen, 1991; Weiss and Klausner, 1990). The term aplastic pancytopenia can also be used depending on the degree of involvement of the cell lines and the severity of the syndrome. Nissen (1991), suggested that aplastic anaemia initially has myelodysplastic traits and a differentiation is often difficult.

Aplastic anaemia may be the result of a number of conditions which may be congenital or acquired. In human beings congenital aplastic anaemia is recognised as Fanconi's anaemia due to an autosomal recessive chromosome which have not yet been reported in animals (Feig, 1980). Acquired anaemias in man may be idiopathic, or caused by chemical agents, irradiation or drug-induced myelotoxicity (Cotran *et al.*, 1994a). Acquired aplastic anaemia occurs in dogs often as a result of toxic insult to the bone marrow, due to drugs such as oestrogens, phenylbutazone, trimethioprim-sulfadiazine, meclofenamic acid, fenbendazole and quinidine and chloramphenicol (Weiss and Klausner, 1990, Weiss and Adams, 1987). In dogs, marrow hypoplasia can also be caused by infections such as chronic Ehrlichiosis and Parvovirus infection (Kuehn and Gaunt 1985; Boosinger *et al.*, 1982). Myelofibrosis and chemotherapy may also cause hypoplasia or aplasia.

The mechanism which cause bone marrow aplasia seems to be the consequence of stem cell damage resulting in the production of cells with poor proliferative and differentiative capacity. If the stem cell damage is permanent then an aplastic anaemia develops (Cotran *et al.*, 1994a). Cline and Golde, (1978) and Nissen, (1991) suggested that idiopathic aplasia is probably immune-mediated.

According to Nissen (1991), in the development of aplasia T-cells, interleukins and genetic factors may be involved. The author also proposes that as the condition is highly responsive to immunosuppressive therapy, the immune system may play a major role in the development of aplastic anaemia. If the immune response is vigorous, the altered stem cells are depleted and marrow aplasia occurs and if it is weak then myelodysplasia with a chronic pancytopenia occurs (Nissen, 1991). Toxic destruction of stem cells, altered bone marrow microenvironment or lack of haemopoietic growth factors may also be involved in the process (Weiss, 1992).

Haematological observations

Pancytopenia is the hallmark of an aplastic anaemia (Feig, 1980). However, due to the different life span of blood cells, cells with shorter survival time, such as platelets and granulocytes will disappear first followed later by the erythrocytes. Therefore, acute aplastic anaemia is characterised by a normocytic-normochromic mild degree of anaemia with severe granulocytopenia and thrombocytopenia. Chronic aplastic anaemia is characterised by severe non-regenerative anaemia and variable degrees of leucopenia and thrombocytopenia (Weiss, 1992). In true aplasia nucleated red cells are not seen in the blood.

Granulocytopenia renders the patient susceptible to infections whereas severe thrombocytopenia may induce haemorrhagic tendencies and affected patients may bruise easily (Feig, 1980). Bone marrow core biopsy is essential for evaluating the

marrow architecture and cellularity. In aplastic anaemia the haemic tissue occupies only 25 percent or less of the marrow and the remainder is replaced by adipose tissue (Weiss, 1992). In severe aplasia only small lymphocytes and adipose tissue are present. Some commonly occurring conditions causing hypoplasia or aplasia of the marrow in dogs are discussed below.

Oestrogens

Oestrogen and its derivatives are often used in clinical practice for the treatment of urinary incontinence, prevention of conception following mismating and for treatment of some adenomas and prolonged anoestrus. Endogenous oestrogens due to testicular tumours such as Sertoli cell tumour may also cause aplasia. Oestrogen toxicity has been well documented in dogs resulting in pancytopenia (Hall, 1992; Shelly, 1988; Legendre, 1976). Frequently, haemorrhagic problems are the first clinical signs. The exact mechanisms are unclear but it is a well established fact that oestrogens act at the stage of stem cell differentiation (Teske, 1986).

Haematological observations

Initially, there is a thrombocytopenia which develops approximately two weeks after the drug administration. In the following 16 to 20 days an enhanced granulopoiesis with a left shift and a normocytic-normochromic anaemia develop. After 22 to 25 days, there is a decrease in granulopoiesis resulting in leucopenia (Teske, 1986).

Ehrlichiosis and parvovirus infections

Ehrlichia canis, a rickettsial organism is known to cause canine tropical pancytopenia. It is transmitted by the tick *Rhiphicephalus sanguineus* and infects canine blood mononuclear cells as an intracellular parasite. There are two phases in

which *Ehrlichia* affects dogs. The acute stage which causes severe thrombocytopenia with a hypercellular bone marrow, and the chronic phase which usually responds poorly to treatment, resulting in pancytopenia and marrow hypoplasia. The dogs usually die due to severe non-regenerative anaemia, haemorrhages (epistaxis), secondary infections and seizures (Meinkoth *et al.,* 1989; Kuehn and Gaunt, 1985). *Hepatozoon canis,* a protozoan parasite may causes moderate degrees of a non-regenerative anaemia, thrombocytopenia and leucopenia and may mimic *Ehrlichiosis.* (Elias and Homans, 1988; Zinkl, 1981).

Bone marrow necrosis has not been frequently reported in dogs but can produce severe anaemia due to the pancytopenia. In the dogs studied by Weiss *et al.,* (1985a), necrosis was suspected to be caused by chronic *Ehrlichiosis,* septicaemia and oestrogen toxicity. Marked amount of necrotic debris and haemorrhage within the marrow was evident in all the dogs in addition to erythroid hypoplasia. In dogs bone marrow necrosis may also be caused by *Parvovirus* infection. *Parvovirus* affects the rapidly dividing cells, initially, the virus replicates in the lymphoid tissue and circulates in the plasma. After initial replication, the virus starts multiplying in the bone marrow and in the intestinal crypts and in neonates in the myocardium (Valli and Parry, 1993). The first report of the effects of *Parvovirus* on morphological alterations in canine bone marrow was by Boosinger *et al.,* (1982). The presence of *Parvovirus* may be associated with red cell aplasia, however and the cause may be an immune-mediated inhibition of erythropoiesis (Weiss, 1986).

The effects on the bone marrow are degenerative changes of the myeloid cells and in the peripheral blood there is a neutropenia rather than pancytopenia (Greene, 1984). However, if the condition is aggravated by secondary infections causing

septicaemia and endotoxaemia, neutropenia and some degree of anaemia are observed (Boosinger *et al.*, 1982).

Erythroid hypoplasia and pure red cell aplasia (PRCA)

The diagnosis of erythroid hypoplasia in human patients is mainly based on the following observations made by Seaman and Kohler (1953),

a. Severe, chronic, normocytic-normochromic or macrocytic-normochromic non-regenerative anaemia.

b. A cellular bone marrow with active granulopoiesis and thrombopoiesis but marked hypoplasia of erythroid cells.

c. Normal total and differential leucocyte counts.

d. Normal platelet count.

c. No evidence of EMH.

This condition must be differentiated from pure red cell aplasia (PRCA), in which there is a complete selective depletion (aplasia) of the erythroid precursors in the bone marrow. Both conditions must be distinguished from an aplastic anaemia in which all the cell lines are affected.

Erythroid hypoplasia has been observed in animals, the aetiology remains unknown, although an immune-mediated cause may be present (Kaplan *et al.*, 1985; Weiss *et al.*, 1982). In human beings, pure red cell aplasia may be congenital (Diamond Blackfan anaemia) or may be acquired. Acquired PRCA may be primary, idiopathic or secondary due to thymoma, infections or neoplasia (Krantz, 1980). In man, primary PRCA seems to be immune-mediated and an IgG auto-antibody directed against the erythroid precursors in the bone marrow seems likely to be involved in its development (Cline and Golde, 1978). Secondary form of PRCA is

most often seen in association with thymoma in man and thymectomy is known to improve this condition (Krantz, 1980). Red cell aplasia and hypoplasia have been seen in dogs which were either Coombs' positive or negative (Stockham *et al.*, 1980; Weiss *et al.*, 1982). According to Weiss (1986), a serum IgG inhibitor directed against the precursors of CFU-E has been detected in vitro in such dogs. As these cases respond to immunosuppressive therapy an immune-mediated mechanism seems to be the most likely cause for acquired PRCA in dogs as well as in man (Gilmour *et al.*, 1991; Weiss, 1986).

Haematological observations

A normocytic-normochromic, non-regenerative anaemia and normal granulocyte and platelet numbers are observed in-addition to a marked decrease or absence of erythroid cells in the bone marrow (Kaplan *et al.*, 1985; Weiss *et al.*, 1982). There is also an increase in the serum iron concentration and in the percentage of transferrin saturation and the level of Epo is high (Krantz, 1980). A differentiating feature of PRCA from the anaemia of chronic disease, in addition to the low erythrocyte numbers and high levels of serum iron, is the PCV which is usually well below 20 percent in PRCA, and above 25 percent in anaemia of chronic disease (Gilmour *et al.*, 1991). Bone marrow examination is essential in diagnosis and this which may reveal a lack or absence of erythroid precursors and the presence of all other cell lines at different stages of development.

Myelophthisic anaemia

This anaemia is the result of replacement of the normal active bone marrow architecture by foreign cells which may be neoplastic or fibrotic affecting all the cells of the blood by causing various degrees of cytopenias. The commonest causes are the

metastases of neoplasms and variants of the myeloproliferative and lymphoproliferative disorders. Myelofibrosis and myelodysplasia are included in the former group of disorders (Raskin, 1996).

Myelofibrosis

Myelofibrosis is characterised by fibroblastic proliferation and varying degrees of collagen deposition within the marrow cavity. Myelofibrosis can be primary (idiopathic) or secondary to the invasion of the marrow, it is frequently seen in chronic myeloproliferative diseases. It is associated with myeloid metaplasia of the liver, spleen and lymph nodes, proliferation of fibroblasts and abnormal growth of haemopoietic cells in the marrow (Dunn *et al.*, 1986). Haematological abnormalities include a normocytic or occasionally, macrocytic-normochromic anaemia. Peripheral blood smear examination reveals mis-shapen cells (ovalocytes or tear drop dacrocytes), anisocytosis, giant platelets and a leuco-erythroblastic blood picture reflecting increased activity of unaffected marrow and EMH (Dunn *et al.*, 1986). Platelet and WBC counts are usually normal or slightly increased. Megakaryocytic hyperplasia is seen particularly in myeloproliferative disorders.

Myelofibrosis has been observed in dogs as a terminal event in oestrogen toxicosis and in chronic myeloproliferative disorders (Weiss *et al.*, 1985b). It has also been recognised as a terminal event in Basenji's and Beagles with erythrocyte pyruvate kinase deficiency (Chapman and Giger, 1990).

Myelodysplasia is a part of the myeloproliferative disorders and may also be associated with anaemia.

Myelodysplastic syndromes

Myelodysplasia is the qualitative and quantitative disorders of myeloid cells which need not be neoplastic. A hypercellular bone marrow and peripheral blood cytopenias are the characteristic features of myelodysplasia (Valli and Parry 1995). Myelodysplasia is a rare disease but it has been reported in dogs and as a condition preceding the development of acute leukaemia (Weiss *et al.,* 1985b; Shull, 1981). In the two dogs studied by Weiss *et al.,* (1985b) dysplastic changes were seen in all the different haemopoietic cell lines causing pancytopenia with a hypercellular marrow. Myelodysplasia as a pre-leukaemic state may have features of both dyserythropoiesis and dysgranulopoiesis.

(B) Extramarrow conditions causing anaemia

Nutritional deficiency anaemias

Nutritional deficiencies are rare in dogs except for iron deficiency. In man however, megaloblastic anaemias are well recognised diseases.

Megaloblastic anaemia is caused by impaired DNA synthesis, cell maturation and cell division, resulting in abnormal erythroid precursors (megaloblasts) showing abnormal nuclear features in the bone marrow, and large erythrocytes (macrocytes) in the blood. The cause for this anaemia is the absence or reduced amounts of vitamin B12 or folic acid which are coenzymes for the DNA synthesis. Reduction in the amounts may be due to inadequate dietary intake or impaired absorption from the gastrointestinal tract. The RNA and protein synthesis in the cytoplasm is unaffected hence there is an asynchrony in the cytoplasmic and nuclear maturation leading to immature large nuclei with open chromatin patterns. A large proportion of

megaloblasts are destroyed in the marrow resulting in severe anaemia (Cotran *et al.*, 1994a). All anaemias due to deficiency of vitamin B12 or folic acid have the common morphological features of megaloblastic erythropoiesis with characteristic abnormal appearance of all nucleated red cells. Vitamin B12 and folate deficiencies usually occur separately and folic acid deficiency is usually dietary.

Addisonian megaloblastic or pernicious anaemia is caused by vitamin B12 deficiency and it is the commonest type of megaloblastic anaemia in man. It is due to the absence from the gastric juice the substance known as intrinsic factor which is essential for the absorption of vitamin B12. Approximately 50% of the patients have auto-antibodies against this intrinsic factor indicating an immune-mediated disorder. The anaemia is normochromic and macrocytic with a high MCV and MCH and normal MCHC. In the peripheral blood there is marked anisocytosis and poikilocytosis, due to the presence of macrocytes, microcytes and pear-shaped cells. Polychromasia is not a feature. Hypersegmented large neutrophils and often low granulocyte and platelet counts are also present reflecting impaired DNA synthesis. In the bone marrow erythroid hyperplasia is present with megaloblasts at all stages of development. Granulopoiesis is also affected and many giant metamyelocytes and band forms can be seen (Rogers, 1995; Thompson 1979; Schalm *et al.*, 1975c).

There have been very few reports of these deficiencies in dogs either in the U.K, or other developed countries. The provision of a well-balanced commercially-prepared diet for dogs does not predispose them to any dietary deficiencies compared to human beings.

Iron deficiency anaemia (IDA)

Iron deficiency anaemia may be either a consequence of increased utilisation of iron, such as during growth and pregnancy or decreased intestinal absorption or chronic blood loss (as discussed before) (Harvey *et al.*, 1982). Milk has low levels of iron and fast growing puppies of large breeds can develop IDA if their diet is not sufficiently supplemented (Evans *et al.*, 1987; Mahaffey, 1986). Iron deficiency results in a non-regenerative, microcytic-hypochromic anaemia. The serum iron concentration is low, with a decrease in the serum ferritin levels. Serum ferritin levels are low in dietary iron deficiency and are elevated in ACD and has been of advantage in distinguishing both types of anaemias (Refer to Table 7) (Stone and Freden, 1990). The above deficiency states and some other conditions may be associated with dyserythropoiesis.

Dyserythropoiesis refers to a qualitative abnormal erythroid maturation in the bone marrow. This is characterised by morphological changes such as multiple nuclear fragmentation, megaloblastic nuclear morphology, very large nuclei, very small erythroblasts and bi-and multinucleated cells due to incomplete cell division. Mild or moderate degrees of dyserythropoiesis may be seen in a number of deficiency states, including vitamin B12, folic acid and iron (Weiss and Reidarson, 1989). Dyserythropoiesis may also be seen in cases of DiGulielmo's syndrome, pre-leukaemic states or acute leukaemias (Evans *et al.*, 1987; Weiss, 1985b). In the latter conditions dyserythropoiesis is only a part of the basic disease process.

Table 7: Haematological comparison of ACD and IDA

Parameters	Iron Deficiency	Chronic Disease
Red cell morphology	Microcytic-hypochromic	Normocytic-normochromic or hypochromic
Serum iron	Decreased	Decreased
Serum ferritin	Decreased	Increased or normal
Total iron binding capacity (TIBC)*	Increased (in other species) or normal in dogs	Decreased or normal
Marrow iron stores	Decreased or absent	High or normal

*Total iron binding capacity = a measure of serum transferrin content based on iron stores. (Adapted from Stone and Freden, 1990).

Anaemia of chronic renal failure (CRF)

The renal cortex is the primary site for the production of the glycoprotein hormone erythropoietin (Epo) which is essential for stimulating erythropoiesis (Krantz, 1991). The kidneys are susceptible to a number of toxins and ischaemic injuries. The number of pathological conditions affecting the kidneys are in many forms which may or may not involve the entire kidney but ultimately may result in acute or chronic renal failure. Renal failure results in an irreversible damage to the nephrons and the prognosis of any case would depend on the extent to which the nephrons are damaged and the functional activity of the kidneys is impaired.

Anaemia is the most common sequel of chronic renal failure in humans beings as well as in dogs (Erslev and Caro, 1990; King *et al.*, 1992). The aetiology of anaemia in chronic renal failure is multifactorial and the deficiency of Epo is of major importance. There are other factors such as haemolysis and toxins which synergistically may contribute to the anaemia.

(A) Haemolysis

The life span of the erythrocytes in patients with chronic renal disease may be shorter than normal (Erslev and Caro, 1990). If this is observed in affected human patients the haemolytic uraemic syndrome should be considered. The anaemia observed is regenerative hence different from the majority of anaemias seen in CRF. This condition is caused by the deposition of fibrin and microthrombi in the glomerular capillaries and arterioles, the cause of which may be the result of viral or bacterial infection. There is also renal cortical necrosis, swelling and fragmentation of the glomerular basement membrane, resulting in a severe MAHA with regenerative features and marked haemolysis (Hammond and Lieberman, 1970). This syndrome of

man has been recently described in three dogs by Holloway *et al.*, (1993). Features in dogs were similar to those seen in man.

(B) Uraemic toxins

Originally, it had been though that several inhibitors of erythropoiesis were present in case of uraemia. Although the mechanisms for the mild haemolysis still remains unclear in affected subjects, no uniform correlation between blood urea or creatinine levels and red cell survival has been established (Eschbach *et al.*, 1990; Eschbach, 1980).

Uraemic metabolites were thought to be responsible for causing erythrocyte changes such as abnormalities in glycolysis and membrane changes leading to burr cell or acanthocyte formation (discussed later) (Erslev and Caro, 1990). It has also been suggested that there is an abnormality of the pentose-phosphate shunt preventing the glutathione balance to move towards the reduced form resulting in an increase in the oxidising process and haemolysis (Cambi and David, 1994). However, haemolysis according to Eschbach and Adamson (1985) cannot be the major cause of anaemia since the erythroid marrow can compensate for a mild degree of haemolysis.

Previously it was also believed that there were inhibitors of erythropoiesis such as spermine, spermidine and parathyroid hormone which were contributing to the anaemia (Radtke *et al.*, 1981; Petrites-Murphy *et al.*, 1989). Spermine and spermidine, however, were shown to be not specific for erythropoiesis in vivo (Cambi and David, 1994). According to King *et al.*, (1992), and Eschbach *et al.*, (1990), in man and dogs, hyperparathyroidism perhaps may co-relate with the severity of anaemia which is proportional to the extent of renal failure.

Another condition, osteitis fibrosa, a complication of hyperparathyroidism, may also be present which may reduce the marrow space. It resolves after

parathyroidectomy (Boxer *et al.*, 1977). If fibrosis becomes severe it can lead to pancytopenia and to EMH and associated splenomegaly (Cambi and David, 1994; Eschbach and Adamson, 1985).

Recently, Eschbach *et al.*, (1990), King *et al.*, (1992), Eschbach and Adamson (1985), and many other researchers summarised the causes of anaemia associated with renal failure and have proposed that the anaemia is primarily due to the lack of Epo. According to Eschbach *et al.*, (1990), there is sufficient evidence that uraemic toxins do not play any significant role as the anaemia improves in vivo after Epo therapy. However, a raised parathyroid hormone level may be significant (Cambi and David, 1994).

(C) Decreased Epo production

Erythropoietin is the primary regulator of erythropoiesis and is secreted by the peritubular capillary lining cells of the kidneys. The production of Epo is inversely related to the oxygen availability to the kidneys (Adamson, 1994). Most anaemic and uraemic patients do not have measurable amounts of Epo in CRF, in contrast to other anaemic conditions, which have detectable high amounts. With the recent development of rHuEpo and its role in the management of anaemia in human CRF, has increased the interest in its application in animals too (Giger, 1992; Bloomberg *et al.*, 1992). Erythropoietin therapy is known to be effective in human patients and in animals in correcting the anaemia of CRF without affecting the renal function, although there is an associated side effect (Refer article 1.3) (Cowgill, 1992b; Giger, 1992).

The levels of iron have also been known to decrease in renal failure. Approximately one half of the dogs and cats with anaemia of CRF have low serum iron concentration and a transferrin saturation below 15 percent (Cowgill, 1992b). It

may occur due to continued repetitive losses of blood associated with uraemic gastritis in which iron loss may exceed the absorption from the diet. In such patients iron must be provided to avoid iron deficiency anaemia (Cambi and David, 1994; Eschbach and Adamson, 1985). In addition to iron deficiency, folate deficiency, malnutrition, blood loss and loss of amino acids during dialysis, aluminium toxicity and hypersplenism may be certain complications (Cambi and David 1994).

Haematological observations

The anaemia of CRF is normocytic-normochromic and non-regenerative implicating impaired erythropoiesis. In a study be King *et al.*, (1992), 71 percent of the dogs with CRF had a normocytic-normochromic anaemia. The anaemia was from a mild to moderate severity with the lowest PCV of 22 percent. Usually, the anaemia is more severe in younger animals than in older ones. Sometimes the anaemia may be masked by dehydration.

The leucocyte and platelet numbers are usually normal but may be affected by the increasing uraemia and in man by dialysis (Erslev and Caro, 1990). Acanthocytes may be present in uraemia which may be caused by the uraemic metabolites affecting the surface of the erythrocytes (Erslev and Caro, 1990). According to Erslev and Caro (1990), burr cell formation in uraemia may be due to artifacts because these type of cells are not always seen in the circulation. But according to Bell (1963), and Dacie and Lewis (1991b), burr cells (red cells with small spiny fragments) may be seen in uraemia.

Anaemia of chronic disease (ACD)

Anaemia of chronic disease (ACD) may be defined as the anaemia associated with chronic infections or inflammatory conditions, or neoplastic illnesses which are

not due to bleeding or marrow replacement by the tumour. It is one of the most frequently encountered types of anaemia as well in man as in dogs (Stone and Freden, 1990; Feldman and Kaneko, 1981; Cartwright, 1966). There are several mechanisms that are involved in the development of ACD which result in a slightly shortened red cell life-span, a moderate disturbance in the iron metabolism and impaired erythropoiesis (Erslev, 1990b; Cartwright, 1966).

The shortened red cell life span may be due to extracorpuscular factors, such as bacterial toxins. In most cases, however, the bone marrow fails to increase its cell production sufficiently and a mild anaemia ensues. The red cell production is inhibited for some unknown reasons (Cartwright, 1966).

In this type of anaemia there is a characteristic disturbance in the iron metabolism despite adequate iron stores (Feldman and Kaneko, 1981). Moderate amounts of iron are absorbed from the intestines in ACD. However, because there is a disturbance in the cellular mobilisation of iron from ferritin or haemosiderin to circulating transferrin, there is an increase in the ferritin levels within tissues and a reduction in the iron binding capacity which is the concentration of transferrin. This results in the reduction of the circulating iron pool for uptake by the erythroid precursors (Cartwright, 1966). During inflammation there is lactoferrin released by the neutrophils, which complexes with iron and is phagocytosed by macrophages which prevents erythroid precursors from obtaining iron. The ACD shows features of iron deficiency, but the degree of anaemia and the extent of hypochromasia or micoryctosis are rarely pronounced as it is in true iron deficiency anaemia (Erslev, 1990b). It seems likely that the low serum iron levels in ACD is caused by impaired release of iron from the monocyte-macrophage system and impaired re-utilisation

According to Means and Krantz (1992), the anaemia of chronic disease is a "bag of cytokines". The amount and release of Epo is not impaired in this condition but the moderate degree of anaemia observed may be due to factors having suppressive effects on the bone marrow. These factors may be a variety of cytokines such as IL-I, IL-6, TNFα, γIFN, that may be released during inflammation and infections causing inhibition of the erythropoiesis.

Haematological observations

The anaemia is moderate and the haemoglobin concentration is usually within normal range. The anaemia is usually non-regenerative, normocytic-normochromic, or it may be normocytic-hypochromic and occasionally microcytic-hypochromic. The severity of the anaemia is mild and usually not very progressive (Cartwright, 1966). The bone marrow is cellular with a normal or increased level of iron stores (Feldman and Kaneko, 1981). In inflammatory conditions there may be leucocytosis and a left shift may indicate a bacterial or fungal infection. But other causes of inflammation should not be ruled out (Kidd, 1991).

Hormonal deficiency anaemia

The anaemia in hormonal dysfunction is rarely of any clinical significance except to distinguish it from other causes of anaemia (Valli and Parry, 1995). Endocrinopathies such as hypothyroidism and Addison's disease are usually associated with a mild normocytic-normochromic, non-regenerative anaemia (Aufderheide, 1981).

Anaemia of malignancy

The commonest complication associated with neoplasia is the development of anaemia which may involve a number of mechanisms that may be primarily due to the malignancy or to complications of the malignancy or it may be the result of chemotherapy. The following types of anaemias may occur:

(i) Hypoproliferative anaemias for example: anaemia of chronic disease, myelophthisic anaemia.

(ii) Haemolytic anaemias: AIHA, MAHA.

(iii) Blood loss anaemias: acute and chronic.

The degree of the anaemia varies depending on the duration of the disease the mechanisms involved and on the type of malignancy. A mild anaemia of the chronic disease is frequently seen in cancer which may be overlooked and noticed only after the cancer is in a far advanced state (Zucker, 1985). In this type of anaemia, increased levels of IL-1, γIFN, TNF all seem to be involved indirectly in causing the suppression of erythropoiesis (Bick, 1995). Replacement of the bone marrow by leukaemic cells, myeloma cells or by metastatic tumour cells can also result in moderate or severe myelophthisic anaemia and other cytopenias (Cotran *et al.*, 1994a).

In man and also in dogs, neoplasia, myeloproliferative or lymphoproliferative disorders, especially chronic lymphocytic leukaemia, may trigger an immune-mediated haemolytic anaemia (Arbaje and Beltran, 1990; Evans and Gorman, 1987; Madewell and Feldman, 1980; Dodds, 1977). Microangiopathic haemolytic anaemia, due to widespread metastasis of carcinomas and particularly due to haemangiosarcomas may also occur (Rebar *et al.*, 1980; Madewell and Feldman 1980). Thrombocytopenia and

associated haemorrhagic anaemia has also been seen in neoplasia as a result of decreased production or increased consumption of platelets i.e. DIC, increased sequestration or immune-mediated destruction of platelets. In a study by Grindem *et al.*, (1994) 61 percent of the dogs had thrombocytopenia related to neoplasia.

Anaemia is frequently caused by chronic blood loss associated with gastrointestinal and urogenital tumours. Acute blood loss may occur after rupture of a haemangioma or haemangiosarcoma (Brown *et al.*, 1985).

Anti-cancer chemotherapy may suppress granulopoiesis and erythropoiesis as these drugs are meant to act on the rapidly dividing cells. The cells with shorter life span would decrease faster than the cells with longer life span such as the erythrocytes. These drugs may cause bone marrow hypoplasia resulting in agranulocytosis and subsequently in a non-regenerative anaemia (Zucker, 1985; Madewell and Feldman, 1980).

The haematological features may vary depending on the type of anaemia and the mechanisms involved. The anaemia may be non-regenerative, normocytic-normochromic or microcytic-hypochromic in case of defective iron utilisation and chronic blood loss. Acute blood loss, haemolytic anaemia and myelophthisic anaemias are usually normocytic or macrocytic and normochromic. Specific features may also be present such as schistocytes in MAHA, spherocytes in AIHA, nucleated red cells in regenerative and myelophthisic anaemias (Madewell and Feldman, 1980).

CHAPTER 3

A DIAGNOSTIC APPROACH TO, AND THE CLINICAL MANIFESTATIONS OF ANAEMIA

3.1 DIAGNOSTIC APPROACH TO ANAEMIA

A systematic approach is essential to diagnose the cause of anaemia which includes obtaining all the information concerning the dog. Taking an accurate history, recording the age, breed and sex is the initial procedure. Identifying the overt clinical signs of anaemia by a thorough physical examination of the animal and taking a blood sample for a complete haematological and biochemical evaluation are the subsequent essential procedures. Other special investigatory tests, such as ultrasonography, radiology may be required to investigate for any organomegaly, infiltrative fluids or effusions, or any other underlying causes. Bone marrow examination may even be necessary in certain cases to assess the cellularity of the bone marrow and the estimation of the myeloid : erythroid ratio. The erythropoietic response to anaemia is best demonstrated by a reticulocyte count. Special tests such as direct or indirect Coombs' tests, clotting profile tests, soluble fibrin monomer complex test (SFMC) and von Willebrands factor (vWF) test may also be required.

When an anaemic patient is approached there are certain parameters such as the environment that needs to be considered. Taking a blood sample from the animal should be done carefully to avoid any stress and excitement because splenic contractions will force the reserve pool of erythrocytes into the circulation, causing a false increase in the PCV and plasma proteins which will mask the anaemia. Severe pain may also produce similar effects on the spleen (Schalm, 1970). Consideration of all these parameters and the presenting clinical signs can help to identify the aetiology and the pathogenesis of the anaemia in question.

3.1.1 History

The history of the animal is essential in any condition and can be very informative. Any history of travel should be considered significant. One of the causes of infectious haemolytic anaemia are the different kinds of parasites such as *Babesia canis or Babesia gibsoni, Haemobartonella canis. Babesia* a protozoal organism, can be acquired from different countries like U.S.A., Australia, Asia and Africa and can result in severe haemolytic anaemia due to the change on the erythrocyte surface by the parasitic organism (Farwell *et al.,* 1982). *Haemobartonella canis,* a rickettsial parasite, is geographically widespread but is of no pathogenic significance, although latent infections can be activated by splenectomy (Middleton *et al.,* 1982). Parasites such as *Dirofilaria immitis* (heartworm) may be acquired from areas of high prevalence which may also cause anaemia in affected dogs.

All these conditions are seldom observed in the United Kingdom and are observed with a higher incidence in other countries. Parasitic infections have been reported to have higher incidence in splenectomised dogs therefore it is important to obtain information regarding any surgery (Middleton *et al.,* 1982).

Any information whether the dog has had any access to poisons or medications is equally essential to be obtained from the owner. Drugs such as corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDS), have been known to cause gastrointestinal blood loss due to ulcerations (Stanton and Bright, 1989). Oestrogen compounds are used frequently and have been known to cause adverse effects on the bone marrow resulting in pancytopenia, although in many cases thrombocytopenia and associated haemorrhagic problems will be the first presenting signs, which may progress to severe marrow aplasia (Hall, 1992). Phenylbutazone,

trimethoprim-sulphadiazine and some other drugs have also been reported to cause severe aplastic anaemia (Weiss, 1992; Weiss and Adams, 1987).

In dogs, methylene blue has been used for identification of parathyroid gland and pancreatic islet-cell tumours in surgical procedures. It is essential to know whether any infusions of this dye has been carried out as it causes severe Heinz body haemolytic anaemia (Fingeroth *et al.*, 1988). It is also necessary to know whether the dog has ingested any foreign objects. Ingestion of coins been reported to result in zinc intoxication, causing a regenerative Heinz body haemolytic anaemia (Luttgen *et al.*, 1990). Onions may be fed by owners to their pets which may lead to adverse effects of a severe Heinz body haemolytic anaemia, due to the toxic factor n-propyl disulphide and presence of haemoglobin, urobilinogen and bilirubin may be observed in the urine (Spice, 1976).

Any recent exposure to vaccines may be of significance since, according to Dodds (1983), there is a possibility that modified live virus vaccines may be the cause of immune mediated haemolytic anaemia. Although this has not been substantiated the possibility should not be ruled out in the history of the dog. In man, an acute transient warm antibody autoimmune haemolytic anaemia can develop in some patients as a sequel to an acute viral infection (Dacie, 1992b). The owners should also be asked if they have observed any overt signs of haemorrhages such as epistaxis melaena, haematuria, which may help to identify the underlying cause. Dehydration associated with pyrexia, persistent vomiting or diarrhoea should be recorded and considered when interpreting laboratory results because dehydration may mask the presence or severity of anaemia.

3.1.2 The Role of Age, Breed and Sex

According to Lee *et al.*, (1976), there is a great amount of destruction of foetal erythrocytes during the first two weeks after birth which seems to result in a neonatal anaemia. The anaemia appears to be in all puppies under ordinary nursing conditions which eventually disappears as the pup grows. In their study, they observed that dogs between birth and one week of age, had a decrease in their blood volumes, whereas the plasma volume remained unchanged. However, from one week to 2-3 months of age, the total blood volume and plasma volumes did not change markedly. Chronic haemorrhage and an associated iron deficiency anaemia is more likely to be caused by tumours in older animals whereas in younger ones especially puppies, hookworm and flea infestations may be the primary cause (Harvey *et al.*, 1982).

Age, breed and sex seem to play an important role in the development of certain type of diseases. For example, the onset of immune-mediated anaemia is found to be between 4 to 5 years; in other words it occurs mainly in middle age dogs, although it has also been observed in puppies (Dodds, 1983). It is known that chronic renal failure and certain neoplasias have higher incidence in older dogs for example, Moulton and Harvey (1990), have observed lymphosarcomas more often between the ages 5 to 11 years indicating that age must be considered.

The incidence of haemangiosarcoma has been reported to be higher in German Shepherd dogs than in other breeds (Pearson and Head, 1976; Kleine *et al.*, 1970; Waller and Rubarth, 1967). The number of German Shepherds affected with haemangiosarcomas are more than the number of Boxers, although the latter are known to be more prone to neoplasia (Moulton and Harvey, 1990; Kleine *et al.*, 1970; Waller and Rubarth, 1967). German Shepherd dogs are also known to have a

higher incidence of Factor VIII (Haemophilia A) deficiency which causes severe bleeding episodes in affected animals which if uncontrolled may lead to anaemia (Littlewood, 1992). A rare inherited lethal disease, cyclic neutropenia has only been observed in very young Grey Collies (Lange *et al.,* 1976). Thrombocytopenia and anaemia may complicate the neutropenic episodes in terminal cases. According to Bennett (1984) and Dodds (1983), immunological disorders such as immune-mediated anaemia occur more often in females, spayed or intact due to stress from pregnancy and hormonal imbalances may precipitate the conditions.

If a young dog is presented with a long history of bleeding episodes which have been difficult to control, inherited haemostatic disorders should be considered. There are a number of rare hereditary conditions which have been recognised in dogs some having similarity to their human counterparts. Inherited coagulopathies have been observed in dogs which can be recognised at an early age, during vaccinations or eruption of teeth or minor surgical procedures. Von Willebrand's disease (vWF) an inherited bleeding disorder with an autosomal dominant pattern has been identified in a number of breeds (Littlewood, 1992). Haemophilia A (Factor VIII deficiency) a rare inherited bleeding disorder has been reported in many breeds of dogs. It has a sex-linked recessive pattern so only males are affected with abnormal bleeding tendency and heterozygote females are carriers and asymptomatic (Littlewood, 1992). A coagulation screening and if necessary specific tests, must be performed and if possible the parents and litter mates of affected dogs should also be checked in order to avoid breeding from the affected animals.

Rare enzyme deficiencies such as phosphofructokinase deficiency and pyruvate kinase deficiency have also been observed in young dogs which are similar to the conditions observed in humans (Giger and Harvey, 1987; Searcy *et al.,* 1971).

3.2 CLINICAL MANIFESTATIONS OF ANAEMIA

The clinical signs associated with anaemia are a result of the reduced oxygen carrying capacity of the blood and physiological adjustments which are designed to increase the efficiency of the remaining erythron, to reduce the workload on the heart and direct the blood to the vital organs (Erslev, 1990a). The consequence of anaemia if sufficiently severe or acute in onset may lead to a marked decrease in tissue oxygenation resulting in a variety of clinical signs.

The development of clinical signs depends greatly on the rapidity of onset, degree, and cause of anaemia (Evans *et al.,* 1987). In dogs with an acute blood loss of 30 to 40 percent, which may be substantial and sudden in onset, there is a decrease in the circulating blood volume resulting in a state of hypovolemic shock (Feldman, 1981). The loss of plasma and red cell mass is in proportionate volume. If the loss is more than 50 percent, muscular weakness, subnormal temperature, terminal respiratory distress, increase in the heart rate and stroke volume to reduce the time taken for circulation by red cells, coma and death may ensue (Rogers, 1995). The plasma volume expansion takes place slowly by mobilisation of the extravascular albumin pool producing a haemodilution resulting in anaemia. Due to this haemodynamic change, the skin and especially the extremities may become pale and cold as a result of peripheral capillary vasoconstriction in an attempt to direct blood to the vital organs to compensate for the ongoing anaemia (Hillman, 1990, Wintrobe *et al.*, 1974).

In chronic blood loss anaemia or in other chronic type of anaemias, whatever the type and cause, signs apart from paleness may not be so evident because the

animal will gradually be able to adapt to the anaemia. Symptoms seem to be more prominent in acutely developing anaemias and in aged patients.

Initially, an increase in Epo production takes place due to tissue hypoxia except in chronic renal failure. This increase in Epo level results in increased erythropoiesis in an attempt to maintain normal numbers of erythrocytes. In human beings, this may be associated with tenderness of the bones and with sternal pain or aches although in animals it may be expressed differently (Erslev, 1990a). There is also a slight decrease in the urinary output as a result of a decrease in the renal blood flow pressure. Even in severe anaemia with the renal blood flow reduced by 50 percent, the renal plasma flow is only moderately curtailed (Erslev, 1990a). Within the next few days of a blood loss, the blood volume may be restored by an influx of electrolytes and proteins which result in an increase in the plasma volume.

While examining a dog, it is essential to identify the overt signs of anaemia which may be helpful to approach a diagnosis. The most commonly recognised signs associated with anaemia are pallor, cardiac murmurs, respiratory symptoms, tachycardia, dizziness or weakness, fatigue, fainting episodes and occasionally jaundice. Seizures have also been observed in a dog due to a chronic blood loss anaemia caused by parasitism. (Curtis, 1977).

3.2.1 Pallor

Pallor has been known to be the most prominent and characteristic sign of anaemia. Pallor is the result of peripheral capillary constriction which is an attempt to direct blood to the vital organs (Wintrobe *et al.*, 1974). Paleness may be mild, moderate or severe depending on the degree and onset of anaemia. Pallor in animals

may be observed on the non pigmented areas of the body such as the mucous membranes, conjuctivae, tongue, gums, vulva, penis and underlying skin. In dogs the pigmentation of the skin may make the diagnosis difficult. On one hand, absence of pallor does not necessarily indicate that anaemia is not present, whereas on the other hand, pallor may be as a result of shock or factors affecting the general circulation (Evans *et al.*, 1987). It is known that jaundice and cyanosis may mask the pallor associated with anaemia, hence it is essential to obtain accurate haematological values for the detection and quantification and characterisation of anaemia (Wintrobe *et al.*, 1974).

3.2.2 Cardiovascular and Respiratory Signs

When PCV is very low a soft systolic "haemic murmur" may be evident due to an increased velocity of blood flow and a decreased viscosity which is known to disappear when anaemia is corrected. In a study of 72 human patients with acute and chronic anaemias, murmurs were present in two thirds of the patients and these murmurs were increasing as haemoglobin levels decreased and correction of cardiac murmurs was achieved by reducing the anaemia (Dawson and Palmer, 1966). In cases of severe anaemia, the murmurs are stronger due to mitral and tricuspid insufficiency resulting from cardiac dilatation (Wintrobe *et al.*, 1974). Muffled heart sounds or abdominal distension may indicate bleeding into the thoracic and or peritoneal cavities and one may consider the possibility of a bleeding tumour (Weiser, 1995).

When anaemia is sufficiently severe signs of dyspnoea, tachypnoea, tachycardia and exercise intolerance, lethargy and weakness due to insufficient

oxygenation are observed (Valli and Parry, 1993). Cerebral hypoxia and marked tachycardia can cause signs of fainting and dizziness (Wintrobe *et al.,* 1974).

3.2.3 Jaundice

It is understood that bilirubin produced by the catabolism of haemoglobin imparts a yellow colour to the plasma. Excess haemolysis may result in the accumulation of unconjugated bilirubin in the plasma causing yellow discoloration of the mucous membranes. According to Jones and Gruffydd-Jones (1991), clinical signs such as jaundice, haemoglobinuria and collapse together could be indicative of an acute haemolytic "crisis" but not all dogs may show these typical signs. It has been observed by Cotter (1995), that destruction of four grams of haemoglobin in 24 hours may cause jaundice even in the presence of normal liver functions. The cause of jaundice must be identified because persisting jaundice may also be due to primary or secondary hepatobiliary disease.

3.2.4 Other Relevant Signs

Any evidence of overt haemorrhage such as haematemesis, melaena, haematuria, epistaxis, haemarthrosis or subcutaneous haemorrhage (petechiae and ecchymoses) must be identified. Examination of the occular fundus may reveal pallor of the retinal vessels in severe anaemia and retinal haemorrhages may also be present (Evans *et al.,* 1987). In human beings, retinal haemorrhages are frequently observed due to aplastic anaemia, pernicious anaemia as well as in anaemia due to leukaemia. Also in human beings, gastrointestinal signs of anorexia, abdominal discomfort, nausea, vomiting, constipation or diarrhoea may be observed in anaemic patients

which however can probably be related to the underlying gastrointestinal disease condition causing the anaemia (Wintrobe *et al.*, 1974).

In dogs these signs are also frequently observed, although dogs tend to get anorexic in many disease conditions without being anaemic. Fever may be present which is often associated with infectious, inflammatory or neoplastic diseases. Organomegaly, if present, may be associated with haemorrhage, neoplastic, infiltrative disease or may be due to extramedullary haemopoiesis in case of the spleen and the liver (Rogers, 1995). Skin lesions, lameness, joint swellings due to autoimmune polyarthritis may also be present in anaemic dogs (Evans *et al.*, 1987). A dermatological examination is necessary for identifying the presence or absence of ectoparasites such as fleas, lice or ticks as they may cause chronic blood loss anaemia and some of them are also responsible for the transmission of some erythrocytic parasites.

In chronic blood loss, the haemoglobin levels may decrease sufficiently without the animal exhibiting signs of hypoxia, unless exerted, making it difficult to diagnose. Regional and generalised lymphadenopathy should be investigated in order to identify the underlying reactive or neoplastic process such as lymphosarcoma or leukaemia. Anaemia in the latter may be the result of bone marrow infiltration by neoplastic cells (Squires, 1993).

Presence of a uraemic breath, oral ulcers, polydipsia, polyuria, dehydration may indicate chronic renal disease and uraemia which may be associated with signs of anaemia due to renal failure. The severity of the above signs may vary between individual animals depending on a number of factors, the pathogenesis and the aetiology of anaemia.

CHAPTER 4

RETROSPECTIVE STUDY OF ANAEMIA IN DOGS DURING THE YEAR 1994

4.1 INTRODUCTION

Anaemia is a clinical sign observed in many diseases and results from an increased rate of destruction of erythrocytes or from loss of erythrocytes or from a decreased rate of production of erythrocytes. Anaemia can be primary or secondary and can cause serious clinical diseases. In addition, anaemia is one of the most frequently encountered laboratory abnormalities in veterinary medicine (Squires, 1993). Once anaemia is detected in an animal its pathogenesis and cause should be thoroughly investigated to determine proper therapeutic management.

The purpose of this study was to investigate the causes of anaemia as represented by the dogs referred to the clinicians at the University of Glasgow Veterinary School during 1994.

These cases were investigated by analysing the clinical diagnosis, haematological findings, and post-mortem reports of the referred cases.

4.2 MATERIAL AND METHODS

4.2.1 Animals

During the period 1st January to 31st December in 1994, 830 dogs were referred to the Veterinary Medicine and Veterinary Surgery Departments of the University of Glasgow Veterinary School. Every dog admitted was given a case number for reference which for the purpose of this thesis was designated by a dog number (DN). A history was obtained for each dog and a clinical examination was carried out by the clinicians responsible for the case. A diagnostic workup was done for most of the dogs which involved a thorough clinical examination along with the

collection of blood samples for biochemistry, haematology, and other diagnostic tests when necessary. All the 830 dogs admitted to the Veterinary School had at least one blood sample taken. The samples from these 830 dogs were submitted to the Veterinary Haematology Laboratory of the Department of Veterinary Pathology for haematological examination.

Of these 830 dogs, 245 had PCV of less than 37% and were therefore considered to be anaemic. These 245 dogs formed the basis of this retrospective study. Of these 245 anaemic dogs with PCV of less than 37%, 52 dogs were studied in more detail since they had undergone a full post-mortem examination in the Department of Veterinary Pathology. The following data were obtained on the 52 anaemic dogs that were examined post-mortem:- breed, age, sex, haematological findings, clinical findings and post-mortem findings. These are listed in Appendix B and Appendix C.

4.2.2 Database Studies

The hospital computing system was used for retrieving the data regarding the dogs admitted in 1994. Every dog admitted was assigned a case reference number which was entered into the computing system of the University of Glasgow Veterinary Hospital Database, with the details of the owner and details about the dog such as age, breed, species, sex. The address of the referring veterinarian and the clinician to be in charge of the case were also entered. A letter from the referring veterinarian was very useful since it provided a brief summary of the previous ailments of the dog which was helpful in the clinical examination. Additional details regarding the dog were not entered on the database but were kept in files stored, in

case reference number sequence, at the case reception where further details regarding the dog could be obtained.

A laboratory reference number was given by the Haematology Laboratory of the Department of Veterinary Pathology to every sample for each day then the data for every blood sample from the dogs was entered in the haematology laboratory database, called "Dataflex". Records of each dog were kept in this database which was accessed to obtain the haematological details of the dogs in this study. The features of the haematology details of "Dataflex" are shown in Figure 16. The post-mortem reports were obtained through the "Dataflex" a database of the Pathology Department. The "Dataflex" is a computer software designed individually for some of the departments.

4.2.3 Haematological Examination

All the blood samples followed the same procedure in the haematology laboratory. Every blood sample (2-3ml) was collected by the clinicians from the veins of the dogs in a potassium ethylene diamine tetra-acetic acid (KE-EDTA) tube, (SARSTEDT, Germany) and details of the name of the dog, date of birth, case reference number, date and time of sample collection were recorded on each tube. A haematological form accompanied each blood sample sent to the laboratory. The above details of the dog and a clinical summary were written on the form which were helpful in providing a diagnosis. Sometimes, this clinical summary was the final diagnosis but often only the clinical signs or a tentative diagnosis was written on the forms. After the blood tubes and forms were received in the laboratory they were given a separate laboratory reference number.

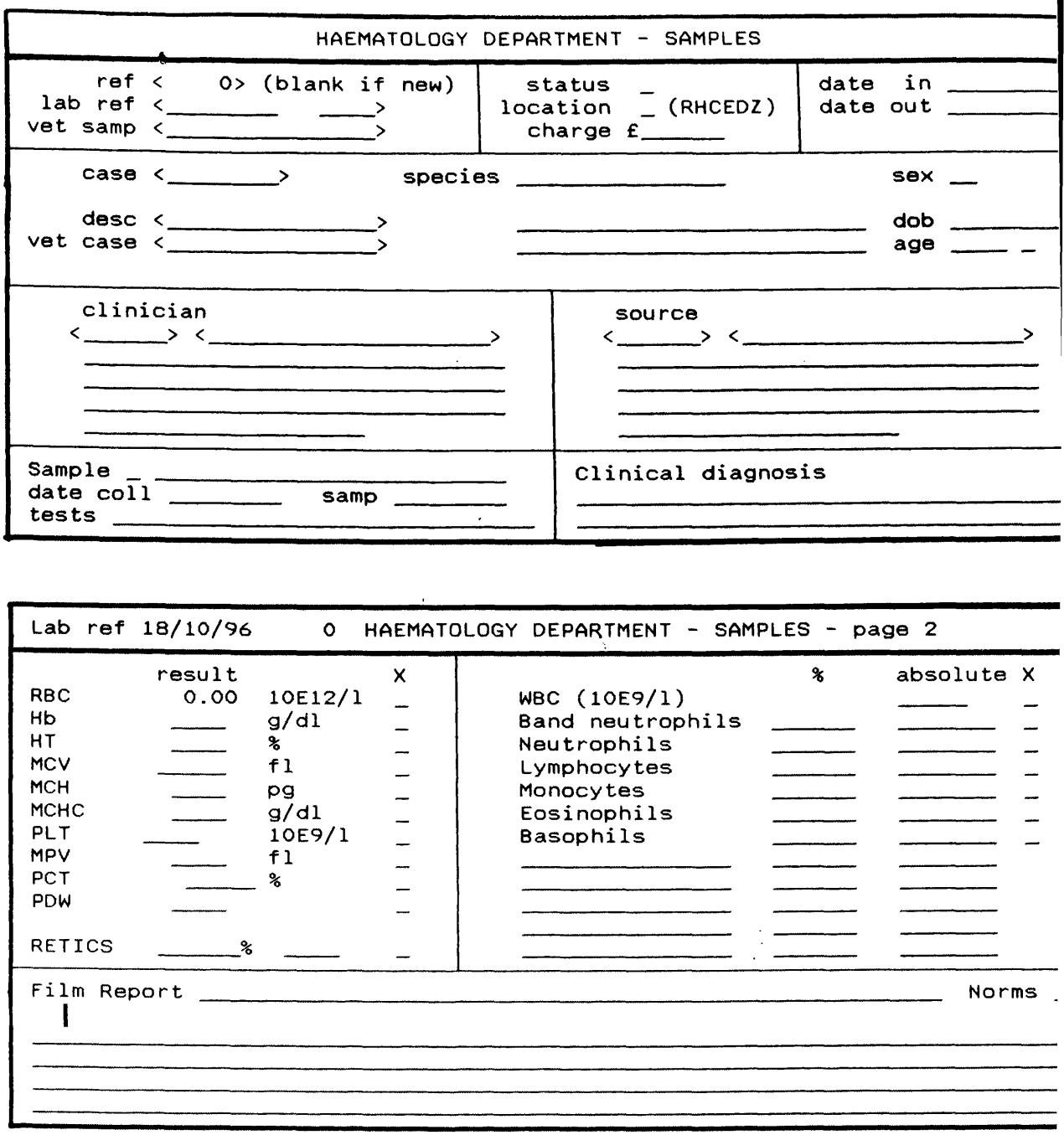

Figure 16: The display on the screen of the Haematology Laboratory Dataflex database is shown describing some of the essential details of the animal that are entered into the database.

The tubes were allowed to mix for about 10 minutes on a mixer so that the anticoagulant mixed well with the blood and aggregation of the blood cells was avoided. A blood smear was then made from every sample using a microcapillary tube of dimensions 75x 1.3-1.5 mm.(technique is described on page 292). On the smear the laboratory reference number, date and case reference number were written. The smears were air dried and then stained using the May Grünwald Giemsa staining procedures (Appendix A). From the remaining sample 25 µl of blood was aspirated for a complete blood count (CBC) profile, using the Roche ABX Minos Veterinary Automated Haematology Analyser. The haematological values and a graph of the platelet distribution width (PDW) for that particular case was produced by an attached printer. After the smears were stained and dried the differential white cell count of 200 cells count was carried out manually and entered into the computer which was connected to another printer. The results from the Roche ABX Minos Veterinary Automated Haematology Analyser were transferred onto the computer for the haematology database "Dataflex", thus producing a complete record of the results.

Morphological examination of every blood smear was also done as a routine procedure by the haematologist and any significant observations were recorded (listed in Appendix B). A reticulocyte count was done manually using a supravital stain of new methylene blue only if requested by the clinician or if it was thought to be necessary by the haematologist.

The following haematological parameters were obtained in the routine procedures:- red blood cells ($RBCx10^{12}/l$), haemoglobin (Hbg/dl), packed cell volume (PCV%), mean corpuscular volume (MCVfl), mean corpuscular haemoglobin (MCHpg), mean corpuscular haemoglobin concentration (MCHCg/dl), platelets

(PLTx10^9/l), mean platelet volume (MPVfl), platelet crit (PCT%), platelet distribution width (PDW), reticulocytes (Reticsx10^{12}/l), white blood cells (WBCx10^9/l), band neutrophils (BNx10^9/l), neutrophils (Neutrox10^9/l), lymphocytes (Lymphox10^9/l), monocytes (Monox10^9/l), eosinophils (Eosx10^9/l), basophils (Basox10^9/l), normoblasts (x10^9/l) Nucleated red cells were included in the total white cell count. However, the platelet parameters (MPV, PCT and PDW) were not considered as a part of this study.

For some dogs depending on their clinical presentation the following special tests were carried out with other tests when necessary:- Direct Coombs' test (ICN Biomedicals, California), detection of soluble fibrinogen monomer complexes (SFMC) (Diagnostica Stago, France), estimation of fibrin degradation products (FDP) (Boehringer Mannheim, United Kingdom),

4.2.4 Criteria for Selection and Classification of Cases

The normal range of canine PCV was considered to be 37%-55%. A dog with a PCV less than 37% was considered to be anaemic. The following criteria were used to classify the severity of anaemia : i) PCV of 36.9%-30% was considered to indicate mild anaemia , ii) PCV of 29.9%-20% was considered to indicate moderate anaemia, iii) PCV of 19.9% and less was considered to indicate severe anaemia.

In cases from which multiple samples were taken the lowest PCV value was used to classify the dogs according to these criteria. When the post-mortem findings were available, the main cause contributing to the anaemia was investigated in more detail.

4.3 RESULTS

There were 830 dogs in 1994 from which blood samples had been taken between 1st January and 31st December. From these 830 dogs, 245 (29.5%) had PCV of less than 37% and were considered to be anaemic according to the criteria stated above. When these anaemic dogs were classified according to the criteria used to grade the severity of the anaemia, the following results were obtained. The results are illustrated in the Table 8. There were 155 (63.3%) which were mildly anaemic, 65 (26.6%) which were moderately anaemic and 25 (10.1%) which were severely anaemic.

When the 245 anaemic dogs, which had been examined clinically in the University of Glasgow Veterinary School in 1994, were classified according to whether they had a regenerative or non-regenerative anaemia, based on the blood picture and other data available, the following results were obtained. There were 74 (30.2%) dogs with a regenerative anaemia and 161 (65.7%) with a non-regenerative anaemia. No data was available to classify the anaemia in 10 (4.1%) dogs from the 245. Of the 74 dogs with regenerative anaemia, 55 (22.4%) dogs were found to have haemorrhagic anaemia and 18 (7.3%) had haemolytic anaemia. In the major proportion (65.7%) of the 161 dogs which had a non-regenerative anaemia, the anaemia was due to a variety of chronic conditions and infections. However, 11 (4.5%) of these dogs had a non-regenerative anaemia due to renal disease and these dogs were considered separately.These results have been tabulated in the Appendix B.

Fifty two anaemic dogs were studied in more detail since there had been a post-mortem examination result to confirm the diagnosis and to explain the underlying cause of the anaemia. This group of 52 anaemic dogs was designated as

Table 8: Degree of Anaemia in 245 Dogs Studied in the University of Glasgow Veterinary School in 1994

Anaemia	Number of Dogs
Mild	155 (63.3%)
Moderate	65 (26.6%)
Severe	25 (10.1%)

Table 9 : Degree of Anaemia in the 52 Dogs Studied in Group A (Anaemic dogs necropsied)

Anaemia	Number of Dogs
Mild	26(50.0%)
Moderate	17(32.7%)
Severe	9(17.3%)

Group A for further reference. These 52 anaemic dogs (Group A) were classified according to the severity of the anaemia by the criteria already stated and there were 26 (50.0%) dogs which were mildly anaemic, 17 (32.7%) moderately anaemic, and there were 9 (17.3%) dogs which were severely anaemic. These results are illustrated in Table 9.

4.3.1 Breed

In Group A, anaemia was most commonly seen in the following breeds : Cocker Spaniels 9(17.3%), Golden Retrievers 8(15.3%), German Shepherd dogs 8(15.38%), Bull Terriers 6(11.53%) and Rottweilers 5(9.61%), 16(30.7%) dogs were other breeds. These results are illustrated in Table 10. The other breeds were represented by one or two animals and these are all listed in the Tables 12, 13 and 14 respectively.

4.3.2 Age

Anaemia of varying degrees is a clinical sign which can be observed at any age and the aetiology of anaemia is often different in different ages of dogs. Consequently, the dogs in Group A were categorised into three age groups, less than 1 to 5 years, young dogs (Group A1); 6 to 10 years, middle aged dogs (Group A2); and dogs of more than 10 years, old dogs (Group A3). This facilitated the study of the causes of anaemia at different stages of the dogs life.

There were 16 dogs (30.7%) which were anaemic between the ages less than 1 to 5 years, 25 dogs (48.1%) which were anaemic between the ages of 6 to 10 years

Table 10: Breeds of 52 Anaemic Dogs Studied in Group A (Anaemic dogs necropsied)

Breed	Number of dog
Cocker Spaniels	9 (17.3%)
Golden Retrievers	8(15.3%)
German Shepherd dogs	8(15.38%)
Bull Terriers	6(11.5%)
Rottweilers	5(9.6%)
Others	16(30.7%)

Table 11: Ages of 52 Anaemic Dogs of Group A

Ages of anaemic dogs.	Number of dogs
up to 5 years (Group A1)	16(30.7%)
6 to 10 years (Group A2)	25(48.1%)
Over 10 years (Group A3)	11(21.2%)

and there were 11 dogs (21.2%) which were anaemic in the age group of more than 10 years. These results are illustrated in Table 11.

4.3.3 Diseases Associated with Anaemia in Group A Dogs in Relation to their Age

Group A1

In the young dogs comprising of Group A1 there were 16 animals, 30.7% of the dogs in Group A. Information on the age, sex and breed, degree of anaemia and the associated diseases found post-mortem in the dogs of Group A1 is listed in Table 12. The average age of this group was 3.3 years. The number of males which were anaemic was 8(50%) and there were 8(50%) anaemic female dogs. When the severity of anaemia was assessed it was seen that there were 10(62.5%) dogs which were mildly anaemic, 4(25%) which were moderately anaemic and only 2(12.5%) dogs which were severely anaemic.

From the pathological reports it was observed that there were five dogs that had renal problems which were considered significant. Three of these dogs, DN13, DN24 and DN230 were Bull Terriers with familial nephropathy which is a condition that has been recognised in this breed. Congenital renal failure was observed in one dog DN206, and one dog, DN133, had pyelonephritis. None of these five dogs were severely anaemic, but inevitably they all died of renal failure.

Two dogs, DN15 and DN142, had lymphosarcoma and of these one had a mild anaemia and the other dog was severely anaemic. There were two severely anaemic dogs, one dog, DN70, with a myeloid leukaemia and the other, DN141, with bone marrow hypoplasia. Cardiovascular disease was seen in two dogs, DN90 and

Table 12: Degree of Anaemia and Pathological Diagnosis in the 16 Group A Dogs up to 5 Years of Age (Group A1)

Dog No. (DN)	Age (yrs)	Sex	Breed	PCV(%) and Degree of Anaemia		Pathological Diagnosis
13	5.4	F	Bull Terrier	30	MI	Familial nephropathy
15	5.9	FS	Bull Terrier	35.6	MI	Multicentric lymphosarcoma, urolithiasis and pulmonary calcinosis
24	2	F	Bull Terrier	31.3	MI	Familial nephropathy
33	1	M	Bull Terrier	32.3	MI	Lethal acrodermatitis
54	2.9	M	German Shepherd dog	30	MI	Small intestine volvulus
55	1.9	FS	Cocker Spaniel	33.6	MI	Sarcoptic mange, focal chronic pneumonia
70	3.5	M	Highland Terrier	27	MO	Myeloid leukaemia
81	5.3	F	German Shepherd dog	33.9	MI	Pancreatic hypoplasia
90	4	M	Cocker Spaniel	32	MI	Dilated cardiomyopathy, pulmonary and systemic thromboembolism
105	1.4	F	Retriever	35.9	MI	Portocaval shunt
133	4	M	Cavalier King Charles Spaniel	22.1	MO	Chronic pyelonephritis, uraemic gastritis
139	4	M	Cocker Spaniel	23.7	MO	AITP
141	5.2	FS	Rottweiler	7.9	S	Bone marrow hypoplasia
142	5.8	M	Cross bred dog	19.1	S	Multicentric lymphoblastic lymphosarcoma
206	0.75	M	Springer Spaniel	28.7	MO	Congenital renal failure
230	1	F	Bull Terrier	30	MI	Familial nephropathy

Abbreviations

M = Male F = Female F (S) = Female Spayed MI = Mild anaemia

MO = Moderate anaemia S = Severe anaemia DN = Dog number

AITP= autoimmune thrombocytopenia

DN105, which were mildly anaemic. In this group, only one dog, DN139, was observed to have anaemia due to autoimmune thrombocytopenia.

It was observed that there were two dogs with dermatological problems DN33 and DN55. One dog, DN54, had small intestinal volvulus and was only mildly anaemic due to the sudden trauma. Pancreatic hypoplasia was observed in a mildly anaemic dog, DN81.

Group A2

In the middle aged dogs of Group A2, there were 25 anaemic dogs, 48.1% of all the dogs of Group A. The details of these cases and the diseases found post-mortem are given in Table 13. The average age for this group was 8.1 years. The number of male dogs were 13(52%) and there were 12(48%) female dogs. The dogs were further classified according to the severity of the anaemia. Nine dogs, that is 36%, were mildly anaemic, 10(40%) moderately anaemic and 6(24%) had severe anaemia. The severity of the anaemia was not related to any specific disease condition. There were three dogs, DN6, DN128 and DN238, with renal disease. One of these dogs, DN6, had familial nephropathy and it was a Bull Terrier.

Many of the dogs, 13 in total, had anaemia related to neoplasia. Four dogs had haemangiosarcoma, two of which, DN58 and DN88, had severe anaemia, and two dogs, DN190 and DN235, were moderately anaemic. Lymphosarcoma was present in three dogs, one dog, DN41, was mildly anaemic and two dogs, DN59 and DN71, were moderately anaemic. Three dogs had adenocarcinomas, one dog, DN149, had a tumour of the pituitary gland and the other two dogs, DN25 and DN49, had tumours of the pancreas. Of the other two dogs with neoplasms, one dog DN56, had a mast cell tumour and the other dog, DN214, had an osteosarcoma.

Table 13: Degree of Anaemia and Pathological Diagnosis in the 25 Group A Dogs Aged 6 to 10 Years (Group A2)

Dog No. (DN)	Age (yrs)	Sex	Breed	PCV(%) and Degree of Anaemia		Pathological Diagnosis
6	10.2	M	Bull Terrier	32	MI	Familial nephropathy
16	6.8	M	Golden Retriever	14.2	S	AIHA and DIC
25	7.8	M	Rottweiler	35.3	MI	Pancreatic adenocarcinoma
41	8.9	M	Cocker Spaniel	24.7	MO	Multicentric lymphosarcoma
45	8.7	FS	German Shepherd dog	24.7	MO	Endocarditis, chronic cystitis
48	7.1	M	Bearded Collie	12.2	S	Dyserythropoiesis, thymoma
49	8.6	M	Cocker Spaniel	34.3	MI	Pancreatic adenocarcinoma
56	8	FS	German Shepherd dog	28.3	MO	Mast cell tumour
58	8	FS	German Shepherd dog	10.5	S	Haemangiosarcoma
59	8	FS	Rottweiler	28	MO	Lymphosarcoma
71	7	F	Collie	33.3	MI	Multicentric lymphosarcoma, hypercalcaemic nephropathy
72	10	FS	German Shepherd dog	30.6	MI	Road traffic accident
88	9	M	Afghan Hound	19.4	S	Haemangiosarcoma
128	9	FS	Airedale Terrier	27.7	MO	Chronic glomerulonephritis
146	8	F	Cocker Spaniel	28.7	MO	Compressive myelopathy
149	8.1	FS	Great Dane	34.3	MI	Pituitary adenocarcinoma
157	8	M	Cocker Spaniel	27.7	MO	Pancreatic hyperplasia
170	9.5	MC	Cocker Spaniel	8.9	S	AITP
190	9	M	German Shepherd dog	29.7	MO	Haemangiosarcoma
199	6.6	FS	Spinone	5.2	S	AIHA
207	8	F	Rottweiler	21.7	MO	AITP
214	8.6	MC	Rottweiler	34.3	MI	Osteosarcoma
226	7	M	Retriever	36.3	MI	Spinal cord compression
235	6	MC	German Shepherd dog	25.4	MO	Haemangiosarcoma
238	9	FS	Border Collie	32	MI	Renal failure

Abbreviations

M = Male F = Female F (S) = Female Spayed MI = Mild anaemia

MO = Moderate anaemia S = Severe anaemia DN=Dog number

AIHA= autoimmune haemolytic anaemia AITP= autoimmune thrombocytopenia

All of these dogs were mildly anaemic except DN56, with mast cell tumour which was moderately anaemic.

Dyserythropoiesis was seen in DN48, with severe anaemia which also had a thymoma. Immune-mediated disease was observed in four dogs in this age group with three of them having severe anaemia and one having a moderate anaemia. A PCV as low as 5.2% was observed in one case DN199, which was the consequence of erythroid hypoplasia.

Anaemia was also observed in a case of endocarditis, DN45, associated with septicaemia. One case was observed with each of the following disease conditions:- road traffic accident, DN72, compressive myelopathy in DN146, pancreatic hyperplasia and spinal cord compression in dogs DN157 and DN226, respectively.

Group A3

There were 11 older dogs in Group A3 which was 21.2% of all the anaemic dogs in Group A, and Table 14, contains detailed information about these dogs.

The average age of this group of dogs was 12.1 years. There were 5(45.4%) males and 6 (54.4%) females in this group. The dogs were further classified according to the severity of the anaemia and there were 7(63.6%) dogs which were mildly anaemic, 3(27.2%) dogs which were moderately anaemic and 1(9%) dog was severely anaemic. There were five dogs with anaemia associated with chronic renal diseases. One dog DN52, with a protein losing nephropathy was severely anaemic and had the second lowest PCV value of 6.2% in this series.

There was one dog, DN93, with mild anaemia due to glomerulonephritis and another dog, DN10, anaemic due to chronic nephropathy. Severe infection causing

Table 14: Degree of Anaemia and Pathological Diagnosis in the 11 Group A Dogs Aged over 11 Years (Group A3)

Dog No. (DN)	Age (yrs)	Sex	Breed	PCV(%) and Degree of Anaemia		Pathological Diagnosis
4	15.2	M	Retriever	29.3	MO	Lymphosarcoma
10	12	FS	Retriever	35.6	MI	Chronic nephropathy, hepatic nodular hyperplasia
37	11.1	FS	Boxer	32.6	MI	Lymphosarcoma but died of gastric torsion
40	12.8	M	Jack Russell Terrier	27.7	MO	Chronic pyelonephritis, Sertoli cell tumour
52	11	FS	Cross bred dog	6.2	S	Protein losing nephropathy
93	11	F	Cairn Terrier	30	MI	Chronic glomerulonephritis, mural endocarditis, aortic thrombosis
124	12.3	M	Retriever	33	MI	Squamous cell carcinoma, endocarditis,chronic pyelonephritis, purulent cystitis
143	12	M	Newfoundland	32.6	MI	AITP
144	11	M	Retriever	32	MI	Acute cardiac failure
234	12	FS	Cross bred dog	25.4	MO	Hepatocutaenous syndrome
244	13	FS	Retriever	33.3	MI	Pulmonary adenocarcinoma

Abbreviations

M = Male F = Female F (S) = Female Spayed MI = Mild anaemia

MO = Moderate anaemia S = Severe anaemia DN = Dog number

AITP=auto-immune thrombocytopenia

pyelonephritis and cystitis was observed in DN40 and DN124. Lymphosarcoma was observed in two cases DN4 and DN37, although the latter, DN37, died of gastric torsion. Other cases of anaemia associated with neoplasia were DN244 with a pulmonary adenocarcinoma and DN124 with a squamous cell carcinoma.

Only one dog, DN143, had anaemia due to autoimmune thrombocytopenia. In one dog, DN234, a moderate anaemia due to hepatocutaenous syndrome was observed.

4.4 DISCUSSION

Epidemiology is the study of the distribution and dynamics of a disease in an animal population, with the objectives of identifying the prevalence of the disease and providing information on which to base preventive measures. For any disease it is important to understand its pattern of occurrence, factors associated with any predisposition to acquire the disease and the aetiology of the disease. The composition of the population in which the disease occurs is an essential element of any study. The population in this study was very selective, and was not a random sample of the general canine population. The dogs studied were referred to the University of Glasgow Veterinary School by veterinarians and this procedure would have created a bias towards certain types of cases. However, the hospital population of referred cases will give some idea about the types of anaemia found in the general population but will not necessarily reflect accurately the prevalence of disease in the population of the whole of the United Kingdom.

In an attempt to obtain information about the prevalence of anaemia in the canine population, particularly in the United Kingdom, a database search on the

"Silver Platter 3.0 and 3.11" VetCD (veterinary computer disc, © Silver Platter International N.V.), designed specifically for veterinary use was done. There were no reports of an overall review of the occurrence of anaemia in dogs in a large population. There were reports regarding the diagnosis of different types of anaemia in dogs for example Squires, (1993), Jain, (1993b) and Weiser, (1995). However, no study on the relative occurrence of the different types of anaemia to be expected the United Kingdom was found. There was one report by Hinton and Jones (1977), recording the analysis of blood samples from 1063 dogs, in the United Kingdom over a period of two years. Of these 1063 dogs, 203 (19.1%) dogs had anaemia (haemoglobin value of less than 11.9g/dl), but no further investigation was done regarding the aetiology of the anaemia and the type of anaemia present. In the work reported in this thesis, 29.5% of 830 dogs were found to have anaemia which was a higher prevalence than that reported by Hinton and Jones (1977).

Anaemia is known to occur as a primary condition or in association with a wide variety of diseases. Characterisation of anaemia frequently leads to identifying the underlying disease (Weiser, 1981). The types of anaemia found in this study were observed to reflect the types of anaemia that have been described in dogs already and recorded in the standard texts on the subject (Weiser, 1995; Valli and Parry, 1993; Schalm *et al.*, 1975c). In this study, certain commonly observed disease conditions were found to be associated with the anaemias usually seen in dogs, although, as was expected some conditions causing anaemia were not observed as they do not occur in the United Kingdom.

There was no case of a blood borne parasite such as babaesia or haemobartonella, causing haemolytic anaemia in any of the dogs studied. Although there have been cases of *Haemobartonella felis* affecting cats, very few cases of

Haemobartonella canis affecting dogs have been reported in the United Kingdom (Bobade, *et al.*, 1988; West, 1979). These infectious causes of haemolytic anaemia are known to have a high prevalence in dogs in tropical countries or in U.S.A. where there is a high tick population for transmission of the parasites (Valli and Parry, 1993).

Enzymopathies such as pyruvate kinase and phosphofructokinase deficiencies are conditions which result in haemolytic anaemia in young dogs of certain breeds and are not very commonly observed (Schaer *et al.*, 1992; Harvey and Smith, 1994). In this study, there were no dogs with any of these conditions. There was also no dog with anaemia due to an inherited coagulopathy, possibly because these conditions have been identified and breeding from animals with the causal genes has been avoided leading to a reduction in the prevalence of these conditions.

Drug-induced toxicity may cause anaemia, and oestrogen toxicity, which results in a severe pancytopenia, is a particularly well recognised problem which has been well documented in dogs (Shelly, 1988). No dog with anaemia due to oestrogen toxicity was observed in this study. This may also be due to an increased awareness of these problems, resulting in better drug administration.

In the 52 dogs studied in Group A, renal disease conditions were the commonest cause of anaemia occurring in 13 of the 52 dogs. The dogs with renal diseases were distributed in all the age groups, however, there were more cases in the very young dogs (Group A1) and in the older dogs (Group A3). The young dogs had mainly renal diseases such as familial nephropathies whereas, the older dogs had other conditions affecting the kidneys such as chronic pyelonephritis and chronic glomerulonephritis.

The next most common cause of anaemia were various neoplasms, which were observed more frequently in the middle aged dogs (Group A2), although all other ages were affected. Haemangiosarcomas and lymphosarcomas were the most common tumours found. Haemangiosarcomas were observed only in the middle aged dogs, 6 to 10 years old, which was consistent with the literature in which Pulley and Stannard (1990), have described the average age to be between 9 to 10 years. According to Moulton and Harvey (1990), dogs with lymphosarcoma are seldom observed at less than one year of age with a high incidence between 5 to 11 years and a decline with increasing age. Similar findings were observed in this study in which seven dogs were found to have lymphosarcoma, three of these were aged 6 to 10 years, two dogs were over 10 years and two were in the younger age group. The prognosis of all these conditions is not very good although proper treatment at the right time may prolong the animal's life. In addition, other neoplasms such as pancreatic adenocarcinomas, osteosarcomas and pituitary adenocarcinomas were seen in the middle aged dogs.

Immune-mediated disease conditions, namely auto-immune thrombocytopenia (AITP)and auto-immune haemolytic anaemia (AIHA), were found, but less frequently than renal disease or neoplasia. There were four dogs between the ages of 6 to 10 years with these conditions of which DN170 and DN207 had auto-immune thrombocytopenia and one dog, DN199, had AIHA. Disseminated intravascular coagulation was observed in one dog, DN16, of this age group. One dog with auto-immune thrombocytopenia was observed in Group A1 and another in Group A2. In the general population of 245 dogs studied, it was observed that 11 dogs had anaemia due to auto-immune conditions of which six were examined post mortem. However, there was a larger number of dogs with auto-immune thrombocytopenia than with

AIHA which were examined post mortem. This may have been due to the fact that many cases of AITP are non-responsive to treatment. The associated bleeding episodes and the amount of blood loss taking place make the mortality rate high in these conditions.

There were several other interesting features observed in the cases examined post-mortem. Auto-immune haemolytic anaemia are usually associated with marked reticulocytosis, although sometimes, they may be non-regenerative due to hypoplastic or aplastic conditions (Jones and Gruffydd-Jones, 1991). Reticulocytopenia is not observed very often and takes place especially when the individual's bone marrow is not able to compensate for the ongoing haemolytic process usually due to immune destruction of erythroid precursors. There was one dog, DN199, (Group A2) with a very severe anaemia and a PCV of 5.2%. This dog had an AIHA which eventually terminated in erythroid hypoplasia, the granulocytic and megakaryocytic cell lineages were not affected. The cause of death in this dog was the severity of the anaemia and the hypoplastic marrow.

A case of chronic myeloid leukaemia was observed in DN70 (Group A1). The clinical and haematological findings with cytochemical stains for myeloid cells in the peripheral blood had confirmed the presence of a myeloid leukaemia. The dog also had a DIC confirmed by a positive test for the soluble fibrin monomer complexes (SFMC) and a low platelet count of Plt.$14x10^9$/l. According to Evans and Gorman (1987), a myeloproliferative disorder may be associated with thrombocytopenia which may be due to an immune-mediated condition.

Another rarely seen case was DN141 (Group A1), which was severely anaemic with a PCV of 7.9%, due to erythroid hypoplasia of the bone marrow,

however, the aetiology was idiopathic in this case. A severe non regenerative anaemia with a PCV of 12.2% was present in DN48 (Group A2), which also had a thymoma. Histological examination of the bone marrow, revealed a hypercellular marrow with increased number of erythroid precursors showing dyserythropoietic features. According to human literature, thymomas may be seen associated with erythroid hypoplasia (Cotran *et al.*, 1994a). Although this dog had dyserythropoiesis the presence of a thymoma may have been significant.

In this study, it was observed that the cases examined had a variety of pathological conditions associated with anaemia. The severity of the anaemia, in the cases studied, ranged from mild anaemia in dogs with PCV's in the low 30's to dogs with severe anaemia and PCV's less than even 10%. Severe anaemia, PCV of less than 20%, had a higher incidence in Group A2 and was observed in six out of 25 dogs. Whereas only two dogs in Group A1, and only one dog in Group A3 were found to have severe anaemia.

The major causes of severe anaemia in the dogs of Group A2 were auto-immune disease conditions which were found in three of the dogs.

Haemangiosarcoma was observed in two dogs with severe anaemia and dyserythropoiesis in one other dog. Dog DN199 in Group A2, had a PCV level of 5.2%, and this was the lowest PCV level in the entire population of 245 dogs in this study. In this dog, the cause was an AIHA which terminated in an erythroid hypoplasia. Disseminated intravascular coagulation due to an immune-mediated cause was observed in DN16 (Group A2) with a severe anaemia and a PCV 14.2%.

Haemangiosarcomas may be associated with a regenerative anaemia and complications of DIC may also occur (Hirsch *et al.*, 1981). This was observed in DN58 (Group A2) of this study. Two dogs, DN58 and DN88, in this age group had

haemangiosarcomas with severe anaemia mainly due to blood loss within the thoracic cavity. Although the blood findings indicated a regenerative anaemia the prognosis in these two dogs was poor due metastasis of the neoplasm to vital organs.

In the younger dogs of Group A1, there was one dog, DN141, with bone marrow hypoplasia and a very severe anaemia with a PCV of 7.9%. A young dog with lymphosarcoma, DN142, was observed to have severe anaemia although the PCV of 19.1% was not as low as that in DN141.

There was only one dog, DN52, with severe anaemia in the group of older dogs (Group A3). This dog had a PCV of 6.2% and this was the second lowest PCV recorded in the entire population studied. The animal had a protein losing enteropathy and eventually also developed an acute renal failure. The severe progressive anaemia was probably due to co-existing marked kidney damage causing increasing uraemia and reduced Epo production.

In all the dogs with severe anaemia, the anaemia was considered to be a significant factor contributing to the animals' death. The severe anaemia was due to factors either directly damaging the erythrocytes membrane surface or affecting the production and release of erythrocytes.

A significant number of dogs, 10 out of 25 dogs, were moderately anaemic in Group A2, while only 4 dogs in the Group A1, and three dogs in Group A3 had moderate anaemias. A variety of pathological conditions were observed causing moderate anaemia in these dogs. The conditions that were found causing moderate anaemia had also been found to be associated with severe anaemia, namely immune-mediated diseases, haemangiosarcomas and lymphosarcomas. Lymphosarcoma was observed in all the age groups which were associated with moderate and severe anaemia depending on the pathogenesis involved. According to Moulton and Harvey

(1990), about two thirds of dogs with lymphosarcoma have anaemia which may be of a normocytic-normochromic type.

According to Eschbach and Adamson (1985), there are many factors producing the anaemia of renal dysfunction. In renal diseases, anaemia may be due to low Epo level and increased loss of erythrocytes through bleeding. Renal problems mainly cause a non-regenerative, normocytic-normochromic anaemia and the severity of the anaemia depends on the amount of damage to the nephrons and on the amount of Epo produced. In all the dogs with renal diseases in this study the degree of anaemia found was not severe, presumably because the disease had not affected Epo production drastically and the erythropoietic process had been maintained.

The major proportion of the dogs in Group A were mildly anaemic, 26 out of 52. There were 10 dogs in Group A1, nine dogs in Group A2 and seven dogs in Group A3. Many of the dogs in Group A1 were mildly anaemic mainly due to familial nephropathy although a few others had skin lesions or cardiac conditions. Neoplasms which were a feature of dogs in Group A2 were associated usually with a mild normocytic-normochromic, non-regenerative anaemia. There was one dog, DN72, (Group A2) with a mild anaemia due to a road traffic accident. In the older dogs (Group A3), mild anaemia was mainly due to chronic infections or other chronic conditions such as neoplasms. This type of anaemia is described collectively as anaemia of chronic disease (ACD).

As expected, in this study, familial renal disease conditions occurred most often in young dogs and these dogs were likely to be mildly anaemic. Skin diseases and congenital cardiac conditions were also important features in the young dogs. Middle aged dogs were more likely to have anaemia due to conditions such as immune-mediated diseases or neoplasms particularly lymphosarcoma and

haemangiosarcoma or chronic inflammatory and infectious conditions. In middle aged dogs, the anaemia was more frequently severe. While the oldest dogs, above 11 years of age, also suffered from chronic diseases such as neoplasia and renal failure, the severity of the anaemia was variable depending on the pathogenesis and the extent to which erythropoiesis and erythropoietin production were affected.

It appears that in clinical practice the age of an anaemic patient and the severity of the anaemia may give some indication as to the disease process causing the anaemia and may aid in the clinical diagnosis. However, certain kinds of disease conditions causing anaemia can affect all ages and the anaemia may not be restricted only to a particular age group. The population investigated in this study was small and care has to be taken in extrapolating these observations to the general population. Nevertheless, these results are interesting and have provided useful information on the occurrence and pathogenesis of anaemia in different age groups of dogs in the United Kingdom.

CHAPTER 5

A PROSPECTIVE STUDY OF SELECTED CASES OF ANAEMIA IN DOGS PRESENTED DURING THE YEAR 1995

5.1 Introduction

During the year 1995 interesting haematological cases were selected for study from the dogs referred by local veterinarians to the University of Glasgow Veterinary School. The selection of cases was carried out to analyse in some detail the haematological abnormalities found in these dogs in order to compare the findings with the information in the literature relating to the pathogenesis of anaemia.

In the cases chosen, the dogs were considered to be anaemic if the haematocrit was less than 37 percent. There were 15 cases selected in order to study more easily and comprehensively the haematological features which may be observed in cases of anaemia. The dogs were grouped according to the classification followed in this thesis (see 4.2.4). Most of these dogs had a moderate to severe degree of anaemia. However, this does not indicate that cases with a lesser degree of anaemia were not referred to the Veterinary School. Most of those cases were associated with chronic diseases or renal diseases and they have been excluded as cases for this study.

Essential data of the dogs in this study such as age, breed, sex, owners name, referring veterinarian were entered into the database called "Dataflex" at the case reception. A case number was given to every dog; and for the purpose of this study, they were designated as prospective cases (PC). The dogs were assigned to the clinician in-charge who took a history of the case and then performed a thorough physical examination. After the clinical examination and history taking, blood samples were taken for haematology and biochemistry. When a diagnosis had been made essential procedures for the treatment were decided by the clinician. In addition, arrangements were made to monitor the progress of the dog by further clinical examinations and blood sampling as considered appropriate.

As part of the diagnostic procedures, blood samples were collected from the dog by the clinician for haematology and biochemistry. The blood samples submitted to the haematology laboratory were accompanied by a clinical summary and details of the dog. The blood samples followed the standard procedures of the haematology laboratory as described in Chapter 4. A special record of the haematology and of the clinical progress of the dogs was made for this study. The haematological interpretations were made by the haematologist and the final outcome of the case was summarised by the clinician. In cases where the dog had a poor prognosis it was euthanased and a post-mortem examination was performed in agreement with the owner. The post-mortem and histological examinations were carried out by the pathologist on duty for that week.

Haematological values of all the blood samples of the cases studied have been recorded in Appendix D. In addition, the biochemistry reports and a report of various clinical signs for each case have been presented in Appendix E and Appendix F, respectively. When multiple blood samples had been taken from a dog, the first blood sample at presentation and the last sample of the series during the follow-up period have been shown here with some representative intervening samples. The complete series of samples has been shown in the Appendix D and Appendix E. The laboratory parameters were compared with the normal reference ranges in Appendix A: haematology in Table A1 and biochemistry in Table A2.

Fifteen cases of dogs with anaemia were selected. There were four cases of haemorrhagic anaemia, four cases of haemolytic anaemia, three cases of hypoproliferative anaemia and four cases of anaemia due to neoplasia.

5.1.1 HAEMORRRHAGIC ANAEMIA-CASE DETAILS

There were four cases in this category Case PC.1 to Case PC.4 and the details of the animals were as follows:

Case PC.1

A Boxer, 8.5 years old, male neutered.

History

The dog had been collapsed and very anaemic according to the history of the referring veterinarian. The dog had made a recovery but was presented at the Glasgow Veterinary School, as being very lethargic, pale and with knuckling of the rear paws.

Clinical Signs on Presentation

The mucous membranes were pale. The dog also had some weight loss, slight anorexia, polydipsia and polyuria. A slight increase in the heart rate (140 beats/minute) with muffled heart sounds was detected. The pulse was regular and there were no respiratory signs.

A neurological examination was done and the local reflexes were found to be very slow in the right hind limb, especially in the left paw.

Radiography

A large mass was present in the ventral abdomen. There was also evidence of some free fluid.

Ultrasonography

An enlarged spleen with a very irregular architecture was detected. There were multiple hypoechoic patches within it and no normal splenic tissue could be visualised.

Laboratory Tests

Biochemistry: These results were all within normal ranges.

Urinalysis: This gave the following results; protein ++, blood +++, ketones +, pH 7.0, specific gravity 1.025.

Haematological Findings and Diagnosis

The haematological findings are shown in Table 15.

A markedly regenerative blood picture with numerous target cells, normoblasts and some schistocytes were seen on the blood smear.

The findings indicated blood loss and the presence of schistocytes suggested microangiopathy possibly due to a haemangioma or haemangiosarcoma.

Outcome

The dog died a few hours after the ultrasonography was done.

Pathological Examination

The carcass was in good body condition but with marked pallor. The abdomen was distended with fresh blood. The site of the abdominal haemorrhage was a large flattened spherical mass (15x13x13cm in size) located in the cranial pole of the spleen. This mass was soft, fluid in consistency and had ruptured at one point. There were extensive omental adhesions to the mass but there was no metastasis. Liver and kidney were pale, consistent with severe acute haemorrhage, but were otherwise normal.

Histopathological Diagnosis

The splenic mass revealed an outer rim of compressed collagenous tissue with the mass itself formed out of blood. There was marked EMH in the adjacent splenic tissue with no evidence of any tumour mass. The splenic mass was considered to be a haematoma.

Diagnosis

Splenic haematoma.

Table 15: Haematology results of PC.1

Sample	1
R.B.C.x10^{12}/l	3.17
Hb.g/dl	8.20
PCV%	24.10
MCVfl	76.00
MCHpg	25.80
MCHCg/dl	34.00
Plt.x10^{9}/l	189.00
Retic.%	Not done
W.B.C.x10^{9}/l	9.60
BNeutro.x10^{9}/l	0
Neutro.x10^{9}/l	6.43
Lympho.x10^{9}/l	1.20
Mono.x10^{9}/l	0.14
Eosino.x10^{9}/l	0.58
Baso. x10^{9}/l	0
Normoblasts	1.25

Case PC.2

A Flat Coated Retriever, 8 years old, female.

History

The dog was presented with a history of anaemia and anorexia.

Clinical Signs on Presentation

On examination the dog had pale conjunctivae and mucous membranes with no evidence of petechiae or ecchymoses. On palpation the abdomen was "lumpy".

Radiography

On radiography the liver was normal but the spleen was slightly enlarged but smooth in contour.

Laboratory Tests

Biochemistry: The total bilirubin concentrations were elevated on the two occasions when the blood sample were taken. The values were 16 μmol/l and 33 μmol/l, respectively.

Haematological Findings and Diagnosis

Haematological findings are presented in Table 16.

Sample 1 (day 0)

Anaemia, thrombocytopenia and a slightly regenerative blood picture with occasional schistocytes, many target cells, and large platelets were present suggesting a blood loss. Possibly an internal blood loss which would explain the raised bilirubin. The direct Coombs' test was negative.

Subsequent samples showed a slightly regenerative blood picture with some large platelets, occasional schistocytes and many target cells. It was suggested that the thrombocytopenia may have been caused by increased splenic sequestration or increased consumption of platelets.

Bone Marrow Aspirate

There was an erythroid hyperplasia due to increased red cell production. Red cell precursors were present at all stages of differentiation.

Spleen Biopsy Report

A splenectomy was done and sent for biopsy. In the red pulp of the spleen a massive histiocytic reaction was found. The histiocytes contained red cells and red cell fragments. Mitotic figures were noted in these cells and there was also marked erythropoiesis and focal thrombosis causing infarcts.

Diagnosis

Hypersplenism and splenic infarction. "Neoplasia should not be ruled out".

Outcome

Although no obvious cause for haemolysis could be found clinically or historically on the basis of the raised bilirubin levels a provisional diagnosis of immune-mediated haemolytic anaemia was made by the clinicians. However, the Coombs' test was negative. Immunosuppressive therapy was carried out with blood transfusions, the dog did not respond and was worsening and therefore it was decided to euthanase the dog.

Pathological Examination

At necropsy the carcase was pale. Liver, kidneys and heart were pale. There was excessive fluid in the abdomen and thorax. Lungs were congested and oedematous. Bone marrow was fleshy and red.

Histopathological Diagnosis

Microscopically, an active marrow with normal red cell maturation was found. There was oedema and haemorrhages in the lung with microthrombi and infarcts in the kidney. The cause of the increasing anaemia and thrombocytopenia could not be established and a diagnosis of DIC causing the anaemia was made.

Diagnosis

Disseminated intravascular coagulation causing anaemia.

Table 16 : Haematology results of PC.2

Sample * Days after Sample 1	1 0	3 3 (before splenectomy)	5 5	6 6	7 10
R.B.C.x10^{12}/l	1.90	1.13	2.57	2.32	1.96
Hb.g/dl	4.40	2.80	6.00	5.30	4.50
PCV%	13.90	9.00	18.60	16.40	14.20
MCVfl	73.00	80.00	72.00	71.00	72.00
MCHpg	23.10	24.70	23.30	22.80	22.90
MCHCg/dl	31.60	31.10	32.20	32.30	31.60
Plt.x10^{9}/l	8.00	6.00	53.00	49.00	14.00
Retic.%	0	0	0.02	0	0
W.B.C.x10^{9}/l	5.90	9.30	12.70	11.50	7.70
BNeutro.x10^{9}/l	0.15	0.28	0.19	0.11	0
Neutro.x10^{9}/l	4.84	7.58	11.43	10.35	7.01
Lympho.x10^{9}/l	0.47	0.32	0.13	0.11	0.27
Mono.x10^{9}/l	0.35	0.98	0.95	0.92	0.19
Eosino.x10^{9}/l	0	0	0	0	0
Baso. x10^{9}/l	0	0	0	0	0
Normoblasts	0.09	0.14	0	0	0.23

*Intervening samples are shown in Appendix D.

Case PC.3

A German Shepherd dog, 3.5 years old, male castrated.

History

The dog had chronic hip dysplasia and in the last 12 months had been on the drug Ibuprofen.

Clinical Signs on Presentation

There were weight loss, lethargy, dullness and reluctance to stand and polydipsia. The mucous membranes were very pale. After rectal examination occult blood was present on the gloves. The hair of the animal's coat was out of condition. A nasal discharge was seen which was more marked from the left nostril than from the right. There was no evidence of any gross haemorrhages. The lymph nodes were slightly enlarged.

Laboratory Tests

Biochemistry: Findings were unremarkable.

Haematological Findings and Diagnosis

Haematological findings are in Table 17.

Examination of the blood samples revealed a microcytic hypochromic anaemia with a slightly regenerative blood picture, pale target cells and reactive thrombocytosis. These findings were consistent with a chronic blood loss.

Outcome

A diagnosis of gastric ulceration due to the long term treatment with Ibuprofen (NSAID) leading to chronic blood loss and iron deficiency anaemia was made. The dog was sent home with medications of cimetidine, iron tablets and sucralfate.

Diagnosis

Drug-induced gastric ulceration leading to chronic blood loss and iron deficiency anaemia.

Table 17 : Haematology results of PC.3

Sample *	1	2	3	4	5	6
Days after Sample 1	0	4	6	8	11	15
R.B.C.x10^{12}/l	4.36	4.140	3.48	3.80	4.330	5.830
Hb.g/dl	6.00	5.30	4.20	5.00	6.30	9.20
PCV%	21.30	19.20	16.60	18.60	21.70	29.90
MCVfl	49.00	46.00	48.00	49.00	50.00	51.00
MCHpg	13.70	12.80	12.00	13.10	14.50	15.00
MCHCg/dl	28.10	27.60	25.30	26.80	29.00	30.00
Plt.x10^{9}/l	360.00	620.00	579.00	593.00	585.00	639.00
W.B.C.x10^{9}/l	25.60	8.90	10.90	11.40	5.50	7.50
BNeutro.	0.13	0	0	0.28	0	0
Neutro.x10^{9}/l	22.53	5.83	8.72	9.46	3.22	4.61
Lympho.x10^{9}/l	0.64	1.42	1.20	1.25	1.13	1.31
Mono.x10^{9}/l	2.18	1.29	0.71	0.34	0.44	0.49
Eosino.x10^{9}/l	0.13	0.36	0.27	0	0.72	1.09
Baso. x10^{9}/l	0	0	0	0	0	0

*Intervening samples are shown in Appendix D.

Case PC.4

A Labrador cross, 11 years old, female spayed, weight 26.5 kgs.

History

The dog had been intermittently anorexic and had been very lethargic with a very slow gait. There were two haematology and one biochemistry tests done by the referring veterinarian. The two haematology test results were enclosed. The first test showed the following results: PCV. 23.3%, Hb. 7.9g/dl, Plts. $73x10^9$/l, leucocytes $12.9x10^9$/l, and Retics. 0.3%. In the second blood sample the haematocrit decreased significantly (16.3%), however, the platelet numbers were within normal ranges $453x10^9$/l.The biochemistry reports showed a very high serum Alk.Phos. concentration of 543 IU/l. The dog had been on some amount of steroid and vitamin B12 treatment, however, the anaemia and anorexia seemed to have got worse and it was thought advisable to refer the dog here to the Veterinary Hospital.

Clinical Signs on Presentation

The dog was dull and lethargic. The abdomen felt ascitic on balottement. The mucous membranes were pale.

Radiography

Significant findings were of a bulge of the caudoventral abdominal wall caused by soft tissue mass. The gastric axis was rotated caudally by a soft tissue density in the cranial abdomen protruding beyond the right costal arch.

Radiography Diagnosis

Hepatomegaly.

Ultrasonography

Enlarged diffuse hepatomegaly, large gall bladder and an increase in the echogenicity around the bile duct.

Laboratory Tests

Biochemistry: The concentrations of the serum ALT were high 180 IU/l and serum alkaline phosphatse was extremely high 6930 IU/l which may have been due to the steroids. Bile acids pre-prandial was 0.1 mmol/l and post-prandial was 44.4 mmol/l. The amylase test was 2534 IU (normals: 510-1589 IU) and lipase was 546 IU (normals: 0-1004).

Urine test: The results were: proteins ++, some haematuria and leucocytes in the urine, pH 7.5 and specific gravity of 1.025.

Haematological Findings and Diagnosis

Haematological findings are in Table 18.

Sample 1

A slightly regenerative blood picture was seen showing marked poikilocytosis and a few schistocytes. The size of the platelets was large and many of them formed aggregates. Blood findings suggested a blood loss and the presence of schistocytes was thought to be due to a neoplasm or thrombosis.

Sample 2 (day 2)

The fibrinogen content of the blood was normal, 370 mg/dl. The normal range is 150-400 mg/dl. Coagulation tests failed to reveal any abnormalities.

Prothrombin time of the blood sample was 8 seconds when compared to the control sample of 12 seconds. This test measures the status of the extrinsic coagulation pathway and it is performed for monitoring patients with hepatic disease or DIC. Kaolin cephalin clotting time of the test blood sample was 21 seconds compared to a control of 22 seconds. This test is essential to detect any abnormalities in the intrinsic coagulation pathway.

The dog had positive (++) soluble fibrin monomer complexes and this confirmed the presence of a thrombotic condition.

Sample 3 (day 7)

The blood picture remained regenerative with poikilocytes, a few schistocytes and with an increase in platelet size. As the anaemia did not improve a blood transfusion was advised.

Sample 4 (day 8)

After the blood transfusion anaemia seemed to have improved but poikilocytes and schistocytes were still present .

The anaemia appeared to be due to a blood loss. However, no tumour mass could be found. The diagnosis of hepatomegaly was made by the clinicians.

Diagnosis

Hepatomegaly unknown aetiology was diagnosed .

Outcome

As the dog seemed to have improved after the blood transfusion, it was sent home on medications and was advised by the clinician to be treated with multivitamin injections and a small dose of prednisolone. No further follow-up was possible.

Table 18 : Haematology results of PC.4

Sample * Days after Sample 1	1 0	2 7	3 8 (after blood transfusion)
R.B.C.x10 12/l	2.53	2.59	4.47
Hbg/dl	7.20	7.90	10.10
PCV%	19.40	19.10	32.60
MCVfl	77.00	74.00	73.00
MCHpg	28.40	30.50	22.50
MCHCg/dl	37.10	41.30	30.90
Plt x10 9/l	410.00	522.00	396.00
Retics.%	0.01	0.05	0.01
W.B.C. x10 ^{9}/l	19.50	22.90	19.00
BNeutro.x10 ^{9}/l	0.49	0.00	0.57
Neutro.x10 ^{9}/l	13.85	20.95	17.77
Lympho.x10^{9}/l	3.80	1.37	0.57
Mono. x10 ^{9}/l	0.88	0.34	0.10
Eosino. x10 ^{9}/l	0.01	0.11	0.00
Baso. x10 ^{9}/l	0.00	0.00	0.00
Normoblasts	0.01	0.11	0.00
Metamyelocytes	0.097	0.00	0.00

*Intervening samples are shown in Appendix D.

5.1.2 HAEMORRHAGIC ANAEMIA-DISCUSSION

Four cases of haemorrhagic anaemia were presented during the course of this study. Splenic haematoma was seen in PC.1, whereas, case PC.2, was unusual and the cause of the anaemia remains unclear. PC.4 was diagnosed of having hepatomegaly, however, no particular cause could be found. Of these three dogs with haemorrhagic anaemia two had a poor prognosis due to the aetiology and severity of the anaemia and one was sent home on medications.

Chronic haemorrhagic anaemia as a result of gastrointestinal ulceration was seen in one case, PC.3. It was drug-induced and the dog had a good prognosis. The conditions causing haemorrhagic anaemia in these dogs are discussed below.

Splenic haematoma

Splenomegaly may be due to a number of diseases which may be inflammatory, infiltrative, suppurative or neoplastic. In a study on splenomegaly, by Couto (1990), of the 42 dogs which were splenectomised, 36 percent had haematomas and according to the author it seemed to be one of the commonest types of splenic masses. According to another study on splenomegaly by Wrigley *et al.*, (1989), of the 89 dogs studied, 10 had splenic haematomas. The authors considered that the attention given to haemangiosarcomas resulted in haematomas being rarely diagnosed.

Splenic haematomas, which are large, are usually formed as a result of intermittent haemorrhages within the mass. This may cause anaemia of a varying severity depending on the extent of blood loss. In PC.1, there was a large abdominal

mass diagnosed as a splenic haematoma on ultrasonography. A sudden rupture of the haematoma resulted in abdominal haemorrhage which was confirmed post-mortem. It is known that a sudden haemorrhage does not cause an immediate fall in the PCV as haemodilution takes time to develop. In this case, the blood loss was subclinical at first, but eventually increased after the rupture of the haematoma causing hypovolaemic shock.

The disease conditions affecting the spleen are known to greatly influence the haematological findings (Couto, 1990). In this case, the anaemia was haemorrhagic with all the features of blood loss. In addition, there was a decrease in the platelet numbers. However, no marked changes in the leucocyte parameters were seen. Blood passing through a splenic haematoma as well as fibrin deposits which could have been present may have caused the fragmentation of the erythrocytes resulting in schistocytes. Extramedullary haemopoiesis may be seen in the conditions affecting the spleen resulting in a leuco-erythroblastic blood picture (Neer, 1996). In PC.1 only normoblasts were present in an increased number and a left shift of neutrophils was not seen.

According to Couto (1990), clinical signs of polydipsia and polyuria are frequently seen in dogs with splenomegaly. He considered these to be probably psychogenic due to the abdominal distension or pain. These observations were also seen in this particular case (PC.1). Case PC.1 did not have any marked changes in the serum biochemistry which is consistent with the observations of other researchers. The haematological findings in this case were similar to those described in dogs with splenic haematomas and the lesion was confirmed by post-mortem examination.

Hypersplenism and DIC

In cases of haemolytic anaemia the clinical signs most frequently seen are haemolysed plasma, hepatosplenomegaly, jaundice and occasional haemoglobinuria. The haematological findings include a markedly regenerative blood picture with normoblastosis, and a marked leucocytosis; spherocytes may also be present. Inaddition, there is often a raised unconjugated bilirubin level (Jones and Gruffydd-Jones, 1991; Dodds, 1983). The above clinical and haematological findings were not present in case PC.2 except for a high total bilirubin level. The initial haematology reports prompted the consideration of an auto-immune haemolytic anaemia and a possible immune-mediated thrombocytopenia. However, the Coombs' test was negative and there were no spherocytes both of which are features of auto-immune haemolytic anaemia. There was also no response to the immunosuppressive therapy where a splenectomy was performed. Splenectomy can have a beneficial effect on dogs with immune-mediated haematological diseases and this had been examined in dogs in a study by Feldman *et al.*, (1985). In their study, the dogs did survive longer after splenectomy although the underlying mechanism was not elucidated.

After splenectomy, in case PC.2 there was no improvement in the anaemia and thrombocytopenia, so the diagnosis of hypersplenism and associated splenic sequestration could not entirely explain the thrombocytopenia. To complicate this case further, the post-mortem findings of haemorrhages and microthrombi in the lungs and infarcts in the kidney were interpreted as the result of an underlying DIC. The mechanism for a DIC to occur is a systemic intravascular activation of the blood coagulation mechanism together with activation of the fibrinolytic system, as the result of the exposure of blood to abnormal surfaces and the entry of thromboplastic

material into the circulation (Slappendel, 1988; Legendre and Krehbiel, 1977). There are a number of factors which will cause DIC and amongst them sepsis, neoplasms, liver diseases, complications of gynaecology are the most important causes (Bell, 1994). The major feature of an uncompensated DIC is haemorrhage which may be in the form of ecchymoses, petechial haemorrhages or frank bleeding from a variety of sites, in addition to shock and hypoxia (Bell 1994). In this complicated case of dog PC.2, no overt signs of haemorrhages were found to explain the anaemia. The dog was not tested for FDP's but the presence of schistocytes reflected microangiopathy caused apparently by a compensated DIC which may have been induced by an underlying neoplastic process. In the biopsy report, histiocytes were present and a histiocytic neoplasm could be a possible cause of the hypersplenism. Taking this into account, the clinical and haematological findings could be best explained if the dog had a systemic malignant histiocytosis type condition which is associated with anaemia and thrombocytopenia caused by the phagocytic activity of the neoplastic histocytes. This condition has been reported in dogs (Madewell and Feldman, 1980).

Chronic haemorrhagic anaemia

Chronic haemorrhagic anaemia is a consequence of an ongoing blood loss which sometimes goes undiagnosed for a long time until other supporting clinical signs appear. There are a number of conditions which can cause chronic haemorrhagic anaemia and gastrointestinal ulceration due to drugs can be one of the commonest causes. Certain drugs are known for their deleterious effects on the gastrointestinal tract, most often resulting in gastroduodenal ulcerations (GDU). The most commonest causes of GDU as a result of drug usage are non-steroidal anti-

inflammatory drugs (NSAID) some of which have been cited by Cosenza (1984). These drugs are known to cause GDU by inhibition of prostaglandin synthesis which decreases the mucosal blood flow and alters mucus production (Stanton and Bright, 1989).

In a study by Stanton and Bright (1989), of 43 dogs with non-neoplastic gastric or duodenal ulcerations 32 had anaemia. In this study, dog PC.3, had a history of hip dysplasia and had been prescribed the drug Ibuprofen, a NSAID. This case had similar clinical signs to those of the cases described by Cosenza (1984). These clinical signs were melaena, pallor, weight loss and polydipsia. According to Stanton and Bright (1989), haematemesis or melaena are strongly suggestive of gastric or duodenal mucosal erosion or ulcerative disease. The history and the haematological observations confirmed the aetiology of this case.

A non-regenerative anaemia was a common finding in the dogs with ulcers in the study by Stanton and Bright (1989), but a microcytic, hypochromic anaemia was seen in one fourth of the cases studied. Case PC.3 had a slightly regenerative anaemia with an increase in platelet numbers, and in the first sample, an increase in the leucocytes, especially band neutrophils, which may have been caused by myeloid hyperplasia which accompanies erythroid hyperplasia in an ongoing blood loss. In subsequent blood samples there was a decrease in the MCV and MCHC and a diagnosis of microcytic hypochromic anaemia was confirmed.

The anaemia in this particular case was a result of chronic blood loss causing loss of iron and the development of iron deficiency. Prophylaxis and supportive treatment with antacids for ulcers usually resolves the problem. Case PC.3 made a good recovery after this type of therapy had been started.

Hepatomegaly

Hepatomegaly in dogs can result from passive congestion, infiltrative disorders, inflammatory disease, reticuloendothelial hyperplasia, neoplasia, extrahepatic bile duct occlusion and cystic liver disease. This may lead to a variety of changes in the haematology of dogs with hepatic disease. The liver is the main organ for the synthesis of the coagulation proteins (Green, 1981). Any disturbance in the normal functioning of the liver may lead to some degree of haemostatic abnormality. In dogs however, only a severe hepatic disease, such as cirrhosis would cause a haemorrhagic problem.

In this particular case, PC.4 the anaemia was always regenerative with a slight neutrophilia which may have been reactive or may been caused by an underlying infection. Small numbers of schistocytes and poikilocytes were also present suggesting a neoplastic or thrombotic condition, but no neoplasm was found in this case.

The platelet numbers were within the normal ranges, although on one occasion it was high possibly due to splenic contraction. Slightly increased fibrin monomer levels were found indicating some degree of fibrinolysis, possibly due to the thrombosis. They were not high enough to consider DIC as a cause of haemorrhage. The normal platelet counts and the normal coagulation tests also excluded DIC. Taking everything into consideration it would seem most appropriate to diagnose a haemorrhagic anaemia and thrombosis although the underlying cause remains unclear.

5.1.3 HAEMOLYTIC ANAEMIA-CASE DETAILS

There were four cases in this category Case PC.5 to Case PC.8 and the details of the animals were as follows:

Case PC.5

A Jack Russell Terrier, 3 years old, female spayed, weight 7.3 kg.

History

Five days ago the dog had become dull, weak anorexic and recumbent. The owners noticed very dark coloured urine and that her eyes were slightly swollen.

Clinical Signs on Presentation

A strong bounding pulse was palpable and on auscultation a grade I and II heart murmur was heard on the right and left side of the chest. The mucous membranes were pale and slightly icteric. The dog was very weak when standing.

Laboratory Tests

Biochemistry: The results that were significant were an increase in the concentrations of urea 9.6 mmol/l, total bilirubin 32 μmol/l, and serum Alk.Phos. 417 IU/l.

Haematological Findings and Diagnosis

The haematological findings are shown in Table 19.

The direct Coombs' test was weakly positive. A markedly regenerative blood picture was detected with normoblasts, spherocytes and auto-agglutination of red cells. The MCV was very high. Neutrophilia with a left shift was seen as well as a monocytosis

and lymphopenia due to haemolysis and steroids. These findings were consistent with IgG type AIHA.

Outcome

This was a typical case of an acute auto-immune haemolytic anaemia. Since the dog was deteriorating in condition and the prognosis was not very good, it seemed better to the clinicians to euthanase the dog.

Pathological Examination

At necropsy the dog was in good bodily condition and the subcutaneous fat and mucous membranes appeared yellow in colour. The spleen was enlarged and fleshy red in texture whereas the gall bladder was distended with normal bile and the liver was apparently normal. Kidneys were normal too.

Histopathological Diagnosis

Microscopic examination of the spleen revealed extensive necrosis of the red pulp with macrophages showing marked erythrophagocytosis and containing haemosiderin. There was marked EMH also in the splenic tissues. Marked erythrophagocytosis was also present in the liver resulting in the formation of haemosiderin which was seen in increased amounts in the Kupffer cells. The pattern of the splenic necrosis together with the limited change in the liver and kidney would fit with the recent and sudden breakdown of the red cells.

Diagnosis

Auto-immune haemolytic anaemia IgG type.

Table 19 : Haematology results of PC.5

Sample	1
R.B.C.x10 12/l	1.07
Hb.g/dl	2.80
PCV%	9.90
MCVfl	93.00
MCHpg	26.10
MCHCg/dl	28.20
Plt x10 9/l	231.00
W.B.C. x10 ^{9}/l	62.10
BNeutro.x10 ^{9}/l	2.48
Neutro.x10 ^{9}/l	56.20
Lympho.x10^{9}/l	0.93
Mono. x10 ^{9}/l	1.24
Eosino. x10 ^{9}/l	0
Baso. x10 ^{9}/l	0
Normoblasts	0.31
Metamyelocytes	0.93

Case PC.6

An Airedale Terrier, 8 years old, male.

History

About a year ago the dog had a history of lethargy, pyrexia, anaemia and was treated symptomatically. The dog was ill again with an enlarged spleen on palpation and with polydipsia, haemoglobinuria, lethargy and anorexia. Anaemia was also detected. The dog was treated again symptomatically, however there was another third relapse and at this time the dog was referred to the Veterinary Hospital with this history.

Clinical Signs on Presentation

The dog's mucous membranes were very pale and the dog had a bounding femoral pulse. No haemic cardiac murmurs were evident. The dog did have some cervical pain and difficulty in walking. It was hyperpnoeic and tachypnoeic with very harsh lung sounds on auscultation. The dog was dysuric too. Abdominal palpation provided a tentative diagnosis of splenic enlargement.

Radiography

Lateral abdomen: The liver shadow extended well beyond the costal arch yet retained a sharp caudal border. The spleen was visible ventrally and although it was not enlarged it did have rounding of its margins. There was a very large mass in the caudal abdomen which was most likely the bladder and a second irregular smaller mass from the pelvic inlet. A diagnosis of hepatomegaly was made.

Ultrasonography

Nothing significant was observed on ultrasound of the spleen and bladder, although the liver revealed an enlarged, diffuse, regular increase in echogenicity. The ultrasound diagnosis of a cellular infiltrate or a generalised fibrous tissue deposition in the liver was made.

Laboratory Tests

Biochemistry: This revealed a moderate increase in urea concentrations (21 mmol/l), along with an increase in liver enzyme serum ALT (289 IU/l).

Urine and faeces examination: Moderate amount of blood which may be haemoglobinuria due to haemolysis

Haematological Findings and Diagnosis

The haematological findings are shown in Table 20.

Sample 1

Examination of the blood film showed marked a regenerative blood picture with marked poikilocytosis and normoblastosis. Neutrophilia with a left shift was also seen probably due to a reactive bone marrow. The Coombs' test was positive.

Sample 2 and subsequent samples

A moderately regenerative blood picture, pale target cells and many schistocytes were found. The differential count probably reflected steroid effect. The blood in the urine and faeces, the presence of schistocytes, pale target cells and the modest regeneration were consistent with blood loss due to neoplasia which may have been lymphoid and may have induced the AIHA too.

Bone Marrow Aspirate

It was decided by the clinicians to examine the bone marrow. There were numerous red cell precursors at all stages of differentiation with adequate numbers of megakaryocytes and granulocyte precursors. The sample had a poor cytology and it was also diluted with blood which showed regenerative feature and some schistocytes.

Outcome

The dog was sent home with treatment for the auto-immune haemolytic anaemia and haemorrhage with a guarded prognosis.

Diagnosis

Auto-immune haemolytic anaemia and blood loss due to an underlying unknown neoplasm was diagnosed.

Table 20 : Haematology results of PC.6

Sample	1	2	3	4
Days after Sample 1	0	16	22	79
R.B.C.x10^{12}/l	2.40	2.26	1.93	5.89
Hb.g/dl	6.70	6.10	5.10	11.70
PCV%	22.00	20.40	16.80	39.90
MCVfl	92.00	90.00	87.00	68.00
MCHpg	28.00	26.60	26.40	19.80
MCHCg/dl	30.00	29.90	30.30	29.30
Plt.x10^9/l	0	292.00	219.00	420.00
Retic.%	not done	not done	0.04	not done
W.B.C.x10^9/l	18.20	27.60	8.90	12.20
BNeutro.x10^9/l	12.00	0.83	0.22	0
Neutro.x10^9/l	11.60	23.87	7.7	8.11
Lympho.x10^9/l	6.00	0.41	0.67	3.11
Mono.x10^9/l	0.40	2.35	0.27	0
Eosino.x10^9/l	0.20	0	0.04	0.47
Baso. x10^9/l	0	0	0	0.12
Normoblasts	0	0.14	0	0

Zero for platelet indicates platelet aggregates.

Case PC.7

A German Shepherd cross, 4 years old, male castrated.

History

During the last three days the dog had been very dull, lethargic, anorexic and had lost a lot of weight.

Clinical Signs on Presentation

The dog was weak and dull with pale mucous membranes. There were hyperpnoea, and tachycardia but no cardiac murmurs.

Radiography

There was slight hepatomegaly.

Laboratory Tests

Biochemistry: There was an increase in the urea 11.8 mmol/l, cholesterol 9.1mmol/l, total bilirubin 16 μmol/l, Alk.Phos. 431 IU/l, ALT 88 IU/l and AST 111 IU/l concentrations.

Haematological Findings and Diagnosis

The haematological findings are shown in Table 21.

Sample 1

The direct Coombs' test was positive indicating IgG class auto-antibodies. The blood picture showed all the features typical of auto-immune haemolytic anaemia i.e. anisocytosis, polychromasia, spherocytosis and auto-agglutination. The latter indicated high antibody concentration.

Fibrinogen was slightly increased, 512 mg/dl. The normal range is 150-400 mg/dl.

Sample 2

The dog was still anaemic. The blood picture still showed regenerative features i.e. Howell-Jolly bodies and occasional normoblasts.

Outcome

The dog was treated here with prednisolone and azathioprine until the haemolytic anaemia resolved and sent home with amoxycillin antibiotics.

Diagnosis

Auto-immune haemolytic anaemia IgG type.

Table 21: Haematology results of PC.7

Sample Days after Sample 1	1 0	2 8	3 22
R.B.C.x10 12/l	1.39	2.87	5.43
Hbg/dl	4.00	8.00	13.50
PCV%	13.00	26.50	43.50
MCVfl	93.50	92.00	80.00
MCHpg	28.70	27.80	24.80
MCHCg/dl	30.80	30.10	31.00
Plt x10 9/l	117.00	207.00	485.00
Retics.%	not counted	20.50	not counted
W.B.C. x10 ^{9}/l	87.00	35.10	11.80
BNeutro.x10 ^{9}/l	13.92	0.53	10.03
Neutro.x10 ^{9}/l	57.85	28.78	0
Lympho.x10^{9}/l	1.31	2.11	0.85
Mono. x10 ^{9}/l	6.96	1.93	0.47
Eosino. x10 ^{9}/l	0.44	0	0.47
Baso. x10 ^{9}/l	0	0	0
Normoblasts	6.55	1.75	0

Case PC.8

A Cross bred dog, 1.5 years old, female spayed, weight 14 kg.

History

The dog had a long history which started initially with a fracture of the left humerus which was stabilised by interosseous bone pining. Recovery was good but the dog seemed in more pain than expected and the pin was removed and replaced with a plate with screws, as the fracture healed. Subsequently the dog was presented at the Veterinary School with a history of pyrexia.

Clinical Signs on Presentation

The dog was very lethargic with anorexia. Mucous membranes were very pale and the dog had a temperature of 39.9°C. On abdominal palpation an enlarged spleen was felt.

Laboratory Tests

Biochemistry: There was a marked increase in the total bilirubin 21 µmol/l, Alk.Phos. 889 IU/l, A.L.T. 217 IU/l and A.S.T 216 IU/l in the first samples. Subsequent serum samples also showed a marked increase in the Alk.Phos., ALT. and AST. levels. Values of subsequent samples are shown in the Appendix E.

Urine Test : proteins and blood were present.

Haematological Findings and Diagnosis

The haematological findings are shown in Table 22.

Sample 1

The direct Coombs' test was positive. A regenerative blood picture was seen with spherocytosis, auto-agglutination of the spherocytes and normoblastosis. Reactive neutrophilia with a left shift was also present. These findings were indicative of an auto-immune haemolytic anaemia of the IgG type. Marked decrease in the platelet numbers was also seen.

Sample 2

There was a markedly regenerative blood picture with a marked increase in the spherocyte numbers. An increase in the MCV was also present due to the reticulocytosis. The platelet numbers were still decreased.

Subsequent samples showed spherocytosis indicating an ongoing auto-antibody activity which seemed to affect the platelet numbers too. A slightly regenerative blood picture was seen but with schistocytes and many target cells. The presence of schistocytes suggested a thrombotic condition and the target cells possibly reflected steroid effect on the liver.

A diagnosis of an auto-immune haemolytic anaemia and an immune-mediated thrombocytopenia was made.

Outcome

The dog was on a high dose of prednisolone and azathioprine. Unfortunately there was a very poor response. It was decided to give the dog a blood transfusion. Following the transfusion there was a gradual improvement in the anaemia and thrombocytopenia and the drug doses were gradually lowered. However, the dog also

developed severe lameness of the left forelimb, and osteomyelitis. It was decided to euthanase the dog.

Pathological Examination

On post-mortem examination the carcase was in a good condition. On examination of the abdomen the jejunum was dark red in colour, swollen, turgid and the contents were blood stained. The mesentry was also dark red and haemorrhagic. The liver was pale and friable. The spleen was small and the splenic vein contained a well organised thrombus.

Histopathological Diagnosis

Microscopy revealed the liver to have some amount of centrilobular fatty change with superimposed marked necrosis. Necrosis was also seen in the kidney. The intestinal architecture was well preserved despite the haemorrhage and congestion. The splenic vein thrombus was well organised and the spleen was small with an acellular red pulp. No evidence of EMH was seen.

The reason for deterioration in the dog's condition was circulatory collapse with pooling of blood in the jejunum. However, the underlying aetiology was unclear. The liver collapse, possibly related to the anaemia and or therapy. The splenic thrombosis has been present for some time.

Diagnosis

Auto-immune haemolytic anaemia and thrombocytopenia. Haemorrhagic enteropathy and shock.

Table 22 : Haematology results of PC.8

Sample *	1	2	3	4	11	14
Days after Sample 1	0	1	5	7	54	72
R.B.C.x10 ^{12}/l	0.83	0.51	1.19	1.44	4.69	4.73
Hbg/dl	3.30	2.60	4.10	4.40	13.00	13.00
PCV%	10.50	9.30	13.30	14.8	37.80	37.50
MCVfl	127.00	182.00	112.00	103.00	81.00	79.00
MCHpg	39.70	50.00	34.40	30.50	27.70	27.40
MCHCg/dl	31.40	27.90	30.80	29.70	34.30	34.60
Plt x10 ^{9}/l	16.00	10.00	5.00	11.00	431.00	257.00
W.B.C. x10 ^{9}/l	89.20	70.20	33.20	36.40	23.70	16.80
BNeutro.x10 ^{9}/l	8.47	2.80	2.98	1.82	0	0
Neutro.x10 ^{9}/l	70.46	54.05	25.89	26.57	21.92	0.08
Lympho.x10^{9}/l	0.89	0.70	0.66	0.73	0.12	15.29
Mono. x10 ^{9}/l	1.78	4.56	1.66	5.46	1.66	0.84
Eosino. x10 ^{9}/l	0	0	0	1.36	0	0.50
Baso. x10 ^{9}/l	0	0	0	0	0	0.084

*Intervening samples are shown in Appendix D.

5.1.4 HAEMOLYTIC ANAEMIA-DISCUSSION

The four cases presented in this study were all examples of auto-immune haemolytic anaemia (AIHA). Auto-immune haemolytic anaemia can be primary (idiopathic) or secondary when it is seen in association with a number of disease conditions (Jain, 1993e; Dodds, 1983; Halliwell, 1978). The primary cases have been described as acute because of their sudden onset whilst the secondary causes are chronic because of the more insidious onset. A history of a sudden onset, was seen in PC.5 and PC.7 and this suggested an idiopathic cause. This was similar to the findings of Halliwell (1978), who describes the dogs as being in an acute haemolytic "crisis" and this was known to have a poor prognosis. In PC.6 however the history was of a slow onset and a suspected neoplasm may have triggered this event. In PC.8 the anaemia seems to have been associated with the bone pinning and antibiotics.

The diagnosis of AIHA is mainly based on the positive direct Coombs' test in addition to certain typical haematological findings. All the dogs in this study had features of a regenerative anaemia, such as polychromasia, anisocytosis, normoblastosis, Howell-Jolly bodies. Neutrophilia with a left shift and increased W.B.C count were also observed which are features of this type of anaemia (Dodds 1983). Spherocytosis and auto-agglutination of red cells are also characteristic findings associated with auto-immune haemolytic anaemias. Warm antibodies can produce auto-agglutination which may be IgM, IgG and IgM or high amounts of IgG alone. Alternatively, agglutination be caused by high amplitude cold antibodies which are always IgM class. Spherocytosis and auto-agglutination were seen in all the cases described here and all of them were direct Coombs' test positive indicating that the AIHA was caused by IgG class auto-antibodies.

The commonest clinical signs in all these cases, were increased dullness and pallor, occasional icterus and haemoglobinuria. These observations were consistent with the clinical signs described in the literature (Dodds 1983; Halliwell, 1978). Distinct cardiac murmurs (Grade I and II) were present in PC.5 which was probably due to the severity of the anaemia resulting in a PCV. 9.9%. This dog (PC.5) was presented in an acute haemolytic "crisis" with a high bilirubin of 32mmol/l. Changes in the serum biochemistry of all these dogs included a significant increase in the bilirubin associated with increased erythrocyte destruction, and the marked increase in the serum alkaline phosphatase may have been due to the effect of steroids on the liver.

Schistocytes were present in two cases PC.6 and PC.8. Schistocytes are usually seen in MAHA and are caused by excess fibrin deposition in the microvasculature which may be systemic (DIC) or localised. In PC.6 a neoplasm was suspected. In PC.8, the schistocytes appearing in the subsequent samples were suggestive of a thrombotic condition and may have been associated with the developing osteomyelitis. It was noted that in PC.7 an increase in the fibrinogen content of the blood was present similar to the cases reported by Ward (1983).

The prognosis of affected dogs is usually difficult to assess and depends not only on the amount of erythrocyte destruction but also on the regenerative capacity of the bone marrow. Treatment of these cases is usually with high doses of steroids or immunosuppressive therapy such as using azathioprine or cyclophosphamide. Splenectomy can also be considered as a form of treatment (Cotter, 1995)

A post-mortem examination was performed on two dogs. In PC.5, evidence of splenic erythrophagocytosis and marked EMH was found. In the case of PC.8

haemorrhage and circulatory collapse due to shock were the main cause of death, in addition to the haemolysis.

According to Jain (1993e), Jackson and Kruth (1985), there is no particular breed predilection for this condition and this was in accord with all these prospective cases. The average age of all the dogs affected was 4.1 years and was similar to the findings by Dodds (1983) and many other researchers. According to the literature females are affected more often than males (Dodds 1983; Halliwell, 1978). In this study there were equal numbers of males and females, however, the samples were too small to be statistically significant.

5.1.5 HYPOPROLIFERATIVE ANAEMIA-CASE DETAILS

There were three cases in this category Case PC.9 to Case PC.11 and the details of the animals were as follows:

Case PC.9

A Newfoundland, 4 years old, male.

History

The dog was referred with a history of eosinophilic gastroenteritis which had been previously been treated with azathioprine and prednisolone. The dog subsequently developed signs of vestibular disease and was found to be thrombocytopenic and lymphopenic and was referred.

Clinical Signs on Presentation

The dog was thin with pale mucous membranes, tachypnoea and irregular heart beats. There was a large ulcer on the centre of the tongue and the front paw was swollen with a bruise especially obvious over the first two digits.

Radiography

This showed only a slight cardiomegaly.

Electrocardiography: Artial fibrillation was seen on ECG.

Laboratory Tests

Biochemistry: There was a very high serum Alk. Phos. 2141 IU/l and high serum ALT 92 IU/l was also seen.

Haematological Findings and Diagnosis

The haematological findings are shown in Table 23.

A non-regenerative blood picture with severe neutropenia and thrombocytopenia was observed. The direct Coombs' test was negative .

The fibrinogen was 576 mg/dl.

Prothrombin time of the test sample was 12 sec compared with a control of 10 sec.

Kaolin cephalin clotting time of the test blood sample was 19 sec and the control was 22 sec.

A diagnosis of pancytopenia was made.

Outcome

It seemed that the bone marrow was depressed secondary to chemotherapy. The serum alkaline phosphatase concentrations were elevated probably due to the steroid therapy. Monitoring of the platelet numbers was considered, however, the numbers did not increase. The following morning the dog became paraplegic and developed mild epistaxis. Blood transfusion was given, but because of the grave prognosis the dog was euthanased.

Pathological Examination

At necropsy the dog was in fair bodily condition but there was a marked degree of autolysis. The carcase was pale and the spleen was small and contracted. The bone marrow was fatty. The liver was slightly enlarged and pale. Several small ulcers were noted in the gastric mucosae at the pylorus. The heart was slightly enlarged with pale muscle.

Histopathological Diagnosis

Microscopic examination confirmed that there were very few haemopoietic cells in the marrow and the spleen. No lesions were detected in the gastric mucosae to confirm the diagnosis of eosinophilic gastroenteritis.

Diagnosis

Bone marrow hypoplasia due to drug induced myelosuppression.

Table 23 : Haematology results of PC.9

Sample Days after Sample 1	1 0	2 1
R.B.C.x10^{12}/l	3.19	3.09
Hbg/dl	7.10	7.40
PCV%	22.00	21.30
MCVfl	69.00	69.00
MCHpg	22.20	23.90
MCHCg/dl	32.30	34.70
Plt.x10^{9}/l	272.00	20.00
W.B.C.x10^{9}/l	7.00	1.40
BNeutro.x10^{9}/l	0.00	0.00
Neutro.x10^{9}/l	5.88	0.95
Lympho.x10^{9}/l	0.70	0.41
Mono.x10^{9}/l	0.28	0.04
Eosino.x10^{9}/l	0.00	0
Baso. x10^{9}/l	0.00	0

Case PC.10

A German Shepherd Dog, 3.5 years old, female.

History

The dog had been presented for assessment of a possible vaginal stricture. She was prescribed oestradiol several times for misalliance. The animal was described as being dull, becoming tired fast and losing weight.

Clinical Signs on Presentation

The dog was very dull and quiet. She was pyrexic with a temperature (40°C), had pale mucous membranes, melaena, a firm mass in the dorsal neck region and a soft swelling dorsal to the anus. A very soft cardiac murmur could be heard on auscultation. Rectal palpation was slightly painful.

Laboratory Tests

Biochemistry: These tests were unremarkable.

Cytology: The fine needle aspirate of the masses revealed only some erythrocytes and white blood cells suggesting that the lumps were haematomas.

Urine test: On examination trace proteins, pH6.0 and a specific gravity of 1.037 were seen. Examination of the urine deposit detected epithelial cells, cell debris and some organisms.

Haematological Findings and Diagnosis

The haematological findings are shown in Table 24.

Sample 1

A non-regenerative blood picture, marked pancytopenia and lymphopenia were present.

Sample 2

A non-regenerative blood picture was still present with marked cytopenias.

Samples 3-5 were the same. The dog was transfused with blood on the day the third sample was taken. A fibrinogen estimation was also carried out and the result was 806 mg/dl which was considered high. A direct Coombs' test was negative.

Sample 6

Occasional reticulocytes and normoblasts were present, along with a slight increase in the platelet numbers. The latter may have been the first signs of marrow recovery or may be due to the transfusion.

Sample 7 and 10

Slightly regenerative blood picture was seen with a slight leucocytosis. A bi-morphic population of erythrocytes i.e. round (young) and poikilocytes (old) cells was also present. There were also some large immature platelets.

Diagnosis

A diagnosis of oestradiol-induced bone marrow hypoplasia was made.

Outcome

A diagnosis of oestrogen induced bone marrow suppression was made based on the history of oestradiol (injectable) administration and the above haematological observations. Supportive therapy was started with antibiotics. The dog improved although her neutrophil count was still severely low. The haematomas resolved on

their own and the dog was sent home with medications. The dog was later presented for a routine check and appeared bright and was eating well.

Table 24 : Haematology results of PC.10

Sample *	1	2	3	6	7	10
Days after Sample 1	0	1	2 (transfusion)	10	14	39
R.B.C.x10^{12}/l	4.64	4.78	3.87	4.26	4.66	6.64
Hbg/dl	11.10	11.50	9.20	9.90	11.40	15.70
PCV%	30.30	32.20	25.40	28.20	31.60	44.90
MCVfl	65.00	67.00	66.00	66.00	68.00	68.00
MCHpg	23.90	240	23.70	23.20	24.40	23.60
MCHCg/dl	36.60	35.70	36.20	35.10	36.00	34.90
Plt.x10^{9}/l	9.00	7.00	9.00	16.00	18.00	158.00
W.B.C.x10^{9}/l	1.20	1.80	1.60	4.20	4.40	6.30
BNeutro.x10^{9}/l	0	0.11	0.06	0	0	0
Neutro.x10^{9}/l	0.24	0.65	0.58	2.12	2.09	3.72
Lympho.x10^{9}/l	0.91	1.05	0.94	1.85	2.02	2.08
Mono.x10^{9}/l	0.05	0.03	0.03	0.23	0.18	0.13
Eosino.x10^{9}/l	0	0	0	0.02	0	0.38
Baso. x10^{9}/l	0	0	0	0	0	0
Normoblasts	0	0	0	0.02	0.11	0

*Intervening samples are shown in Appendix D.

Case PC.11

A Rottweiler, 5 years old, female spayed, weight 41.4 kgs.

History

The dog had been very lethargic and had been looking very pale. It had a slight temperature and recently had developed a mild cough.

Clinical Signs on Presentation

The oral mucous membranes were pale and the dog was very lethargic. It had a Grade II haemic cardiac murmur and a bounding pulse. Her appetite was normal and there was no weight loss. On abdominal palpation a cranial abdominal mass was felt which may have been splenomegaly or hepatomegaly.

Radiography

There was a suggestion of a cranial abdominal mass, however, it was not conclusive.

Abdominal Ultrasound

Spleen and liver were normal with normal echogenicity, normal size and no evidence of a mass.

Laboratory Tests

No abnormal findings were present in the urine test, biochemistry or faecal examination.

Haematological Findings and Diagnosis

The haematological findings are shown in Table 25.

Sample 1

A slightly regenerative blood picture with a marked macrocytosis was seen. The platelets were aggregated.

Sample 2

There was a slightly regenerative blood picture. Although the number of platelets was normal, very large platelets were seen and also some as aggregates. A small number of blast cells were also seen. The abnormally large platelets were suggestive of a myeloproliferative disorder e.g. myelodysplasia.

Sample 3

The direct Coombs' test was negative and it was thought advisable to check bone marrow at this stage. There were some blasts present but the result was inconclusive. An increase in the platelet size was still present. A blood transfusion was given.

Sample 5

Very large platelets, normoblasts and many atypical lymphoid cells and lymphoblasts were seen. The latter were suggestive of a lymphoid neoplasia. The blood picture was slightly more regenerative than that of the previous samples which may have been due to the previous blood transfusion. Another blood transfusion was advised.

Sample 6

A regenerative blood picture with marked anisocytosis and some schistocytes were seen. The platelet and neutrophil counts were normal.

As seen from the reports it became apparent that the anaemia was caused by abnormal erythropoiesis which may have been due to myelodysplasia. In spite of haematological evidence no lymphoid tumour was found.

Bone Marrow Aspirate

This was attempted on two occasions and on both occasions only a fatty marrow was seen with a small number of erythroid precursors and granulocytes. The marrow did not seem to be hypercellular excluding myelophthisis. The erythrocytes that were present showed slight polychromasia, marked shistocytosis and poikilocytosis.

Diagnosis

Erythroid hypoplasia due to myelodysplasia.

Outcome

The dog was given a chemotherapy protocol which consisted of: vincristine, cyclophosphomide and prednisolone, assuming that erythroid hypoplasia may be caused by an underlying tumour. The dog was presented for the treatments necessary and was sent home on a maintenance dose. However, the prognosis of the dog was guarded.

Table 25: Haematology results of PC.11

Sample Days after Sample 1	1 0	2 9	3 9 (transfusion)	4 12	5 16 (transfusion)	6 19
R.B.C.x10^{12}/l	2.40	1.90	2.25	1.89	1.89	3.19
Hbg/dl	6.40	5.80	5.10	5.00	4.70	7.10
PCV%	21.00	17.00	17.4.0	15.10	14.80	22.00
MCVfl	89.00	88.00	77.00	80.00	78.00	69.00
MCHpg	27.00	30.00	22.60	26.40	24.80	22.20
MCHCg/dl	31.00	34.00	29.30	33.10	31.70	32.20
Plt.x10^9/l	0	0	89.00	230.00	0	272.00
Retic%.	0.6	0.3	not done	not done	not done	not done
W.B.C.x10^9/l	5.7	6.4	6.2	7.6	8.3	7
BNeutro.x10^9/l	0.17	0	0.12	0.15	0.25	0
Neutro.x10^9/l		0	3.81	4.978	4.90	5.90
Lympho.x10^9/l	1.5	0	1.86	2.01	2.78	0.70
Mono.x10^9/l	0.4	0	0.19	0.30	0.25	0.28
Eosino.x10^9/l	0.2	0	0.15	0.08	0.12	0.07
Baso.x10^9/l	0	0	0	0	0	0
Normoblasts	0	0	0.06	0.08	0	0.07

Zero for platelet indicates platelet aggregates.

5.1.6 HYPOPROLIFERATIVE ANAEMIA-DISCUSSION

Hypoplasia and aplasia of the bone marrow is known to occur in dogs and may be the result of toxic injury to the bone marrow cells and their environment or it may be due to immune-mediated injury or it may be idiopathic (Weiss and Klausner, 1990; Cline and Golde, 1978). In dogs, marrow hypoplasia can also be caused by infections such as chronic Ehrlichiosis and parvovirus infection (Kuehn and Gaunt 1985; Boosinger *et al.,* 1982). Acquired aplastic anaemia occurs in dogs and often as a result of toxic insult to the bone marrow, due to drugs such as oestrogens, phenylbutazone, trimethioprim-sulfadiazine, meclofenamic acid, fenbendazole and quinidine and chloramphenicol (Weiss and Klausner, 1990, Weiss and Adams, 1987). Myelofibrosis and chemotherapy may also cause hypoplasia or aplasia.

In this study, there were three cases with marrow hypoplasia that were particularly interesting. The first one, PC.9, had pancytopenia which seemed to be due to the drug azathioprine resulting in severe myelosuppression. The second dog, PC.10, had thrombocytopenia and anaemia caused by oestrogen induced myelotoxicity, and the third dog, PC.11, had erythroid hypoplasia which was thought to be caused by an underlying neoplasia.

In marrow hypoplasia or aplasia the blood picture is usually non-regenerative and cytopenias are always seen, which may vary reflecting the suppression of one or more cell lines (Shelly, 1988). Dogs with marrow hypoplasia or aplasia caused by chemotherapeutic drugs may develop severe neutropenia within 5-7 days of drug administration and thrombocytopenia may develop 7 to 10 days later and there may be haemorrhages due to the markedly decreased platelet number (Weiss, 1992). In Case PC.9, the dog had a non-regenerative anaemia, severe neutropenia and

thrombocytopenia i.e. pancytopenia. The coagulation tests were within normal ranges. The dog developed epistaxis due to the low platelet numbers which decreased markedly to $20x10^9$/l. An increased alkaline phosphatase concentration was also seen which may have been due to the treatment. On post-mortem examination no particular cause for the hypoplasia could be identified indicating that the cause may have been either drug-induced or idiopathic.

According to Teske (1986), there is no breed or sex predilection for oestrogen-induced bone marrow toxicity, but the condition seems more common in older dogs probably due to the less responsive bone marrow with increasing age. However, in this study, PC.10, was a young dog and the accurate history and haematological observations confirmed that the pancytopenia was oestrogen-induced.

The clinical course of oestrogen toxicity usually, starts with petechiae and haemorrhages due to the lack of platelets followed by pallor, most patients eventually die as a consequence of haemorrhagic diathesis (Teske, 1986). Despite the severe thrombocytopenia in PC.10, which had a platelet count less than 20 x 10^9/l, no signs of bleeding was observed. This situation was similar to the case presented by Hall (1992). Therapy in this case was aimed to prevent secondary infections while the animal was recovering normal bone marrow function.

In bone marrow hypoplasia or aplasia caused by oestrogens in addition to thrombocytopenia there may be initially a hyperplasia of the leucocytes followed by neutropenia due to myeloid suppression (Teske, 1986; Weiss and Klausner, 1990; Legendre, 1976). Dog PC.10 was presented in a hypoplastic state with pancytopenia. The mild to moderate severity of the anaemia was similar to the descriptions of other investigators. There was a progressive recovery in this case after the blood

transfusion and this was similar to the observations made by Weiss and Klausner (1990). In their study, three dogs were studied, two were euthanased and the one dog that survived had been given a blood transfusion. This was significant, suggesting that even prolonged bone marrow hypoplasia is potentially reversible with supportive therapy.

Erythroid hypoplasia is known to occur in animals but the aetiology is unclear although it is thought to be immune-mediated or caused by parvovirus infection (Kaplan *et al.,* 1985; Weiss *et al.,* 1982). Case PC.11, was presented with a history of anaemia. There was no particular cause that could have been identified for the initial moderate degree of anaemia seen in this case. Gradually, the severity of anaemia increased. In erythroid hypoplasia, the blood picture is non-regenerative. The anaemia is due to an inadequate production and release of erythrocytes from the bone marrow although the other cells are unaffected (Kaplan *et al,* 1985). The blood picture in PC.11, was however, slightly regenerative, although the anaemia was normocytic-normochromic. Occasional normoblasts, schistocytes and atypical lymphoblasts were also present. The regenerative blood picture may have been due to the blood transfusions. The bone marrow aspirate was fatty and may be best described as hypocellular. Hence the diagnosis of pure red cell aplasia could not be confirmed.

However, based on the haematological observations a diagnosis of erythroid hypoplasia seemed to be most appropriate for PC.11. History and clinical evaluation of PC.11 did not reveal any obvious cause for the development of erythroid hypoplasia and an immune-mediated mechanism, although the dog was Coombs' negative could not be ruled out. Alternatively, it may have been idiopathic.

5.1.7 ANAEMIA DUE TO NEOPLASIA-CASE DETAILS

There were four cases in this category Case PC.12 to Case PC.15 and the details of the animals were as follows:

Case PC.12

A German Shepherd Dog, 11 years old, female spayed, weight 38 kg.

History

The dog had a history of back problems since 1989. The animal was presented with right hind limb lameness and hip pain but reduced proprioception in both hind legs. The dog had been on prednoleucotropin since 1989, but had increasing bouts of hind limb weakness and cramps which persisted for 10-15 minutes. She was presented in 1995, with the same problem but was worsening.

Clinical Signs on Presentation

The dog was dull, slightly deteriorating in condition and lethargic, with a decreased appetite. There was diarrhoea with some melaena from the lower intestinal tract. A swaying gait consistent with her neurological problem was still present.

Radiography

Nothing abnormal was diagnosed.

Ultrasonography

There was some peritoneal effusion and a very mottled spleen with areas of decreased echodensity throughout the spleen. There were also some areas which were

suggestive of haemorrhages. Ultrasound diagnosis of a bleeding splenic mass was made.

Laboratory Tests

Biochemistry: The test revealed only an increase in the concentrations of ALT.

Faecal Culture and Sensitivity: The only potential pathogen isolated was Clostridium perfringens Type A.

Haematological Findings and Diagnosis

Haematological findings are in Table 26.

There were no significant changes in the first blood sample except a reduction in the platelet numbers and the presence of some normoblasts.

In the subsequent blood sample, examination of the blood smear detected features of a regenerative anaemia and some schistocytes and normoblasts. A low platelet count and slight leucocytosis were also present.

Diagnosis of anaemia due to a blood loss was made. The presence of schistocytes suggested a haemangioma or a haemangiosarcoma.

Outcome

Given the findings of a bleeding splenic mass on ultrasound and the haematological profile, it seemed probable that the dog had a splenic neoplasm, most likely a splenic haemangiosarcoma. Taking into consideration the poor prognosis the dog was euthanased.

Pathological Examination

There was a large amount of blood free in the abdominal cavity. On the ventral tip of the spleen there was a dark red irregularly shaped mass (approximately 6.0cm in diameter). There were in addition a number of secondary small masses on the liver.

The spinal cord was removed to be examined for chronic degenerative radiculomyelopathy.

Histopathological Diagnosis

Microscopic examination of the spleen confirmed the presence of haemangiosarcoma and the spinal chord examination confirmed the chronic degenerative radiculomyelopathy.

Diagnosis

Chronic Degenerative Radiculomyelopathy.

Haemangiosarcoma and associated haemorrhagic anaemia .

Table 26: Haematology results of PC.12

Sample Days after Sample 1	1 0	2 29
R.B.C.x10^{12}/l	6.03	4.18
Hb.g/dl	13.80	8.80
PCV%	40.20	28.70
MCVfl	67.00	69.00
MCHpg	22.80	21.00
MCHCg/dl	34.20	30.60
Plt.x10^{9}/l	88.00	98.00
Retic.%.	not done	not done
W.B.C.x10^{9}/l	14.10	20.10
BNeutro.x10^{9}/l	0	0.10
Neutro.x10^{9}/l	11.70	17.48
Lympho.x10^{9}/l	1.06	1.20
Mono.x10^{9}/l	1.13	0.80
Eosino.x10^{9}/l	0.07	0.10
Baso. x10^{9}/l	0	0
Normoblasts	0.14	0.40

Case PC.13

A Labrador, 10 years old, male castrated, weight 37.2 kg.

History

The dog had decreased appetite with weight loss over a four month period. He had also developed haematuria, diarrhoea and a cough in the past two weeks prior to referral to the University of Glasgow Veterinary School. Haematemesis had occurred on two occasions. The dog was on medication prescribed by the previous veterinarian.

Clinical Signs on Presentation

The dog was thin due to chronic weight loss and had a decreased appetite. No pallor of the mucous membranes was seen, although the dog tired very easily. Frequent vomiting, some blood-stained diarrhoea, and on tracheal palpation a productive cough were found. On examination of the mouth there were severe halitosis, tartar and an ulcer on the soft palate. Haematuria was also present.

Radiography

The thoracic radiograph revealed a globular heart shadow and some pulmonary miliary neoplasia which was probably metastatic.

Laboratory Tests

Biochemistry: Nothing remarkable was seen.

Urine Test: The following results were seen: protein ++ , blood +++, pH 7.5, specific gravity 1.024, urea 395 mmol/l.

Haematological Findings and Diagnosis

Haematological findings are shown in Table 27.

A slightly regenerative blood picture was observed, with schistocytes and echinocytes. A reduction in the platelet numbers and many target cells were also found. Blood loss was considered as a possible explanation for the findings.

Outcome

The dog started deteriorating and it was thought advisable to euthanase it.

Pathological Examination

On gross examination the carcase was thin in condition with some disseminated neoplasms in internal organs. The primary tumour mass was an irregularly nodular lesion (3x2x2 cm in size), firm reddish-cream in colour located in the wall of the right atrium. Smaller nodules of similar or more haemorrhagic tissues were present on the adjacent dorsal pericardium with a nodule on the left atrial wall (1cm) . The pericardial sac contained a slight excess of blood tinged fluid. Multiple dark red to black, rounded, firm nodules (1-2mm to 1cm), were scattered throughout the lung lobes Nodules present were composed of clotted blood and there was some subcapsular and pelvic haemorrhage.

Histopathological Diagnosis

Microscopy confirmed that the tumour deposits in the heart, the pericardial sac, the lungs and the kidneys to be a highly malignant haemangiosarcoma.

Diagnosis

Atrial haemangiosarcoma with multiple metastases and associated haemorrhagic anaemia.

Table 27: Haematology results of PC.13

Sample Days after Sample 1	1 0
R.B.C.x10^{12}/l	4.38
Hb.g/dl	10
PCV%	30.6
MCVfl	70
MCHpg	22.8
MCHCg/dl	32.6
Plt.x10^{9}/l	93
Retic.%.	not done
W.B.C.x10^{9}/l	13.7
BNeutro.x10^{9}/l	0
Neutro.x10^{9}/l	11.44
Lympho.x10^{9}/l	0.82
Mono.x10^{9}/l	0.75
Eosino.x10^{9}/l	0.55
Baso. x10^{9}/l	0
Normoblasts	0.14

Case PC.14

A Boxer, 6 years old, female spayed, weight 27kg

History

The dog was diagnosed having multicentric lymphosarcoma a year ago and was referred with a history of feeling "unwell" and was coming out of remission. Persistent vomiting, dullness, anorexia and intermittent diarrhoea were seen and recently the dog became polydipsic. The owners also noticed a progressive weight loss. The dog had been on medication of prednisolone and cyclophosphamide.

Clinical Signs on Presentation

The lymph nodes were all enlarged, the popliteal lymph nodes were 3 to 4cm and the submandibular nodes was approximately 4 to 5cm large. The dog appeared to be dull and on examination the mucous membranes appeared to be pale in colour.

Laboratory Tests

Biochemistry: This test showed an increase in the serum Alk.Phos., serum ALT concentrations and serum AST in some samples. Increase in urea and creatinine concentrations were also seen.

Urine Test: It was positive for proteins, pH 6.5 , a few white blood cells and epithelial cells were present.

Haematological Findings and Diagnosis

The haematological findings are shown in Table 28

Sample 1 (day 0)

A regenerative blood picture was observed and the anaemia was most likely to be due to the neoplasia.

Sample 2 (shown in the appendix)

This sample was also regenerative, in addition, there were quiet a few schistocytes and target cells. The thrombocytopenia and schistocytes may have been due to a blood loss or DIC.

Subsequent blood samples (as shown in Appendix D), showed a regenerative blood picture, target cells, some schistocytes and a few normoblasts. Occasional lymphoblasts were also seen which may have been be exfoliating extramedullary or bone marrow-derived tumour cells. It was thought advisable to check the bone marrow. The coagulation profile was also checked.

Fibrinogen was estimated to be 370 mg/l which was within the normal ranges (normal=150-400mg/dl).

A diagnosis of haemorrhagic anaemia due to neoplasia was considered.

Bone Marrow Aspirate

The bone marrow aspirate was diluted with blood and only small numbers of erythroid and granulocytic precursors were seen in the sample. No megakaryocytes or significant number of blast cells were seen excluding lymphoid leukaemia. The sample was suggestive of a drug-induced myelosuppression.

Outcome

The dog was under medication for lymphosarcoma. The chemotherapy protocol consisted of vincristine, cyclophosphomide and prednisolone. In addition to these

drugs the dog was also given cimetidine and metoclopropamide to treat the developing diarrhoea and vomiting.

After regular checks and routine haematology and biochemistry the dog was finally presented with a very painful thymic mass. Unfortunately, the dog was deteriorating in condition and it was advised in consultation with the owner to euthanase the dog. It was not possible to conduct a post-mortem examination to determine the location and spread of the tumour and the cause of the blood loss.

Diagnosis

Lymphosarcoma and associated haemorrhagic anaemia.

Table 28 : Haematology results of PC.14

Sample *	1	8	9	10	17	18	19
Days after Sample 1	0	53	60	68	116	123	130
R.B.C.x10^{12}/l	4.96	3.71	3.76	3.96	5.07	5.05	5.16
Hb.g/dl	12.00	8.80	9.00	9.40	11.70	12.90	12.10
PCV%	36.90	26.30	27.20	29.40	35.00	34.80	35.30
MCVfl	74.00	71.00	72.00	74.00	69.00	69.00	68.00
MCHpg	24.10	23.70	23.90	23.70	23.00	25.50	23.40
MCHCg/dl	32.50	33.40	33.00	31.60	33.40	37.00	34.20
Plt.x10^{9}/l	102.00	66.00	82.00	91.00	284.00	263.00	248.00
Retic.%.	not done	0.09	0.10	not done	0.00	0.00	0.00
W.B.C.x10^{9}/l	6.70	6.00	3.30	4.70	7.10	3.60	8.40
BNeutro.x10^{9}/l	0.00	0.09	0.05	0.00	0.07	0.00	0.04
Neutro.x10^{9}/l	3.38	3.72	0.33	1.97	4.90	2.646	7.22
Lympho.x10^{9}/l	2.65	1.02	1.15	1.13	1.42	0.76	1.01
Mono.x10^{9}/l	0.30	0.15	0.18	0.14	0.46	0.11	0.08
Eosino.x10^{9}/l	0.00	0.03	0.02	0.00	0.07	0.04	0.04
Baso. x10^{9}/l	0.00	0.00	0.00	0.00	0.18	0.05	0.00
Normoblasts	0.37	0.99	1.57	1.32	0.00	0.00	0.00

*Intervening samples are shown in Appendix D.

Case PC.15

A Golden Retriever, 8 years old, male castrated, weight 35 kgs.

History

The dog was referred to the veterinary hospital at the Glasgow Veterinary School, with small lumps over the eyes, on the neck and on the head which were lately growing in size.

Clinical Signs on Presentation

On physical examination there were warts present all over the head, near the eyes and on the neck. According to the owner the dog was also lethargic and had lost a significant amount of weight and was weaker. The dog was also polydipsic and polyuric. Mucous membranes were normal in appearance.

Laboratory Tests

Haematological Findings and Diagnosis

The haematological findings are shown in Table 29.

Sample 1:

The blood picture was initially non-regenerative and there was increased rouleaux formation of red cells which may have been caused by an increase in plasma proteins. However, no protein estimation and electrophoresis were done so the presence or absence of a gammopathy was not established.

Sample 2

The blood picture became regenerative with many megaplatelets which were suggestive of an anaemia due to blood loss.

Sample 3

A non-regenerative blood picture and a number of target cells were seen. It was advised to check liver functions.

Sample 4

This sample was taken immediately after a meal and hence the Hb, MCH and MCHC values were invalid due to hyperlipidaemia. However, there were some plasmacytoid lymphocytes and occasional blast cells present suggesting a reactive and /or neoplastic process.

Sample 5

A regenerative blood picture was present.

It was concluded that the dog had a possible myeloma or lymphosarcoma.

Bone Marrow Aspirate

A bone marrow aspirate was taken the next day after the first blood sample. On examination the aspirate was hypercellular and large proportion of the cells were plasma cells. In addition, there were normal numbers of erythroid and myeloid precursors. Megakaryocyte numbers appeared normal too. The presence of numerous plasma cells in the marrow may have been the result of a developing myeloma or infection. However, no further investigations were done in order to clarify the situation.

Skin Biopsy Report

An ellipsoidal skin biopsy of 1cm long x 0.5 cm in diameter was taken. On microscopic examination the nodule was composed of sheets of large uniform round cells infiltrating through the dermis. The cells had rounded or slightly cleaved nuclei,

small nucleoli, a very coarse chromatin pattern and frequent mitotic figures, some of which were bizarre. The cells also had small amounts of poorly defined slightly basophilic cytoplasm. The skin biopsy was indicative of a cutaneous lymphosarcoma.

Diagnosis

Cutaneous lymphosarcoma and haemorrhagic anaemia of unknown cause.

Outcome

The dog was sent home on regular medications with no response from the owners regarding further follow ups.

Table 29 : Haematology results of PC.15

Sample	1	2	3	4	5
Days after Sample 1	0	30	36	43	71
R.B.C.x10^{12}/l	4.99	3.32	3.96	3.98	4.80
Hbg/dl	11.10	7.90	9.00	0.00	11.30
PCV%	35.30	25.40	27.50	27.90	33.70
MCVfl	71.00	77.00	69.00	70.00	70.00
MCHpg	22.20	23.70	22.70	0.00	23.50
MCHCg/dl	31.40	31.10	32.70	0.00	33.50
Plt.x10^{9}/l	221.00	588.00	695.00	646.00	391.00
Retic%.	0.00	0.04	not done	0.25	not done
W.B.C.x10^{9}/l	5.20	13.70	8.60	6.60	6.80
BNeutro.x10^{9}/l	0.00	0.14	0.00	0.00	0.07
Neutro.x10^{9}/l	3.51	12.33	7.10	4.88	4.93
Lympho.x10^{9}/l	1.59	0.41	0.39	1.19	0.92
Mono.x10^{9}/l	0.08	0.82	0.21	0.36	0.61
Eosino.x10^{9}/l	0.03	0.00	0.00	0.16	0.07
Baso. x10^{9}/l	0.00	0.00	0.00	0.00	0.00
Normoblasts	0.00	0.00	0.00	0.00	0.20

5.1.8 ANAEMIA DUE TO NEOPLASIA-DISCUSSION

Haemangiosarcoma

Haemangiosarcoma, also called malignant haemangioendothelioma or angiosarcoma, is a malignant tumour of the endothelial cells. It is seen more frequently in dogs than in any other domestic animal (Pulley and Stannard, 1990, Waller and Rubarth, 1967). According to Brown *et al* (1985), in a retrospective study, 62% of the dogs with haemangiosarcoma had a primary splenic involvement. The heart and lung have also been identified as primary sites (Oksanen, 1978). The right atrium seems to be the primary site for cardiac haemangiosarcomas. In a study done by Kleine *et al.*, (1970), 31 dogs had haemangiosarcoma of the heart. In man, haemangiosarcoma is the least commonly found cardiac tumour, unlike domestic animals were there has been an increase in the number of cases identified (Kleine *et al.*, 1970). In this study, there were two dogs with haemangiosarcoma which had haematological changes. Dog PC.13 had haemangiosarcoma of the right atrium and dog PC.12 had the tumour in the spleen. Other organ involvement such as the liver, subcutaneous tissues, the brain, the kidney and the urinary bladder have also been observed (Waller and Rubarth, 1967; Kleine *et al.*, 1970). Bingel *et al.*, (1974), have studied haemangiosarcomas in the bones, and found osseous haemangiosarcomas in 21 dogs which is an unusual location. However, since this is a tumour of endothelial origin, the primary tumour could theoretically arise in any organ (Pulley and Stannard, 1990). It is usually difficult to estimate the size of these tumours due to the extensive intralesional haemorrhages which also causes difficulty in identifying the primary site (Bingel *et al.*, 1974).

The malignancy of these tumours seems to be extremely high as it may become disseminated. It has a very rapid, effective and high rate of metastasis especially by the haematogenous route to various organs making the primary site difficult to identify (Waller and Rubarth, 1967, Brown *et al.*, 1985). Metastasis to the lungs, liver, spleen, kidneys and brain has been reported to be frequent in cardiac haemangiosarcomas (Kleine *et al.*, 1970). This was similar to case PC.13 in which the dog in addition to the primary tumour of the heart had multiple metastasis to the vital organs such as the pericardial sac, lungs and kidneys. Whereas, according to Waters *et al* (1988), the metastatic pattern in dogs with splenic haemangiosarcomas was mainly to the liver, omentum, mesentery, kidneys, adrenals and lymph nodes, and to a lesser extent to the diaphragm, myocardium and brain. Dog PC.12 had metastasis only to the liver which could explain the increase in the serum ALT levels.

The German Shepherd dog is the breed most frequently reported to be susceptible to this tumour (Hirsch *et al.*, 1981; Kleine *et al.*, 1970; Waller and Rubarth 1967). Haemangiosarcomas have also been observed in Boxers although this breed has been reported to be more liable to develop carcinomas than any other breed recorded (Waller and Rubarth 1967; Bingel *et al.*, 1974). Case PC.12 was a German Shepherd dog, and was a female. According to the literature male dogs are affected more frequently than female dogs and the other dog in this study was a male Labrador (Keline *et al.*, 1970; Waller and Rubarth, 1967). The ages of PC.13 and PC.12 were 11 and 10 years respectively, which corresponds with the findings of Pulley and Stannard (1990), who described the average age to be usually 9-10 years.

The commonest clinical sign observed is a marked anaemia, weakness, abdominal distension or sudden collapse. Indistinct signs may be cardiac insufficiency, cough, dyspnoea, polydipsia and apathy. Haemoperitoneum is also an important

finding associated with haemangiosarcomas. (Oksanen, 1978, Kleine *et al.*, 1970). The common signs in cases PC.12 and PC.13 were anorexia and lethargy. Blood-stained diarrhoea was also seen in both these dogs which may have been due to the blood loss along the intestinal tracts. Marked haematuria was present in dog PC.13 as a result of acute blood loss probably due to the multiple metastasis.

Several factors may contribute to the development of anaemia in a dog with haemangiosarcoma. Blood loss, either from rupture of the tumour into the body cavities or lungs or haemorrhage within the tumour mass, may result in a mild to severe anaemia. This mainly depends on the extent of blood loss and the compensatory erythroid hyperplasia of the bone marrow. According to Kleine *et al.*, (1970), dogs with haemangiosarcoma have active haemopoiesis unlike the anaemia associated with other types of neoplasia.

Haematological findings associated with haemangiosarcoma are of a wide variety. Haemangiosarcoma results in haemorrhagic anaemia which may be complicated with haemostatic abnormalities. The anaemia is usually regenerative and normocytic and normochromic. Anisocytosis, poikilocytosis, reticulocytosis, normoblastosis, increase in the number of target cells, Howell-Jolly bodies and neutrophilia with a left shift have been some commonly made observations (Hirsch *et al.*, 1981; Ng and Mills, 1985; Kleine *et al.*, 1970). The leucocytosis with neutrophilia present may be due to the erythroid hyperplasia affecting the myeloid elements but the degenerative tumour changes may also have an effect.

Schistocytosis and acanthocytosis have also been typical findings associated with haemangiosarcomas (Hirsch *et al.*, 1981, Rebar *et al.*, 1980). Microangiopathic haemolytic anaemia (MAHA), may also be observed in haemangiosarcomas, resulting in the formation of schistocytes. Schistocytes are formed due to the mechanical

damage caused by the passage of the erythrocytes through the fibrin deposits within the neoplastic vasculature. However, in some cases if the lesion is too small, schistocytes may not be present in the peripheral blood (Rebar *et al.*, 1980). Schistocytosis was most prevalent in dogs with haemangiosarcomas of the liver and heart (Rebar *et al.*, 1980). Interestingly, some spherocytes were seen in dog PC.12 which according to Kleine *et al.*, (1970), and Rebar *et al.*, (1980), have been observed in dogs which haemangiosarcoma.

In dogs PC.13 and PC.12, features of a regenerative blood picture together with schistocytes and normoblasts were seen. There was also a reduction in the platelet numbers and there was a slight leucocytosis in both dogs. These observations were consistent with the findings reported in the literature.

According to Hirsch *et al.*, (1981), there is a definite association between acanthocytes and haemangiosarcomas. Acanthocytes are often seen in hepatic diseases due to known membrane changes, but their occurrence in haemangiosarcomas is unexplained. In case PC.12 some acanthocytes were observed which may have been as a result of the hepatic metastases of the tumour which was diagnosed post-mortem.

Thrombocytopenia may also be present in cases of haemangiosarcoma suggesting an increased consumption of platelets in association with MAHA or due to blood loss. An increased number of large platelets along with an increase in erythrocyte production reflects a sequestration or haemorrhage into the tumour sinuses or body cavities (Ng and Mills, 1985). According to Hammer *et al.*, (1991), haemangiosarcomas may also be associated with haemostatic abnormalities especially due to DIC. The evaluation of the coagulation profiles of 24 dogs in their study, revealed that 12 (50%) of the dogs had DIC.

Dogs with haemangiosarcoma usually have a poor prognosis irrespective of the location of the tumour. The duration of signs before diagnosis is short and dogs may die suddenly from the rupture of the primary or metastatic tumour (Brown *et al.*, 1985).

In the two cases studied, the findings were typical and were consistent with the findings reported in the literature. Due to the poor prognosis both dogs were euthanased.

Lymphosarcoma

Canine lymphosarcoma is one of the commonest lymphoid neoplasm observed in dogs. The incidence in the general dog population is reported to be as high as 24 per 100,000 dogs (Dorn *et al.*, 1967). Classification of lymphosarcomas can be either based on a combination of the cytology, pattern of growth i.e. diffuse or follicular, immunomorphological or anatomical presentation. In animals the classification is commonly based on the anatomical form such as multicentric, thymic, alimentary, cutaneous and others. The multicentric form seems to be most commonly encountered in dogs (Dobson and Gorman, 1993).

Lymphosarcoma has no breed predilection, however, Boxers and pure bred dogs have been reported to be at a relatively higher risk (Dorn *et al*, 1967). In a survey by Dorn *et al.*, (1967), of the 83 dogs with lymphosarcoma the average age was calculated as 8-10 years. Lymphosarcoma seldom occurs in dogs less than 1 year old although a few cases have been reported in pups as young as 4 months of age. Dogs 1-4 years old make up about 10% of the cases, and the incidence rises steeply at 5-11 years (80% cases) (Moulton and Harvey, 1990). In this study two dogs were

selected with lymphosarcoma, a Boxer, PC.14, with a multicentric form and a Golden Retriever, PC.15, presented with a cutaneous form of lymphosarcoma.

The ages of both these dogs were similar to the ages cited in literature for lymphosarcoma to develop.

Lymphosarcoma can be diagnosed by cytological or by histopathological examination. The skin biopsy taken from PC.15, confirmed the growing skin lesions to be a cutaneous form.

According to Moulton and Harvey (1990), about two thirds of the dogs with lymphosarcoma have anaemia usually with a normocytic-normochromic erythrocyte morphology. However, the type of anaemia observed in lymphosarcoma can vary. The anaemia due to neoplasia can vary from being markedly haemorrhagic to a non-regenerative anaemia of chronic disease. A haemolytic anaemia may also be seen (Arbaje and Beltran, 1990). The severity of anaemia observed in PC.14, was mild to moderate, and it was regenerative. Schistocytes were also seen in the blood smear which is a frequent finding in anaemia due to neoplasia. On some occasions the dog was moderately anaemic with a decreased number of platelets. This finding was suggestive of episodes of blood loss due to the tumour.

In PC.15, the anaemia was initially regenerative with megaplatelets and then non-regenerative. This was probably caused by haemorrhagic lesions. In the absence of a post-mortem examination the exact cause of the blood loss could not be established. However, it is safe to assume that the tumour may have metastasised and may have caused ulcerative lesions.

5.2 Discussion

It is essential to know the history of the patient and to identify the presenting clinical signs in order to diagnose the cause of the anaemia. In the prospective cases presented in this study the history and the clinical presentation formed an important part in the diagnosis of anaemia. In cases where an accurate history was present, such as a history of certain drug treatment, it was usually easy to identify the cause of anaemia and to assess the prognosis of the case. Clinical presentation of these anaemic dogs also revealed some common findings (Appendix E). Dogs with anaemia seemed to be presented with common signs of dullness, lethargy, pallor and anorexia. However, vomiting, pyrexia and polydipsia seemed to be less common clinical signs. Dogs with haemolytic anaemia in addition to the above, had more severe anaemia or icterus, haemoglobinuria, increased pulse rate and cardiac murmurs. It was also observed that anaemia could be associated with a variety of disease conditions. The causes of anaemia included conditions such as haematoma of the spleen, drug-induced chronic blood loss, immune-mediated conditions, haemangiosarcoma, lymphosarcoma and many others. The classification of anaemias was based upon the aetiology involved as discussed previously in this study. The severity of the anaemia and the prognosis can vary depending on the aetiology involved and the regenerative capacity of the bone marrow as was seen in these cases. It can thus be concluded that anaemia is commonly observed in dogs and it is usually a feature of an underlying disease process. The cases described in this study emphasised that to a variety of disease conditions associated with anaemia can be anticipated in clinical practice.

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GLOSSARY

Erythrocyte Abnormalities

1. Size

Anisocytosis

Variation in size of erythrocytes which may be due to the presence of cells larger and smaller than normal. Can be seen in auto-immune haemolytic anaemia.

Microcytes

These are cells smaller than the normally-sized erythrocytes. These cells can be observed in iron deficiency anaemia and may also be the result of spherocytosis or fragmentation.

Macrocytes

These are cells larger than the normally-sized erythrocytes. Can be seen if there is an increase in erythropoiesis resulting in reticulocytosis.

2. Shape

Poikilocytes

A non-specific term describing red cells which show deviation from the normal shape,

Specific Diagnostic Forms

Spherocytes

These are cells which are more spheroidal that is less disc-shaped than the normal erythrocytes. Their diameter is less and their thickness is greater than normal. They appear in a smear as small, round densely staining cells without a central pallor. They are formed either due to inherited membrane abnormalities as in hereditary spherocytosis or due to loss of membrane surface as in IgG type auto-immune haemolytic anaemia.

Elliptocytes

These are red cells that are oval or elliptical shaped due to primary membrane abnormality. They occur in hereditary elliptocytosis.

Stomatocytes

Invaginated, cup shaped cells due to membrane abnormalities They can be seen in hereditary stomatocytosis and are best observed in wet preparations.

Echinocytes or Crenated Cells

Red cells with numerous, small, peripheral protrusions caused by dysproteinaemia which may alter the cell membrane. These cells may be observed in some cases of uraemia, hepatobiliary or pancreatic diseases. Can also be artefactual.

Acanthocytes or Spurr Cells

These are red cells with few, irregular, asymmetrical, spine-like projections. They may be seen in uraemia or in cases of haemangiosarcoma. In severe cirrhosis, red cells

accumulate excess cholesterol from abnormal cholesterol rich plasma lipoproteins resulting in spurr cell and target cell formation.

Burr Cells

Small red cells or cell fragments with one or a few fine spine-like protrusions representing damaged or fragmented cells seen particularly in uraemia.

Schistocytes

Red cell fragments of less than 3μm in diameter. Fragmentation is caused by excessive trauma or abnormal shear forces within the circulation due to fibrin deposition in small blood vessels. They may be associated with disseminated intravascular coagulation, haemangiosarcoma, haemangioma, myelofibrosis or may be due to cardiac disorders such as valvular disease.

3. Haemoglobinisation or Chromasia

Anisochromasia

This is an exaggerated variation in the colour of red cells. Can be seen in iron deficiency anaemia responding to iron therapy.

Hypochromasia

An increase in the central pallor of the cells which may be due to lowered haemoglobin concentration or abnormal thinness of the red cells. This is most commonly seen in iron deficiency anaemia.

Polychromasia

Diffuse, bluish cytoplasmic tint due to the presence of RNA, indicating immaturity of the cells. Polychromatic cells are the reticulocytes which can only be seen when stained with new methylene blue.

Specific "Diagnostic " Forms

Target cells or "Mexican Hat" cells

a. Microcytic hypochromic cells with central blob and peripheral ring of haemoglobinisation. These are observed in iron deficiency anaemia.
b. Macrocytic hypochromic, polychromatic target cells seen in the case of markedly increased erythropoeisis such as in auto immune haemolytic anaemia.
c. Normochromic, normocytic target cells. These are due to increased cell surface area caused by excessive cholesterol in the cell membrane. They occur mainly in hepatic disease.

Stomatocytes

In these cells, the normal central pallor is replaced by a slit or mouth like unstained area corresponding to the invagination of the membrane. Can be best seen in wet preparations. A few may be seen in any chronic condition.

4. Inclusions

Punctate basophilia or basophilic stippling

Numerous, fine blue dots in the erythrocytes either due to the presence of RNA in young cells or iron. Seen as a normal regenerative feature in cattle. In dogs, it is seen associated with lead poisoning whereas in cats it is observed during active erythrocyte regeneration and should not be interpreted as a lead poisoning.

Howell-Jolly Bodies

Round, small densely staining structures representing nuclear remnants and containing DNA. They occur usually as single but may be present as multiple remnants in the erythrocytes. The number of Howell-Jolly bodies increase in regenerative anaemias reflecting increased erythropoiesis. After corticosteroid treatment and post splenectomy the increase is caused by impairment or loss of the pitting function of the spleen.

Reticulum in reticulocytes

Cells containing ribonucleic acid precipitate into granules or filaments are called as punctate or aggregate reticulocytes. These are best observed by supra-vital dyes such as new methylene blue.

Heinz-Bodies

Large rounded refractile inclusions, usually solitary, protruding from the surface of the erythrocytes due to irreversibly oxidised, denatured haemoglobin attaching to the red cell membrane's, reduced gluthathione groups. They stain pale blue with supra-vital stain and may be due to oxidant drug effect.

5. Other Abnormalities

Increased Rouleaux Formation

On standing, the erythrocytes in a blood sample aggregate and lie face to face on their sides like a pile of coins forming a rouleau formation. This is a non-specific phenomenon. Increased rouleaux formation can be seen due to the increase in plasma high-molecular weight proteins such as high levels of fibrinogen or globulins. It may

be associated with infections and multiple myeloma. Pseudoagglutination due to massive rouleaux formation should either disperse completely or transform itself into typical rouleaux.

Auto-agglutination of erythrocytes

Red cells form large clumps which are unable to disperse after the addition of three to four volumes of 9g/l of sodium chloride to the blood. Can be seen in immune-mediated haemolytic anaemias.

Normoblastosis or normoblastaeamia

This term describes the presence of nucleated erythrocyte precursors, that is erythroblasts (normoblasts) in the blood. They are mainly derived from extramedullary foci of erythropoiesis (EMH) that is from the spleen and the liver. A few normoblasts may be found after corticosteroid treatment, in septicaemia or in cyanotic heart failure. Their number will significantly increase in regenerative anaemia, particularly in haemolytic anaemia, and in acute leukaemia. After splenectomy, if there is extramedullary haemopoiesis, their number may be very large. They may also be observed in myeloproliferative disorders involving the erythroid series.

Regenerative blood picture

This term is used to describe the changes in the blood reflecting increased rate of erythropoiesis and consequently increased number of young red cells (reticulocytes). These changes may be slight, moderate or marked and may be described as: "slight/moderate/marked anisocytosis, polychromasia and few/many/numerous/

Howell-Jolly bodies and normoblasts are present". Reticulocytes are larger in diameter than normal red cells, their volume is increased and if present in high enough numbers they increase the MCV. None of the above changes are present in a non-regenerative blood picture reflecting the inactivity of the bone marrow in spite of anaemia, so it will appear as a normal blood picture in health.

Granulocyte Abnormalities

1. Nuclear

Giant cell forms

These may be seen as metamyelocytes representing abnormal granulopoiesis in toxaemia associated with bacterial infection.

2. Cytoplasmic

Toxic granulation

Many purple granules appear in the cytoplasm of neutrophils representing disturbance of granule formation. Can be seen in bacterial infections or toxaemia.

Döhle-bodies

Round or elliptical, pale blue staining inclusions about 5nm long in the cytoplasm of neutrophils which develop in immature granulocytes in the bone marrow and represent depolymerized ribosomes. May be single or multiple. These cells are observed in bacterial infections.

A regenerative left shift

The term "regenerative left shift" is used to describe neutrophilia and the appearance of immature neutrophils in the blood. Depending on the degree of immaturity, the left shift can be slight, moderate or marked. Neutrophilia with band forms only represents a slight left shift while the presence of band forms and metamyelocytes will indicate moderate and the presence of additional myelocytes and promyelocytes a marked left shift. Regenerative left shift occurs in bacterial, particularly pyogenic infections and in auto-immune haemolytic anaemia.

A degenerative left shift

In acute septicaemic infections bacterial endotoxins may suppress granulopoiesis in the bone marrow and instead of neutrophilia, normal or lower than normal neutrophil numbers and immature neutrophils are present in the blood which represent a "degenerative left shift" and usually carries a poor prognosis.

Leuco-erythroblastic blood picture

This term is used to describe the presence of numerous nucleated red cells and immature granulocytes in the blood. It is seen in myelophthisis, auto-immune haemolytic anaemia, septicaemia, severe haemorrhage.

Lymphocyte Abnormalities

Atypical lymphoid cells

Non specific term describing a variety of cell forms which are usually larger than normal lymphocytes.

Reactive lymphoid cells or immunocytes

These are larger than normal lymphocytes with dark nuclei and plasmacytoid appearance. May be seen in high numbers after vaccination. Some may appear in infections, particularly in viral infections.

Lymphoblasts

Large, immature cells with high nuclear cytoplasmic ratio, pale staining nuclei with single or multiple nucleoli and small amount of basophilic cytoplasm. High number of lymphoblasts are present in the blood in acute lymphoblastic leukaemia (ALL). In lymphosarcoma, none, occasional or numerous lymphoblasts may be found.

Lymphosarcoma-type cells

Very large, atypical cells with abnormal nuclear structure and shape, such as irregular nuclear outline or nuclear clefting, one or multiple large nucleoli, increased amount of cytoplasm which may contain vacuoles. These cells may appear in the blood in low or high numbers due either to direct shedding from peripheral tumour masses (overspill leukaemia) or to secondary involvement of the bone marrow.

APPENDIX A

Table A1: Normal haematological values in dogs.

Features	Range	Abbreviations
Red Blood Cells	5.50-8.50 x 10^{12}/l	R.B.C.x10^{12}/l
Haemoglobin	12.00-18.00 g/dl	Hb g/dl
Packed Cell Volume	37.00-55.00 %	PCV %
Mean Corpuscular Volume	60.00-77.00 fl	MCV fl
Mean Cell Haemoglobin	19.50-24.50 pg	MCH pg
Mean Cell Haemoglobin Concentration	32.00-36.00 g/dl	MCHC g/dl
Reticulocytes	1.00-1.50%	Retic.%
Platelets	200-500 x 10^9/l	Plt. x10^9/l
White Blood Cells	6.00-12.00 x 10^9/l	W.B.C.x10^9/l
Neutrophils	3.00-11.80 x 10^9/l	Neutro.x10^9/l
Lymphocytes	1.00-4.80 x 10^9/l	Lympho.x10^9/l
Monocytes	0.15-1.35 x 10^9/l	Mono.x10^9/l
Eosinophils	0.10-1.25 x 10^9/l	Eosino.x10^9/l
Basophils	Rare	Baso. x10^9/l

The normal ranges followed at the University of Glasgow, Veterinary School for canine red blood cell count, white blood cell count and platelet numbers were obtained from the literature (Schalm *et al.*, 1975a). Zero for platelet indicates platelet aggregates and other cells are usually the normoblasts or any other nucleated cell.

Table A2: Normal biochemistry values in dogs.

Assays	Mean	Range
Urea mmol/l	4.98	0.00-7.47
Creatinine µmol/l	88.40	44.00-132.00
Sodium mmol/l	148.00	136.00-160.00
Potassium mmol/l	4.40	3.40-5.80
Chloride mmol/l	105.00	95.00-115.00
Calcium mmol/l	2.61	2.34-3.03
Phosphates mmol/l	1.80	1.29-2.9
Magnesium mmol/l	0.94	0.61-1.19
Cholesterol mmol/l	3.87	1.80-6.96
Total Bilirubin µmol/l	3.42	0.00-10.26
Alkaline Phosphatase IU/l	less than 233	4.00-233
Alanine Aminotransferase IU/l	22.00	0.00-35.00
Aspartate Aminotransferase IU/l	23.00	0.00-35.00
Total Protein g/l	62.00	50.00-78.00
Glucose mmol/l	3.88	2.49-4.99

Preparation of the May-Grünwald Giemsa Stain used at the University of Glasgow, Department of Veterinary Pathology, Haematology Laboratory

Preparation of a good stain for the examination of certain features of a blood smear is one of the most essential aspects in haematology. A good smear with evenly spread cells and a good stain is essential to enhance certain features for identification of cells. A blood sample (25µl) is aspirated through the Roche ABX Minos Vet Automated Haematology Analyser for an automated blood cell count. Subsequently, a blood smear is made by a 75x 1.3-1.5 mm microcapillary tube containing blood from the rest of the blood in the EDTA tube. The smear is air dried and then stained with the May-Grünwald Giemsa stain. The method of stain preparation followed at the haematology laboratory is described as follows:

Materials

Fixative: Methanol.

Buffered distilled water (pH 6.8): This is prepared by dissolving one tablet of pH 6.8 (BDH, England) in one litre of water.

May Grünwald stain (Gurr® BDH, England): The May Grünwald stain is prepared by freshly diluting it to 50 percent with buffered distilled water (pH 6.8 prepared same as above) and it is then filtered with Whatman® filter paper number 113V before use.

Giemsa's Stain (Gurr® BDH, England): Giemsa's stain is prepared freshly by diluting it to 7.5 percent with buffered distilled water (pH 6.8 prepared same as above) and filtering it with Whatman® filter paper number 113V before use.

Methods

1. Fix air dried blood smears in methanol for 5 minutes.
2. The smear is then immersed in May-Grünwald stain for 6 minutes.
3. The smear is transferred for washes into clean buffered water (pH 6.8) for 1 minute for a wash.
4. The third method is then repeated with fresh buffered water (pH 6.8).
5. It is then placed into Giemsa's stain for 8 minutes.
6. Step 4 is repeated for another 8 minutes using another fresh staining jar with Giemsa stain.
7. Step 3 is repeated but by using fresh buffered distilled water, for removing the excess stain.
8. Allow slide to stand in buffered distilled water for 1minute to let differentiation take place.
9. The slide is taken out and then made to stand upright to dry.

Result

Fixation of a blood film in methanol is essential to preserve the cell structure and prevent disruption of morphology by the subsequent procedures. This is a Romanowsky stain and its properties depend on the interaction of methylene blue, azure and eosin components. The methylene blue, a basic dye, is absorbed by the acidic groupings of the nucleic acids and proteins of the cell nuclei and primitive

cytoplasm. Basophil granules contain heparin, which is acidic having affinity for this dye and stain blue in colour. Methylene blue azure stains the azurophil granules of leucocytes.

Eosin, which imparts red colour, is an acidic dye which reacts with basic groupings example haemoglobin molecule. The granules of eosinophils contain a spermine derivative which react strongly with eosin.

Each film is examined under the low power (x25), high power (x40) and oil immersion (x100) of the microscope some blood samples follow a different procedure in which different staining procedures may be required.

Technique of making a blood smear:

It is essential to know the correct technique of making a blood smear for examination. Two clean slides and a microcapillary tube of dimensions 75x 1.3-1.5mm are necessary. One of the clean slides is placed on a levelled surface. Then a very small drop of thoroughly mixed blood, is deposited on one end but not at the edge of the slide with the help of a microcapillary tube. This is called as the "head" of the smear. The drop of blood on the slide is then spread by means of the other slide which has a shortened edge as is called the "spreader". The spreader slide is placed in front of the drop of blood and after they make contact, the blood will spread along the edge of the spreader slide. Then the spreader slide is pushed forward along the far end of the slide in a smooth and continuous glide pulling the blood behind it. The feathered end is called the "tail" of the smear. The angle in which the spreader slide is held determines the thickness of the smear. The greater the angle the thicker the film. Generally, about a 30° angle is used. The slide is then dried rapidly in air either by holding it under a fan or waving rapidly.

Appendix B: Data of 245 dogs with anaemia admitted to the University of Glasgow Veterinary School from 1st January to 31st December 1994.

DN.	Age(yrs)	PCV%	Severity	Clinical summary	Film report	Type of anaemia
1	10.0	26.10	MO	Lymphosarcoma	Non-regenerative blood picture	Secondary anaemia of chronic disease
2	9.3	34.60	MI	No data	No data available	No data available
3	8.6	15.80	S	Post splenectomy	Non-regenerative blood picture many Howell-Jolly bodies and schistocytes.	Acute blood loss/ Haemorrhagic anaemia
4*	15.2	29.30	MO	Skin tumours	Non-regenerative blood picture	Secondary anaemia of chronic disease
5	5.0	35.90	MI	Chronic elbow joint effusion, lame	Non-regenerative blood picture	Secondary anaemia of chronic disease
6*	10.2	32.00	MI	Congestive cardiac failure	Non-regenerative blood picture	Secondary anaemia of chronic disease
7	4.0	33.90	MI	Check prior to breeding	Regenerative blood picture	Haemorrhagic anaemia
8	8.1	34.90	MI	Amputation 24 hrs ago	Non-regenerative blood picture	Acute blood loss/ Haemorrhagic anaemia
9	7.0	36.60	MI	Atopy?	Non-regenerative blood picture	Secondary anaemia of chronic disease
10*	12.0	35.60	MI	Diabetes mellitus	Non-regenerative blood picture	Secondary anaemia of chronic disease
11	9.1	32.60	MI	Diabetes mellitus	Non-regenerative blood picture	Secondary anaemia of chronic disease
12	5.0	35.30	MI	Demodex and anaemia	Non-regenerative blood picture	Secondary anaemia of chronic disease
13*	5.4	30.30	MI	Chronic renal failure protein, loosing nephropathy	Non-regenerative blood picture	Secondary anaemia of renal failure
14	0.0	28.70	MO	Had adrenalectomy and is polyphagic	Non-regenerative blood picture	Secondary anaemia of chronic disease
15*	5.9	35.60	MI	Lymphosarcoma	Non-regenerative blood picture,occassional atypical lymphoid cells	Secondary anaemia of chronic disease
16*	6.8	14.20	S	Autoimmune haemolytic anaemia	Regenerative blood picture, and schistocytes	Microangiopathic haemolytic anaemia
17	7.0	33.00	MI	Back pain.	Non-regenerative blood picture,numerous target cells	Secondary anaemia of chronic disease
18	4.8	35.90	MI	Pain of unknown origin	Non-regenerative blood picture	Secondary anaemia of chronic disease
19	9.4	22.40	MO	Hepatocutaneous syndrome and Diabetes mellitus	Moderately regenerative blood picture	Secondary anaemia of chronic disease
20	3.0	34.90	MI	Not given	Non-regenerative blood picture. Neutrophils show Dohle bodies.	Secondary anaemia of chronic disease
21	5.9	19.80	S	Diabetes mellitus and liver cirrhosis	Non-regenerative blood picture, numerous target cells	Secondary anaemia of chronic disease

*Dogs examined post mortem whose detailed haematology is in Appendix C.
DN=dog number, Yrs=years, MI=mild, MO=moderate, S=severe.

Appendix B: Data of 245 dogs with anaemia admitted to the University of Glasgow Veterinary School from 1st January to 31st December 1994.

DN.	Age	PCV%	Severity	Clinical summary	Film report	Type of anaemia
22	0.0	35.30	MI	Diabetes mellitus	Non-regenerative blood picture	Secondary anaemia of chronic disease
23	0.0	36.30	MI	Demodex	Non-regenerative blood picture	Secondary anaemia of chronic disease
24*	2.0	31.30	MI	Renal failure and lethal acrodermatitis	Non-regenerative blood picture	Secondary anaemia of renal failure
25*	7.8	35.30	MI	Diabetes mellitus	Non-regenerative blood picture	Secondary anaemia of chronic disease
26	6.3	7.00	S	Autoimmune haemolytic anaemia	Regenerative blood picture	Autoimmune haemolytic anaemia
27	4.6	34.30	MI	Deep pyoderma.	Non-regenerative blood picture	Secondary anaemia of chronic infection
28	4.6	31.90	MI	Splenic tumour?	Non-regenerative blood picture	Secondary anaemia of chronic disease
29	2.0	33.60	MI	Cervical pain	Non-regenerative blood picture	Secondary anaemia of chronic disease
30	8.5	33.00	MI	Skin problem	Non-regenerative blood picture	Secondary anaemia of chronic disease
31	4.0	9.90	S	Autoimmune haemolytic anaemia	Regenerative blood picture, spontaneous agglutination of red cells	Autoimmune haemolytic anaemia
32	9.7	34.30	MI	Hypothyroid	Non-regenerative blood picture	Secondary anaemia of chronic disease
33*	1.0	32.30	MI	Lethal acrodermatitis	Non-regenerative blood picture	Secondary anaemia of chronic disease
34	14.6	36.90	MI	Collapse	Non-regenerative blood picture	Secondary anaemia of chronic disease
35	2.6	32.30	MI	Pulmonary interstitial empysema	Slightly regenerative blood picture,numerous eosinophils	Haemorrhagic anaemia
36	0.0	36.70	MI	Hypocortisolaemia	Non-regenerative blood picture	Secondary anaemia of chronic disease
37*	11.1	32.60	MI	Lymphosarcoma on chemotherapy	Non-regenerative blood picture	Secondary anaemia of chronic disease
38	5.9	35.60	MI	Addison's disease	Non-regenerative blood picture	Secondary anaemia of chronic disease
39	12.5	33.60	MI	Chronic cough	Non-regenerative blood picture	Secondary anaemia of chronic disease
40*	12.8	27.70	MO	Diabetes mellitus Cushings' disease and urinary tract infection	Slightly regenerative blood picture	Secondary anaemia of chronic disease
41*	8.9	24.70	MO	Lymphosarcoma	Moderately regenerative blood picture	Haemorrhagic anaemia
42	13.1	28.70	MO	Skin?	Non-regenerative blood picture, numerous lymphoblasts	Secondary anaemia of chronic disease
43	10.7	34.60	MI	Meningitis?. Pyrexic	Non-regenerative blood picture	Secondary anaemia of chronic infection

*Dogs examined post mortem whose detailed haematology is in Appendix C.

DN=dog number, Yrs=years, MI=mild, MO=moderate, S=severe.

Appendix B: Data of 245 dogs with anaemia admitted to the University of Glasgow Veterinary School from 1st January to 31st December 1994.

DN.	Age	PCV%	Severity	Clinical summary	Film report	Type of anaemia
44	3.5	32.10	MI	Chronic weight loss	Slightly regenerative blood picture	Haemorrhagic anaemia
45*	8.7	24.70	MO	Jaundiced, anaemia	Slightly regenerative blood picture	Autoimmune Haemolytic anaemia
46	4.0	29.00	MO	Vomiting	Slightly regenerative blood picture,numerous target cells	Haemorrhagic anaemia
47	9.1	30.30	MI	Lymphosarcoma	Non-regenerative blood picture,many normoblasts	Secondary anaemia of chronic disease
48*	7.1	12.20	S	Idiopathic non regenerative anaemia	Non-regenerative blood picture	Hypoplastic anaemia
49*	8.6	34.30	MI	Pancreatitis.	Non-regenerative blood picture	Secondary anaemia of chronic disease
50	2.0	26.00	MO	Lameness, generalised stiffness	Non-regenerative blood picture	Secondary anaemia of chronic disease
51	10.0	36.90	MI	Epistaxis, possible nasal tumour	Slightly regenerative blood picture	Haemorrhagic anaemia
52*	11.0	6.20	S	Protein losing nephropathy	Slightly regenerative blood picture, schistocytes.	Haemorrhagic anaemia
53	5.0	34.60	MI	Dilated cardiomyopathy?	Non-regenerative blood picture	Secondary anaemia of chronic disease
54*	2.9	30.00	MI	Sudden collapse. GI foreign body?.	Non- regenerative blood picture	Secondary anaemia of chronic disease
55*	1.9	33.60	MI	Polyarthritis, skin problems?bacterial endocarditis	Non-regenerative blood picture	Secondary anaemia of chronic disease
56*	8.0	28.30	MO	Dyspnoeic 4 days with pleural effusion	Non-regenerative blood picture	Secondary anaemia of chronic disease
57	0.0	35.90	MI	Paraparetic	Non-regenerative blood picture	Secondary anaemia of chronic disease
58*	8.0	10.50	S	Haemangiosarcoma	Regenerative blood picture, schistocytes	Haemorrhagic anaemia
59*	8.0	28.00	MO	Lethargy, anaemic.	Non-regenerative blood picture	Secondary anaemia of chronic disease
60	5.0	12.80	S	Bleeding in Gastrointestinal tract, disseminated intravascular coagulation	Regenerative blood picture schistocytes	Haemorrhagic anaemia
61	9.0	36.60	MI	Mast cell tumour.	Non-regenerative blood picture	Secondary anaemia of chronic disease
62	12.0	36.60	MI	Cruciate rupture.	Non-regenerative blood picture	Acute haemorrhagic anaemia
63	3.0	33.30	MI	Not given	No data available	No data available
64	9.0	31.60	MI	Pyrexia of unknown origin	Non-regenerative blood picture	Secondary anaemia of chronic disease
65	7.0	34.60	MI	Cardiomyopathy	Non-regenerative blood picture	Secondary anaemia of chronic disease
66	9.0	36.30	MI	Haemorragic diarrhoea	Slightly regenerative blood picture	Haemorrhagic anaemia

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DN=dog number, Yrs=years, MI=mild, MO=moderate, S=severe.

Appendix B: Data of 245 dogs with anaemia admitted to the University of Glasgow Veterinary School from 1st January to 31st December 1994.

DN.	Age	PCV%	Severity	Clinical summary	Film report	Type of anaemia
67	8.0	35.60	MI	Neurological deficits	Non-regenerative blood picture	Secondary anaemia of chronic disease
68	7.0	35.60	MI	Pelvic fracture	Non-regenerative blood picture	Haemorrhagic anaemia
69	7.0	28.00	MO	Retrobulbar abscess	Non-regenerative blood picture, neutrophils show Döhle bodies	Secondary anaemia of chronic disease
70*	3.5	27.00	MO	Possible myeloid leukaemia?	Regenerative blood picture leukaemic cells of myeloid series, schistocytes	Myelophthisic anaemia
71*	7.0	33.30	MI	Multicentric lymphosarcoma	Non-regenerative blood picture	Secondary anaemia of chronic disease
72*	10.0	30.60	MI	Road traffic accident	Non-regenerative blood picture	Acute haemorrhagic anaemia
73	5.2	27.00	MO	Lymphocytic nodular dermatitis	Slightly regenerative blood picture	Haemorrhagic anaemia
74	0.0	22.60	MO	Congestive cardiac failure	Regenerative blood picture, schistocytes	Microangiopathic haemolytic anaemia
75	10.0	33.60	MI	Hepatic encephalopathy?	Non-regenerative blood picture	Secondary anaemia of chronic disease
76	3.0	27.80	MO	Paraparetic	Non-regenerative blood picture	Secondary anaemia of chronic disease
77	9.8	30.20	MI	Increased respiratory effort,ascites liver mass	Slightly regenerative blood picture , target cells schistocytes	Haemorrhagic anaemia
78	5.0	31.00	MI	Porto sytemic shunt?	Non-regenerative anaemia, numerous acanthocytes	Secondary anaemia of chronic disease
79	2.0	36.90	MI	Enlarged lymph nodes. Lymphosarcoma?	Non-regenerative blood picture	Secondary anaemia of chronic disease
80	0.0	35.60	MI	Diarrhoea	Non-regenerative blood picture	Secondary anaemia of chronic disease
81*	5.3	33.90	MI	Pancreatic hypoplasia	Non-regenerative blood picture	Secondary anaemia of chronic disease
82	10.1	36.90	MI	Immune-mediated skin disease	Non-regenerative blood picture	Secondary anaemia of chronic disease
83	15.0	29.70	MO	Pelvic fracture,road traffic accident	Non-regenerative blood picture	Acute haemorrhagic anaemia
84	6.1	33.30	MI	Fore limb ataxia	Non-regenerative blood picture	Secondary anaemia of chronic disease
85	6.0	34.70	MI	Chronic skin problem	Non-regenerative blood picture	Secondary anaemia of chronic disease
86	3.0	8.80	S	Pale, lethargic, tachycardia	Non-regenerative blood picture,large platelets .	Acute haemorrhagic anaemia
87	0.0	34.60	MI	Liver problems?	Non-regenerative blood picture,numerous target cells	Secondary anaemia of chronic disease
88*	9.0	19.40	S	Pale, collapsed, tachycardia	Regenerative blood picture,schistocytes and large platelets present	Haemorrhagic anaemia

*Dogs examined post mortem whose detailed haematology is in Appendix C.

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Appendix B: Data of 245 dogs with anaemia admitted to the University of Glasgow Veterinary School from 1st January to 31st December 1994.

DN.	Age	PCV%	Severity	Clinical summary	Film report	Type of anaemia
89	0.0	36.90	MI	Vomiting.	Non-regenerative blood picture	Secondary anaemia of chronic disease
90*	4.0	32.00	MI	Dilated cardiomyopathy, anorexia,slow capillary refill	Regenerative and leucoerythroblastic blood picture	Autoimmune haemolytic anaemia
91	10.3	24.00	MO	Multicentric lymphosarcoma	Non-regenerative leukaemic picture with lymphoblasts	Secondary anaemia of chronic disease
92	2.9	36.60	MI	Dyspnoeic	Non-regenerative blood picture	Secondary anaemia of chronic disease
93*	11.0	30.00	MI	Glomerulonephritis	Non-regenerative blood picture	Secondary anaemia of chronic disease
94	6.5	36.30	MI	Neck pain	Non-regenerative blood picture	Secondary anaemia of chronic disease
95	6.0	28.70	MO	Had Lymph node biopsy Lymphosarcoma?	Slightly regenerative blood picture	Haemorrhagic anaemia
96	0.0	36.30	MI	Diabetes mellitus	Non-regenerative blood picture	Secondary anaemia of chronic disease
97	2.7	22.10	MO	Lumpectomy, bleeding post op	Slightly regenerative blood picture	Haemorrhagic anaemia
98	5.0	33.30	MI	No data available	No data available	No data available
99	5.0	31.60	MI	No data available	No data available	No data available
100	5.0	31.00	MI	Intervertebral disc protrusion	Non-regenerative blood picture	Secondary anaemia of chronic disease
101	0.0	30.00	MI	No data available	No data available	No data available
102	2.0	36.00	MI	Fluid in chest	Non-regenerative blood picture	Secondary anaemia of chronic disease
103	0.0	34.40	MI	Intermittent pyrexia	Non-regenerative blood picture	Secondary anaemia of chronic disease
104	8.0	29.70	MO	Elbow arthrodesis, wound breakdown	Non-regenerative blood picture	Secondary anaemia of chronic disease
105	1.4	35.90	MI	Portocaval shunt?	Non-regenerative blood picture,numerous target cells	Secondary anaemia of chronic disease
106	0.0	30.00	MI	Wound breakdown. Infection?	Non-regenerative blood picture	Secondary anaemia of infection
107	8.0	32.30	MI	Uterine pathology	Non-regenerative blood picture	Secondary anaemia of chronic disease
108	9.2	35.60	MI	Congestive heart failure.	Non-regenerative blood picture	Secondary anaemia of chronic disease
109	12.0	26.00	MO	Anorexic, dehydrated and has a corneal ulcer	Non-regenerative anaemia,many target cells	Secondary anaemia of chronic disease
110	7.0	33.00	MI	Lymphosarcoma on treatment	Slightly regenerative blood picture with target cells	Haemorrhagic anaemia
111	9.0	34.30	MI	No clinical details supplied	Non-regenerative blood picture	No data available
112	5.1	33.90	MI	Cushings' disease	Non-regenerative blood picture	Secondary anaemia of chronic disease

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DN=dog number, Yrs=years, MI=mild, MO=moderate, S=severe.

Appendix B: Data of 245 dogs with anaemia admitted to the University of Glasgow Veterinary School from 1st January to 31st December 1994.

DN.	Age	PCV%	Severity	Clinical summary	Film report	Type of anaemia
113	10.0	35.60	MI	Renal failure one month ago.	Non-regenerative blood picture	Secondary anaemia of chronic renal failure
114	9.9	36.80	MI	Dull, anorexia. Lame hind legs	Non-regenerative blood picture	Secondary anaemia of chronic disease
115	4.0	21.00	MO	Autoimmune haemolytic anaemia	Regenerative blood picture ,spherocytes	Autoimmune haemolytic anaemia
116	10.0	32.00	MI	Oral neoplasia	Moderately regenerative blood picture	Haemorrhagic anaemia
117	6.9	30.30	MI	Pyrexia of 104 - 106 F arthritis	Non-regenerative blood picture	Secondary anaemia of infection
118	4.6	35.90	MI	Myasthenia gravis ?	Non-regenerative blood picture	Secondary anaemia of chronic disease
119	0.0	26.80	MO	Abdominal mass	Non-regenerative blood picture	Secondary anaemia of chronic disease
120	4.4	34.60	MI	Chronic vomiting	Non-regenerative blood picture	Secondary anaemia of chronic disease
121	2.0	36.70	MI	Check pre-treatment.	Non-regenerative blood picture	Secondary anaemia of chronic disease
122	2.1	36.90	MI	Atopic	Non-regenerative blood picture	Secondary anaemia of chronic disease
123	10.0	29.30	MO	Lymphosarcoma?	Non-regenerative blood picture	Secondary anaemia of chronic disease
124*	12.3	33.00	MI	Lingual squamous cell carcinoma,deteriorating	Non-regenerative blood picture	Secondary anaemia of chronic disease
125	7.0	25.10	MO	Myeloproliferative disorder	Non-regenerative blood, neutrophils with Döhle bodies	Secondary anaemia of infection
126	0.0	34.30	MI	Addisons' disease.	Non-regenerative blood picture	Secondary anaemia of chronic disease
127	0.0	17.40	S	AIHA on treatment	Regenerative blood picture	Autoimmune haemolytic anaemia
128	9.0	27.70	MO	Chronic glomerulonephritis	Slightly regenerative blood picutre with normoblasts, schistocytes	Secondary anaemia of renal failure
129	5.0	9.50	S	Allergic skin disease . Anaemic	Slightly regenerative blood picture, large platelets	Haemorrhagic anaemia
130	5.0	29.70	MO	Dullness, hypothyroid	Non-regenerative blood picture	Secondary anaemia of chronic disease
131	11.4	25.70	MO	Mass/cyst/abscess behind eye	Slightly regenerative blood picture ,many target cells, large platelets.	Haemorrhagic anaemia
132	7.8	31.60	MI	Lymphosarcoma	Non-regenerative blood picture	Secondary anaemia of chronic disease
133	4.0	22.10	MO	Acute or chronic renal failure	Non-regenerative picture with numerous target cells	Secondary anaemia of renal failure
134	8.0	11.00	S	Anaemia, jaundice, dull auto immune hemolytic anaemia	Regenerative blood picture, spherocytes,target cells	Autoimmune haemolytic anaemia
135	15.0	35.90	MI	Jaw pain, polydipsic, weight loss	Non-regenerative blood picture	Secondary anaemia of chronic disease

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DN=dog number, Yrs=years, MI=mild, MO=moderate, S=severe.

Appendix B: Data of 245 dogs with anaemia admitted to the University of Glasgow Veterinary School from 1st January to 31st December 1994.

DN.	Age	PCV%	Severity	Clinical summary	Film report	Type of anaemia
136	11.0	30.10	MI	Lameness	Non-regenerative blood picture	Secondary anaemia of chronic disease
137	17.0	21.70	MO	Gastric bleeding	Regenerative blood,increased neutrophils,large platelets	Haemorrhagic anaemia
138	3.1	30.30	MI	Pyrexia, Chronic skin condition	Non-regenerative blood picture	Secondary anaemia of infection
139	4.0	23.70	MO	Haematuria,haematemesis melaena	Regenerative blood picture	Haemorrhagic anaemia
140	2.5	33.00	MI	Seizures, possible encephalitis	Non-regenerative blood with numerous target cells	Secondary anaemia of chronic disease
141	5.2	7.90	S	Chronic gastrointestinal bleeding	Non-regenerative blood picture	Dyserythropoietic anaemia
142	5.4	19.10	S	Lymphosarcoma	Slightly regenerative blood picture, many lymphoblasts,schistocytes	Microangiopathic haemolytic anaemia
143	12.0	32.60	MI	Possible thrombocytopenia	Regenerative blood picture	Immune mediated anaemia
144	11.0	32.00	MI	Inspiration pneumonia?	Non-regenerative blood picture	Secondary anaemia of chronic disease
145	4.9	33.20	MI	Meningitis?	Non-regenerative blood picture	Secondary anaemia of infection
146	8.0	28.70	MO	Compressive myelopathy	Non-regenerative blood picture	Secondary anaemia of chronic disease
147	11.0	36.80	MI	Portosystemic shunt.?	Non-regenerative anaemia, reactive lymphoid cells	Secondary anaemia of chronic disease
148	5.0	32.20	MI	Uveitis,enlarged lymph nodes	Non-regenerative anaemia,leukaemic blood picture with lymphoblasts	Secondary anaemia of chronic disease
149	8.1	34.30	MI	Pituitary adenocarcinoma	Non-regenerative blood picture	Secondary anaemia of chronic disease
150	4.0	31.00	MI	VII/VIIIth cranial nerve deficits	Non-regenerative blood picture	Secondary anaemia of chronic disease
151	0.0	20.70	MO	Urethrostomy 2-3wks ago	Slightly regenerative blood picture,large platelets	Haemorrhagic anaemia
152	3.9	34.30	MI	Severe respiratory distress	Non-regenerative blood picture	Secondary anaemia of chronic disease
153	8.0	31.00	MI	Pyrexia, liver problem?	Slightly regenerative blood picture	Haemorrhagic anaemia
154	4.0	32.60	MI	Polyneuropathy.	Non-regenerative blood picture with numerous target cell	Secondary anaemia of chronic disease
155	10.0	22.70	MO	Haemangiosarcoma ?	Regenerative blood picture, numerous schistocytes.	Haemorrahgic anaemia
156	9.0	33.30	MI	Ataxic / liver damage.	Non-regenerative blood picture	Secondary anaemia of chronic disease
157	8.0	27.70	MO	Liver failure, pancreatitis	Slightly regenerative blood picture, neutrophils show Döhle bodies.	Haemorrhagic anaemia

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DN=dog number, Yrs=years, MI=mild, MO=moderate, S=severe.

Appendix B: Data of 245 dogs with anaemia admitted to the University of Glasgow Veterinary School from 1st January to 31st December 1994.

DN.	Age	PCV%	Severity	Clinical summary	Film report	Type of anaemia
158	6.5	33.90	MI	Anaemia	Non-regenerative blood picture, many eosinophils.	Secondary anaemia of chronic disease
159	0.0	31.00	MI	Coughing blood. Primary lung tumour?	Slightly regenerative blood picture, large platelets	Haemorrahgic anaemia
160	0.0	35.30	MI	Anorexia, polydipsic,polyuric	Non-regenerative blood picture	Secondary anaemia of chronic disease
161	0.0	30.60	MI	Acute renal failure	Non-regenerative blood picture,numerous target cells and schistocytes.	Secondary anaemia of renal failure
162	0.0	36.60	MI	No data available	No data available	No data available
163	7.0	10.50	S	Autoimmune haemolytic anaemia,thrombocytopenia	Regenerative blood picture, spherocytes, few platelets	Autoimmune haemolytic anaemia
164	0.0	35.30	MI	Sarcoma / foreign body in ventral neck	Non-regenerative blood picture	Secondary anaemia of chronic disease
165	6.0	22.70	MO	Bile duct tear repaired last week	Slightly regenerative blood picture,many target cells, few schistocytes	Haemorrhagic anaemia
166	0.0	35.60	MI	Not given	Non-regenerative blood picture	Secondary anaemia of chronic disease
167	14.0	33.30	MI	Paraparetic	Non-regenerative blood picture	Secondary anaemia of chronic disease
168	3.5	29.00	MO	Sub-aortic stenosis	Non-regenerative blood picture	Secondary anaemia of chronic disease
169	12.0	36.60	MI	Polyarthritis	Non-regenerative blood picture	Secondary anaemia of chronic disease
170	9.5	8.90	S	Multpile bleeding sites immune mediated thrombocytopaenia	Regenerative blood picture,schistocytes	Haemorrhagic anaemia
171	13.0	36.30	MI	Laryngeal problem,on prednisolone since 4 yrs	Non-regenerative blood picture	Secondary anaemia of chronic disease
172	10.0	26.40	MO	Appears to be blind	Slightly regenerative blood picture	Haemorrhagic anaemia
173	0.0	9.50	S	Anaemia	Slightly regenerative blood picture,marked poikilocytosis with schistocytes	Haemorrhagic anaemia
174	3.2	20.80	MO	Hepatopathy	Regenerative and leucoerythroblastic blood picture,target cells, large platelets	Haemorrhagic anaemia
175	0.0	28.00	MO	Thyroid carcinoma?	Non-regenerative blood picture	Secondary anaemia of chronic disease
176	3.0	32.60	MI	Pelvis fracture	Non-regenerative blood picture	Haemorrhagic anaemia of acute blood loss
177	0.0	14.00	S	Autoagglutination and liver disease	Regenerative blood, many spherocytes,target cells.	Autoimmune haemolytic anaemia

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DN.	Age	PCV%	Severity	Clinical summary	Film report	Type of anaemia
178	5.9	30.00	MI	Liver damage	Slightly regenerative blood picture	Haemorrhagic anaemia
179	9.6	35.60	MI	Chronic degenerative radiculomyopathy	Non-regenerative blood picture	Secondary anaemia of chronic disease
180	11.5	32.60	MI	Exercise intolerance, stiffness	Non-regenerative blood picture	Secondary anaemia of chronic disease
181	8.0	28.30	MO	Had a gastroduodenostomy	Non-regenerative blood picture	Acute haemorrhagic anaemia
182	9.0	29.00	MO	Epistaxis	Regenerative blood picutre, schistocytes	Haemorrhagic anaemia
183	18.0	33.00	MI	Incontinence.	Non-regenerative blood picture	Secondary anaemia of chronic disease
184	12.1	29.70	MO	Repeat. Had high white cell count	Non-regenerative blood picture	Secondary anaemia of infection
185	0.0	35.00	MI	Episodes of masticary and facial spasm	Non-regenerative blood picture	Secondary anaemia of chronic disease
186	0.0	30.70	MI	Heart murmur .Endocarditis?	Regenerative blood picture, schistocytes	Microangiopathic hemolytic anaemia
187	0.0	36.60	MI	Prostatic enlargement.Prostatic cyst.?	Non-regenerative blood picture	Secondary anaemia of chronic disease
188	12.0	31.40	MI	Pancreatitis ?Peritonitis?	Slightly regenerative blood picture	Haemorrhagic anaemia
189	6.0	28.70	MO	Bizarre skin disorder.Pemphigus?	Slightly regenerative blood pciture	Haemorrhagic anaemia
190	9.0	29.70	MO	Abdominal haemangiosarcoma	Moderately regenerative blood picture	Haemorrhagic anaemia
191	10.0	30.30	MI	No clinical details supplied	Non-regenerative blood picture	Secondary anaemia of chronic disease
192	12.0	35.20	MI	Diabetes mellitus	Non-regenerative blood picture	Secondary anaemia of chronic disease
193	7.0	36.30	MI	Renal failure? vomiting	Non-regenerative blood picture	Secondary anaemia of renal failure
194	0.0	9.00	S	Slight pyrexia 103oF. Pallor, weakness	Slightly regenerative blood picture	Haemorrahgic anaemia
195	0.0	29.70	MO	Skin problems	Non-regenerative anaemia ,many target cells	Secondary anaemia of chronic disease
196	10.0	35.60	MI	Chronic vomiting	Non-regenerative blood picture	Secondary anaemia of chronic disease
197	10.0	33.30	MI	Mass on limb	Non-regenerative blood picture	Secondary anaemia of chronic disease
198	0.0	35.30	MI	Chronic renal failure or neoplasia?	Non-regenerative blood picture	Secondary anaemia of renal failure
199	6.6	5.20	S	Anaemia.	Non-regenerative blood picture	Immune mediated anaemia
200	0.0	33.00	MI	Lymphosarcoma	Slightly regenerative blood picture	Haemorrahgic anaemia
201	0.0	16.70	S	Anaemia	Slightly regenerative blood, schistocytes,large platelets	Haemorrhagic anaemia
202	9.0	31.60	MI	Bizzare behaviour.Porto systemic shunt?	Non-regenerative blood picture	Secondary anaemia of chronic disease

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Appendix B: Data of 245 dogs with anaemia admitted to the University of Glasgow Veterinary School from 1st January to 31st December 1994.

DN.	Age	PCV%	Severity	Clinical summary	Film report	Type of anaemia
203	4.6	31.30	MI	Otitis	Non-regenerative blood picture	Secondary anaemia of infection
204	12.7	30.60	MI	Paraparesis, spinal problem	Non-regenerative blood picture	Secondary anaemia of chronic disease
205	0.0	22.40	MO	Skin disease, possible Zn deficiency	Slightly regenerative blood picture with numerous target cells present	Haemorrhagic anaemia
206	0.7	28.70	MO	Congenital renal disease.	Non-regenerative blood picture	Secondary anaemia of renal failure
207	8.0	21.70	MO	Spontaneous haemorrhage, ecchymoses	Regenerative blood picture, few large platelets	Haemorrhagic anaemia
208	0.0	36.60	MI	Road traffic accident	Non-regenerative blood picture	Acute haemorrhagic anaemia
209	5.6	36.60	MI	Nasal aspergillosis	Non-regenerative blood picture	Secondary anaemia of infection
210	22.8	36.30	MI	Deep pyoderma	Non-regenerative blood picture	Secondary anaemia of infection
211	12.0	28.30	MO	Paraparesis	Non-regenerative blood picture	Secondary anaemia of chronic disease
212	10.0	33.90	MI	Presence of ascites	Non-regenerative blood picture	Secondary anaemia of chronic disease
213	11.0	30.00	MI	Seizures	Non-regenerative blood picture	Secondary anaemia of chronic disease
214	8.6	34.30	MI	Coughing, tachypnoeic	Slightly regenerative blood picture, reactive lymphocytes	Secondary anaemia of infection
215	9.0	29.00	MO	Weight loss	Non-regenerative blood picture	Secondary anaemia of chronic disease
216	9.0	21.40	MO	Peritonitis following enteropathy	Non-regenerative blood,neutrophils with Dohle bodies	Secondary anaemia of infection
217	3.0	19.40	S	Chronic renal failure.	Non-regenerative blood picture, schistocytes.	Secondary anaemia of renal failure
218	19.0	29.00	MO	Severe weight loss,hookworm infestation	Slightly regenerative blood picture	Haemorrhagic anaemia
219	0.0	36.30	MI	No clinical details supplied	No data available	No data available
220	15.0	36.90	MI	Chronic skin/ear/eye problems	Non-regenerative blood picture	Secondary anaemia of chronic disease
221	5.0	29.40	MO	Scuffing front feet	Non-regenerative blood picture	Secondary anaemia of chronic disease
222	10.6	20.10	MO	Multiple haematoma, pyelonephritis, splenic enlargement	Regenerative blood picture, schistocytes,megaplatelets normoblasts	Haemorrhagic anaemia
223	10.0	36.00	MI	Mammary tumour	Non-regenerative blood picture	Secondary anaemia of chronic disease
224	8.0	36.30	MI	Rupture cruciate repair 2weeks ago	Non-regenerative blood picture	Secondary anaemia of chronic disease
225	12.0	30.60	MI	Congestive heart failure	Non-regenerative blood picture	Secondary anaemia of chronic disease

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DN.	Age	PCV%	Severity	Clinical summary	Film report	Type of anaemia
226	7.0	36.30	MI	Ataxia. Pain in cervical/lumbar?	Non-regenerative blood picture	Secondary anaemia of chronic disease
227	4.0	29.70	MO	Mass in abdomen	Regenerative blood picture, large platelets	Haemorrhagic anaemia
228	5.8	36.60	MI	Weight loss	Non-regenerative blood picture	Secondary anaemia of chronic disease
229	4.0	25.30	MO	Pyrexia,anaemia ?	Slightly regenerative blood picture and occassional Howell Jolly bodies	Haemorrhagic anaemia
230	1.0	30.00	MI	Chronic renal failure	Non-regenerative anaemia	Secondary anaemia of renal failure
231	5.3	30.30	MI	Osteomyelitis	Non-regenerative blood picture	Secondary anaemia of chronic disease
232	0.0	29.30	MO	Hindleg ataxia	Non-regenerative blood picture	Secondary anaemia of chronic disease
233	0.0	23.40	MO	Tachycardia.Hepatomegaly?	Moderately regenerative blood picture, large platelets	Haemorrhagic anaemia
234*	12.0	24.00	MO	Pododermatitis	Slightly regenerative blood picture with many target cells	Haemorrhagic anaemia
235	6.0	25.40	MO	Lethargy	Regenerative blood picture numerous schistocytes	Microangiopathic hemolytic anaemia
236	0.0	32.60	MI	Abscess on left leg	Non-regenerative blood picture	Secondary anaemia of infection
237	0.0	26.00	MO	Bone tumour	Non-regenerative blood picture	Secondary anaemia of chronic disease
238	9.0	32.00	MI	Hepatic failure.	Non-regenerative blood picture	Secondary anaemia of chronic disease
239	0.0	35.30	MI	Prostatic neoplasia	Non-regenerative blood picture	Secondary anaemia of chronic disease
240	12.0	36.90	MI	Brain tumour/hydrocephaly.	Non-regenerative blood picture	Secondary anaemia of chronic disease
241	0.0	30.00	MI	Abdominal pain.	Non-regenerative blood picture	Secondary anaemia of chronic disease
242	4.0	13.20	S	Addison's disease	Slightly regenerative blood picture, schistocytes and normoblasts	Haemorrhagic anaemia
243	10.0	24.40	MO	Vomiting	Slightly regenerative blood picture,Howell Jolly bodies, numerous schistocytes	Microangiopathic hemolytic anaemia
244	13.0	33.30	MI	Lymphosarcoma?	Non-regenerative blood picture	Secondary anaemia of chronic disease
245	10.0	35.60	MI	Routine		Normal blood picture

*Dogs examined post mortem whose detailed haematology is in Appendix C.

DN=dog number, Yrs=years, MI=mild, MO=moderate, S=severe.

Appendix C: Haematological data of 52 anaemic dogs (PCV<37%), examined post-mortem at the University of Glasgow Veterinary School in 1994.

DN.*	Age(Yrs)	Breed	Sex	Severity	Sample	RBC	Hb	PCV	MCV	MCH	MCHC	PLT	WBC	BN	Neutro	Lympho	Mono	Eo	Baso	Other*
4	15.2yrs	Retriever	M	MO	1	4.32	10.30	29.30	68.00	23.80	35.10	280.00	6.00	0.15	4.83	0.60	0.24	0.18	0.00	0.00
6	10.2 yrs	Bull Terrier	M	MI	1	6.17	13.00	35.30	57.00	21.00	36.80	332.00	13.30	0.00	12.37	0.47	0.47	0.00	0.00	0.00
					2	5.46	11.30	32.00	59.00	20.60	35.30	313.00	14.00	0.14	13.02	0.35	0.49	0.00	0.00	0.00
10	12yrs	Retriever	FS	MI	1	5.33	12.30	35.60	67.00	23.00	34.50	301.00	29.90	1.20	27.21	0.75	0.75	0.00	0.00	0.00
13	5.4yrs	Bull Terrier	F	MI	1	4.80	11.80	33.30	69.00	24.50	35.40	405.00	11.80	0.00	9.44	1.24	0.18	0.89	0.06	0.00
					2	4.41	11.00	30.30	69.00	24.90	36.30	398.00	14.20	0.00	11.72	1.56	0.43	0.50	0.00	0.00
					3	4.70	11.40	32.00	68.00	24.20	35.60	478.00	16.10	0.00	14.49	1.13	0.24	0.24	0.00	0.00
					4	4.58	10.30	30.30	66.00	22.40	33.90	459.00	10.90	0.00	8.45	1.36	0.55	0.55	0.00	0.00
					5	4.43	10.50	30.00	68.00	23.70	35.00	424.00	9.80	0.00	8.72	0.69	0.25	0.15	0.00	0.00
					6	4.65	11.20	31.30	67.00	24.00	35.70	406.00	10.50	0.00	8.93	1.10	0.16	0.32	0.00	0.00
					7	4.78	11.30	31.60	66.00	23.60	35.70	396.00	11.90	0.00	10.23	1.01	0.30	0.36	0.00	0.00
15	5.9yrs	Bull Terrier	FS	MI	1	5.25	14.10	35.60	68.00	26.80	39.60	338.00	3.70	0.04	2.92	0.44	0.11	0.00	0.00	0.19
					2	5.37	0.00	36.60	68.00	0.00	0.00	343.00	5.10	0.05	4.23	0.61	0.20	0.00	0.00	0.00
16	6.8yrs	Retriever	M	MO	1	4.20	9.90	29.70	71.00	23.50	33.30	8.00	7.90	1.30	5.14	0.59	0.47	0.08	0.00	0.32
				S	2	2.65	6.50	19.40	73.00	24.50	33.50	8.00	28.10	0.70	22.34	3.23	1.12	0.28	0.00	0.42
				S	3	2.27	5.70	17.40	77.00	25.10	32.70	8.00	56.60	3.96	49.24	1.70	1.13	0.00	0.00	0.57
				S	4	1.24	4.20	14.20	115.00	33.80	29.50	8.00	90.60	27.18	32.62	3.62	6.34	0.00	0.00	8.15
24	2 yrs	Bull Terrier	F	MI	1	4.47	10.90	31.30	70.00	24.30	34.80	257.00	33.40	2.67	27.72	1.00	1.67	0.33	0.00	0.00
*Other refers to normoblasts and nucleated cells in the entire series																				

Units:RBCx10 ^{12}/l, Hb g/dl, PCV %, MCV fl, MCH pg, MCHC g/dl, Plt. 10 ^{9}/l
(zero= platelet aggregates),WBC and differentials x10 ^{9}/l,
BN=band neutro.,DN*=Dog number,MI=mild, MO=moderate,S=severe
M=male, C=castrated, F=female, S=spayed

Appendix C: Haematological data of 52 anaemic dogs (PCV<37%), examined post-mortem at the University of Glasgow Veterinary School in 1994.

DN.*	Age(Yrs)	Breed	Sex	Severity	Sample	RBC	Hb	PCV	MCV	MCH	MCHC	PLT	WBC	BN	Neutro	Lympho	Mono	Eo	Baso	Other*
25	7.8yrs	Rottweiler	M	MI	1	5.61	12.50	35.30	63.00	22.20	35.40	221.00	14.40	0.00	10.08	2.02	1.51	0.79	0.00	0.00
33	1yr	Bull Terrier	M	MI	1	4.95	11.00	32.30	65.00	22.20	34.00	299.00	23.70	2.84	17.54	0.71	2.13	0.24	0.00	0.24
37	11.1yr	Boxer	FS	MI	1	5.54	13.10	36.90	67.00	23.60	35.50	232.00	9.70	0.15	6.50	1.36	0.82	0.78	0.00	0.10
					2	5.00	11.50	32.60	65.00	23.00	35.20	309.00	9.50	0.05	6.56	1.47	0.62	0.48	0.33	0.00
40	12.8yrs	Jack Russell	M	MI	1	5.67	13.10	35.90	63.00	23.10	36.40	716.00	6.10	0.18	3.63	1.68	0.52	0.09	0.00	0.00
		Terrier			2	5.25	12.10	34.60	66.00	23.00	34.90	783.00	9.70	0.15	6.94	2.18	0.29	0.15	0.00	0.00
					3	4.29	10.20	29.30	68.00	23.70	34.80	653.00	14.00	0.00	11.34	2.03	0.35	0.14	0.00	0.14
					4	4.56	10.40	30.60	67.00	22.80	33.90	640.00	10.70	0.11	5.51	3.16	0.70	1.23	0.00	0.00
					5	4.62	10.50	31.00	67.00	22.70	33.80	669.00	8.70	0.00	4.70	2.96	0.30	0.74	0.00	0.00
					6	4.76	10.50	31.30	66.00	22.00	33.50	579.00	5.60	0.06	1.85	2.72	0.25	0.76	0.00	0.00
					7	4.27	9.10	27.70	65.00	21.30	32.80	466.00	23.40	1.64	18.95	1.64	1.17	0.00	0.00	0.00
41	8.9yrs	Cocker	M	MI	1	5.54	12.90	36.30	66.00	23.20	35.50	337.00	14.20	0.43	10.22	1.35	1.28	0.50	0.00	0.43
		Spaniel		MO	2	5.19	12.50	33.00	64.00	24.00	37.80	360.00	14.50	0.36	11.89	0.58	1.16	0.29	0.00	0.07
				MO	3	4.62	10.30	29.70	64.00	22.20	34.60	237.00	15.40	0.15	12.47	0.62	2.00	0.15	0.00	0.00
				MO	4	3.74	8.90	24.70	66.00	23.70	36.00	97.00	51.00	4.08	43.61	0.13	2.93	0.26	0.00	0.00
45	8.7yrs	Cocker	FS	MO	1	3.29	7.90	24.70	75.00	24.00	31.90	62.00	7.00	0.00	5.11	0.81	0.14	0.32	0.00	0.63
		Spaniel		MO	2	3.62	9.10	28.30	78.00	25.10	32.10	96.00	5.60	0.17	4.62	0.53	0.11	0.00	0.00	0.00
				MI	3	4.34	11.00	33.30	77.00	25.30	33.00	106.00	6.80	0.07	5.64	0.48	0.48	0.07	0.00	0.07
*Other refers to normoblasts and nucleated cells in the entire series																				

Units:RBCx10 ^{12}/l, Hb g/dl, PCV %, MCV fl, MCH pg, MCHC g/dl, Plt. 10 ^{9}/l
(zero= platelet aggregates),WBC and differentials x10 ^{9}/l,
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Appendix C: Haematological data of 52 anaemic dogs (PCV<37%), examined post-mortem at the University of Glasgow Veterinary School in 1994.

DN.*	Age(Yrs)	Breed	Sex	Severity	Sample	RBC	Hb	PCV	MCV	MCH	MCHC	PLT	WBC	BN	Neutro	Lympho	Mono	Eo	Baso	Other*
48	7.1 yrs	Bearded	M	S	1	2.15	5.40	15.80	73.00	25.10	34.10	145.00	5.40	0.08	2.86	1.08	0.70	0.68	0.00	0.00
		Collie		S	2	1.69	4.20	12.20	72.00	24.80	34.40	102.00	5.30	0.24	2.92	1.01	0.27	0.82	0.00	0.00
49	8.6yrs	Cocker	M	MI	1	4.99	12.10	34.30	69.00	24.20	35.20	216.00	18.40	1.84	13.98	1.47	0.74	0.37	0.00	0.00
		Spaniel																		
52	11yrs	Cross	FS	S	1	1.89	4.80	14.10	75.00	25.30	34.00	16.00	3.00	0.03	2.58	0.12	0.27	0.00	0.00	0.00
					2	4.32	10.80	29.00	67.00	25.00	37.20	216.00	9.90	0.00	8.46	0.89	0.54	0.00	0.00	0.00
					3	4.62	11.20	31.60	68.00	24.20	35.40	306.00	8.10	0.00	6.08	1.30	0.53	0.20	0.00	0.00
					4	4.71	11.60	31.60	67.00	24.60	36.70	450.00	12.50	0.06	8.94	1.75	0.75	1.00	0.00	0.00
					5	1.11	2.10	6.20	56.00	18.90	33.80	444.00	4.70	0.02	3.01	1.18	0.28	0.19	0.00	0.02
					6	5.00	12.30	31.40	63.00	24.60	39.10	352.00	8.30	0.04	5.85	1.70	0.29	0.42	0.00	0.00
					7	4.97	10.60	31.60	64.00	21.30	33.50	353.00	6.70	0.03	3.79	1.34	0.34	1.21	0.00	0.00
54	2.9yrs	GSD	M	MI	1	4.77	11.50	30.00	63.00	24.10	38.30	16.00	3.60	1.66	1.64	0.07	0.07	0.00	0.02	0.00
55	1.9yrs	Cocker	FS	MI	1	5.54	13.70	36.90	67.00	24.70	37.10	44.00	19.90	0.00	17.91	1.39	0.60	0.00	0.00	0.00
		Spaniel			2	4.89	11.60	33.60	69.00	23.70	34.50	329.00	42.60	0.00	39.83	1.28	0.43	0.43	0.00	0.64
56	8	GSD	FS	MO	1	4.08	10.00	28.30	69.00	24.50	35.30	225.00	20.70	0.00	18.00	0.62	1.04	0.21	0.10	0.10
58	8yrs	GSD	FS	S	1	1.78	3.20	10.50	59.00	17.90	30.40	14.00	19.20	0.19	14.78	0.58	0.00	0.00	0.00	3.65
59	8yrs	Rottweiler	FS	MO	1	4.29	9.60	28.00	65.00	22.30	34.20	244.00	16.80	0.50	14.11	0.17	1.85	0.17	0.00	0.00
*Other refers to normoblasts and nucleated cells in the entire series																				

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(zero= platelet aggregates),WBC and differentials x10 ^{9}/l,
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DN.*	Age(Yrs)	Breed	Sex	Severity	Sample	RBC	Hb	PCV	MCV	MCH	MCHC	PLT	WBC	BN	Neutro	Lympho	Mono	Eo	Baso	Other*
70	3.5yrs	W.H.W.Terrier	M	MO	1	3.06	9.30	27.00	88.00	30.40	34.40	14.00	144.70	11.58	111.42	2.89	10.13	0.00	0.00	7.24
71	7yrs	Collie	F	MI	1	4.91	12.10	33.30	68.00	24.60	36.30	248.00	4.60	0.00	3.54	0.41	0.46	0.14	0.00	0.05
72	10yrs	GSD	FS	MI	1	4.97	11.10	30.60	62.00	22.30	36.20	181.00	16.90	0.42	14.79	0.85	0.68	0.00	0.00	0.17
81	5.3yrs	GSD	F	MI	1	5.91	13.30	36.70	62.00	22.50	36.20	258.00	9.60	0.00	6.43	2.11	0.72	0.34	0.00	0.00
					2	5.33	11.90	33.90	64.00	22.30	35.10	265.00	8.60	0.00	4.95	2.41	0.39	0.86	0.00	0.00
88	9yrs	GSD	M	S	1	2.79	6.70	19.40	70.00	24.00	34.50	20.00	23.20	0.35	20.30	0.58	0.58	0.23	0.00	0.70
90	4yrs	Cocker Spaniel	M	MI	1	5.12	11.00	32.00	62.00	21.40	34.30	78.00	48.60	4.62	39.37	1.46	1.22	0.24	0.00	0.24
93	11yrs	Cairn terrier	F	MI	1	5.28	13.10	36.90	70.00	24.80	35.50	257.00	26.50	0.27	24.91	0.53	0.80	0.00	0.00	0.00
				MI	2	4.29	9.90	30.00	70.00	23.00	33.00	236.00	28.00	0.00	26.18	0.56	1.26	0.00	0.00	0.00
105	1.4	Retriever	F	MI	1	5.82	12.00	35.90	62.00	20.60	33.40	255.00	13.60	0.14	7.89	3.13	0.68	1.63	0.00	0.14
124	12.3	Retriever	M	MI	1	5.42	10.80	33.00	61.00	19.90	32.70	232.00	7.10	0.00	4.72	1.63	0.28	0.28	0.00	0.18
				MI	2	6.03	11.40	33.30	55.00	18.90	34.20	224.00	18.10	0.00	15.93	1.00	1.18	0.00	0.00	0.00
128	9yrs	Airedale	FS	MO	1	4.50	9.40	27.70	62.00	20.80	33.90	163.00	20.00	0.70	16.10	1.20	1.80	0.10	0.00	0.10
*Other refers to normoblasts and nucleated cells in the entire series																				

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DN.*	Age(Yrs)	Breed	Sex	Severity	Sample	RBC	Hb	PCV	MCV	MCH	MCHC	PLT	WBC	BN	Neutro	Lympho	Mono	Eo	Baso	Other*
133	4yrs	Cavalier	M	MO	1	3.29	7.70	22.10	67.00	23.40	34.80	166.00	24.20	0.36	22.39	0.48	0.85	0.12	0.00	0.00
		Spaniel																		
139	4yrs	Cocker	M	MO	1	3.49	8.60	23.70	68.00	24.60	36.20	7.00	25.30	0.25	23.53	0.51	1.01	0.00	0.00	0.00
		Spaniel																		
141	5.2	Rottweiler	FS	S	1	2.15	4.10	14.10	66.00	19.00	29.00	434.00	13.20	0.59	10.43	0.92	0.86	0.33	0.00	0.07
				S	2	2.10	3.80	12.50	60.00	18.00	30.40	492.00	7.00	0.07	3.82	2.17	0.56	0.39	0.00	0.00
				S	3	1.72	2.90	10.50	61.00	16.80	27.60	464.00	9.10	0.00	7.01	1.55	0.23	0.32	0.00	0.00
				S	4	3.90	8.70	25.70	66.00	22.30	33.80	329.00	10.50	0.00	6.56	2.63	0.37	0.95	0.00	0.00
				S	5	2.56	4.70	15.50	61.00	18.30	30.30	368.00	7.70	0.00	5.93	0.92	0.23	0.62	0.00	0.00
				S	6	1.32	2.40	7.90	60.00	18.10	30.30	178.00	11.50	0.00	9.49	1.61	0.40	0.00	0.00	0.00
142	5.4yrs	Cross	M	MI	1	4.68	10.40	30.30	65.00	22.20	34.30	605.00	9.30	0.00	7.67	0.93	0.56	0.14	0.00	0.00
				MO	2	4.38	9.90	28.30	65.00	22.60	34.90	339.00	6.90	0.00	6.21	0.48	0.17	0.03	0.00	0.00
				MO	3	4.11	9.40	28.70	70.00	22.80	32.70	239.00	5.20	0.00	4.03	0.75	0.42	0.00	0.00	0.00
				MO	4	3.73	8.20	24.00	64.00	21.90	34.10	252.00	5.90	0.00	4.93	0.50	0.24	0.06	0.00	0.18
				MO	5	4.43	10.00	29.70	67.00	22.50	33.60	43.00	21.60	0.22	17.71	2.48	0.97	0.00	0.00	0.22
				MO	6	3.54	7.90	26.70	75.00	22.30	29.50	32.00	5.20	0.03	1.85	1.95	0.00	0.00	0.00	0.21
				MO	7	3.18	6.70	21.40	67.00	21.00	31.30	11.00	2.80	0.00	1.62	0.81	0.27	0.00	0.00	0.10
				S	8	2.66	6.50	19.10	72.00	24.40	34.00	14.00	7.00	0.21	5.88	0.91	0.00	0.00	0.00	0.00
143	12yrs	New	M	MI	1	4.83	11.20	32.60	67.00	23.10	34.30	14.00	24.10	0.00	20.49	1.21	1.93	0.00	0.00	0.48
		Foundland																		
*Other refers to normoblasts and nucleated cells in the entire series																				

Units:RBCx10 ^{12}/l, Hb g/dl, PCV %, MCV fl, MCH pg, MCHC g/dl, Plt. 10 ^{9}/l
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DN.*	Age(Yrs)	Breed	Sex	Severity	Sample	RBC	Hb	PCV	MCV	MCH	MCHC	PLT	WBC	BN	Neutro	Lympho	Mono	Eo	Baso	Other*
144	11yrs	Retriever	M	MI	1	4.88	11.40	32.00	66.00	23.30	35.60	307.00	6.50	0.10	5.23	0.46	0.65	0.07	0.00	0.00
146	8yrs	Cocker Sp.	F	MO	1	4.32	9.90	28.70	66.00	22.90	34.40	135.00	2.60	0.00	0.00	0.00	0.00	0.00	0.00	0.00
149	8.1yrs	Great Dane	FS	MI	1	5.12	12.90	35.60	70.00	25.10	36.20	308.00	4.80	0.00	3.74	0.67	0.36	0.02	0.00	0.00
				MI	2	5.03	12.20	34.30	68.00	24.20	35.50	270.00	7.10	0.00	6.04	0.78	0.28	0.00	0.00	0.00
157	8yrs	Cocker Sp.	M	MI	1	5.04	11.50	33.30	66.00	22.80	34.50	140.00	12.90	0.00	9.93	1.03	1.03	0.90	0.00	0.00
				MI	2	5.40	12.50	34.60	64.00	23.10	36.10	239.00	11.50	0.00	9.03	1.09	1.04	0.35	0.00	0.00
				MO	3	3.56	8.10	27.70	78.00	22.70	29.20	174.00	50.40	10.08	34.52	2.27	1.76	1.01	0.00	0.76
170	9.5yrs	Cocker	MC	S	1	1.19	2.80	8.90	75.00	23.50	31.40	7.00	25.90	1.30	18.13	2.46	0.39	0.00	0.00	3.37
		Spaniel		S	2	1.36	3.80	12.20	90.00	27.90	31.10	8.00	36.60	4.58	26.54	1.28	0.55	0.00	0.00	3.48
				S	3	1.36	3.60	12.20	90.00	26.40	29.50	100.00	38.50	4.81	29.07	1.16	1.93	0.00	0.00	0.19
				S	4	1.65	4.50	15.50	94.00	27.20	29.00	147.00	49.00	0.25	41.65	0.74	1.96	0.00	0.00	0.49
				S	5	1.08	3.10	11.20	104.00	28.70	27.60	205.00	47.80	4.06	34.42	1.67	1.43	0.00	0.00	6.21
190	9yrs	GSD	M	MO	1	4.61	9.50	29.70	64.00	20.60	31.90	0.00	28.90	0.00	26.15	0.58	1.45	0.14	0.00	0.00
199	6.6yrs	Spinone	FS	S	1	1.61	3.30	10.50	65.00	20.40	31.40	0.00	28.40	0.00	26.41	1.14	1.14	0.00	0.00	0.00
				S	2	1.55	3.20	10.50	68.00	20.60	30.40	0.00	11.50	0.52	8.80	1.90	0.29	0.00	0.00	0.00
				S	3	1.72	3.30	11.80	69.00	19.10	27.90	177.00	28.40	0.00	21.02	1.99	5.40	0.00	0.00	0.00
				S	4	0.82	1.20	5.20	63.00	14.60	23.00	148.00	12.10	0.06	10.47	0.42	1.15	0.00	0.00	0.00
206	9months	Welsh Springer	M	MO	1	4.32	10.00	28.70	66.00	23.10	34.80	113.00	9.40	0.00	7.66	1.18	0.24	0.33	0.00	0.00
		Spaniel																		

Units:RBCx10 ^{12}/l, Hb g/dl, PCV %, MCV fl, MCH pg, MCHC g/dl, Plt. 10 ^{9}/l
(zero= platelet aggregates),WBC and differentials x10 ^{9}/l,
BN=band neutro.,DN*=Dog number,MI=mild, MO=moderate,S=severe
M=male, C=castrated, F=female, S=spayed

Appendix C: Haematological data of 52 anaemic dogs (PCV<37%), examined post-mortem at the University of Glasgow Veterinary School in 1994.

DN.*	Age(Yrs)	Breed	Sex	Severity	Sample	RBC	Hb	PCV	MCV	MCH	MCHC	PLT	WBC	BN	Neutro	Lympho	Mono	Eo	Baso	Other*
207	8yrs	Rottweiler	F	MO	1	2.94	7.20	21.70	74.00	24.40	33.10	6.00	23.20	0.00	16.59	1.86	1.04	0.46	0.00	3.25
				MO	2	3.52	8.20	25.40	72.00	23.20	32.20	10.00	30.70	0.46	28.09	0.61	1.38	0.00	0.00	0.15
214	8.6yrs	Rottweiler	MC	MI	1	5.91	12.70	36.30	61.00	21.40	34.90	311.00	12.70	0.00	10.41	1.08	0.76	0.44	0.00	0.00
				MI	2	5.47	11.20	34.30	63.00	20.40	32.60	407.00	18.40	0.18	16.74	0.55	0.37	0.55	0.00	0.00
226	7yrs	Retriever	M	MI	1	4.91	12.20	36.30	74.00	24.80	33.60	193.00	12.30	0.00	10.89	0.74	0.68	0.00	0.00	0.00
230	1yr	Bull Terrier	F	MI	1	5.29	12.60	36.30	69.00	23.80	34.70	298.00	11.50	0.00	8.80	2.24	0.29	0.12	0.06	0.00
				MI	2	5.14	12.70	35.90	70.00	24.70	35.30	282.00	10.50	0.00	7.98	1.94	0.21	0.37	0.00	0.00
				MI	3	4.36	10.50	30.00	69.00	24.00	35.00	315.00	8.00	0.00	5.88	1.64	0.20	0.28	0.00	0.00
234	12yrs	Cross	FS	MO	1	4.31	7.20	24.00	56.00	16.70	30.00	306.00	17.30	0.00	16.52	0.17	0.00	0.26	0.09	0.26
235	6yrs	GSD	MC	MO	1	4.04	8.30	25.40	63.00	20.50	32.60	160.00	19.50	0.00	18.14	1.07	0.29	0.00	0.00	0.00
238	9yrs	Border Collie	FS	MI	1	5.02	10.50	32.00	64.00	20.90	32.80	293.00	36.30	0.73	28.50	0.73	6.35	0.00	0.00	0.00
244	13yrs	Retriever	FS	MI	1	5.84	11.30	33.30	57.00	19.30	33.90	310.00	8.50	0.09	6.04	1.53	0.77	0.04	0.00	0.04
*Other refers to normoblasts and nucleated cells in the entire series																				

Units:RBCx10 ^{12}/l, Hb g/dl, PCV %, MCV fl, MCH pg, MCHC g/dl, Plt. 10 ^{9}/l
(zero= platelet aggregates),WBC and differentials x10 ^{9}/l,
BN=band neutro.,DN*=Dog number,MI=mild, MO=moderate,S=severe
M=male, C=castrated, F=female, S=spayed

Appendix D: Haematological data of anaemic dogs (PCV<37%) studied as Prospective Cases at the University of Glasgow Veterinary School in 1995.

PC.	Age(Yrs)	Breed	Sex	Severity	Sample	RBC	Hb.	PCV	MCV	MCH	MCHC	Plt.	WBC	BN.	Neutro	Lympho	Mono.	Eos.	Baso	Others*
1	8.5yrs	Boxer	MN	MO	1	3.17	8.20	24.10	76.00	25.80	34.00	189.00	9.60	0.00	6.43	1.20	0.14	0.58	0.00	1.25
2	8yrs	Flat	F	S	1	1.90	4.40	13.90	73.00	23.10	31.60	8.00	5.90	0.15	4.84	0.47	0.35	0.00	0.00	0.09
		Coated		S	2	1.30	3.30	10.20	78.00	25.30	32.30	8.00	10.30	0.00	9.11	0.31	0.77	0.00	0.00	0.10
		Retriever		S	3	1.13	2.80	9.00	80.00	24.70	31.10	6.00	9.30	0.28	7.58	0.32	0.98	0.00	0.00	0.14
				S	4	2.63	6.50	18.90	72.00	24.70	34.30	27.00	10.70	0.16	9.68	0.32	0.53	0.00	0.00	0.00
				S	5	2.57	6.00	18.60	72.00	23.30	32.20	53.00	12.70	0.19	11.43	0.13	0.95	0.00	0.00	0.00
				S	6	2.32	5.30	16.40	71.00	22.80	32.30	49.00	11.50	0.11	10.35	0.11	0.92	0.00	0.00	0.00
				S	7	1.96	4.50	14.20	72.00	22.90	31.60	14.00	7.70	0.00	7.00	0.27	0.19	0.00	0.00	0.23
3	3.5yrs	GSD	MN	MO	1	4.36	6.00	21.30	49.00	13.70	28.10	360.00	25.60	0.13	22.53	0.64	2.18	0.13	0.00	0.00
				S	2	4.14	5.30	19.20	46.00	12.80	27.60	620.00	8.90	0.00	5.83	1.42	1.29	0.36	0.00	0.00
				S	3	3.48	4.20	16.60	48.00	12.00	25.30	579.00	10.90	0.00	8.72	1.2	0.71	0.27	0.00	0.00
				S	4	3.80	5.00	18.60	49.00	13.10	26.80	593.00	11.40	0.28	9.46	1.25	0.34	0	0.00	0.00
				MO	5	4.33	6.30	21.70	50.00	14.50	29.00	585.00	5.50	0.00	3.22	1.13	0.44	0.72	0.00	0.00
				MO	6	5.83	9.20	29.90	51.00	15.00	30.00	639.00	7.50	0.00	4.61	1.31	0.49	1.09	0.00	0.00
4	11yrs	Labrador	FS	MO	1	2.53	7.20	19.40	77.00	28.40	37.10	410.00	19.50	13.845	0.50	3.80.	0.88	0.10	0.00	0.10
		cross		MO	2	2.59	7.90	19.10	74.00	30.50	41.30	522.00	22.90	20.953	0.00	1.37	0.34	0.11	0.00	0.11
				MI	3	4.47	10.10	32.60	73.00	22.50	30.90	396.00	19.00	17.765	0.57	0.57	0.10	0.00	0.00	0.00
5	3yrs	Jack Russel	FS	S	1	1.07	2.80	9.90	93.00	26.10	28.20	231.00	62.10	2.48	56.20	0.93	1.24	0	0	0.31
*Other refers to normoblasts and other nucleated cells																				

Units: RBCx10 ^{12}/l, Hb g/dl, PCV%, MCV fl, MCH pg, MCHC g/dl, Plt. 10 ^{9}/l (zero indicates platelet aggregates), WBC and differentials x 10 ^{9}/l, BN=band neutrophils
PC= Prospective Case, MI=mild, MO=moderate, S=severe.

Appendix D: Haematological data of anaemic dogs (PCV<37%) studied as Prospective Cases at the University of Glasgow Veterinary School in 1995.

PC.	Age(Yrs)	Breed	Sex	Severity	Sample	RBC	Hb	PCV	MCV	MCH	MCHC	Plt.	WBC	BN	Neutro	Lympho	Mono	Eo	Baso	Others*
6	8yrs	Airedale	M	MO	1	2.40	6.70	22.00	92.00	28.00	30.00	0.00	18.20	12.00	11.60	6.00	0.40	0.20	0.00	0.00
		Terrier		MO	2	2.26	6.10	20.40	90.00	26.60	29.90	292.00	27.60	0.83	23.87	0.41	2.35	0.00	0.00	0.14
				S	3	1.93	5.10	16.80	87.00	26.40	30.30	219.00	8.90	0.22	7.70	0.67	0.27	0.00	0.00	0.00
					4	5.89	11.70	39.90	68.00	19.80	29.30	420.00	12.20	0.00	8.11	3.11	0.00	0.47	0.12	0.00
7	4yrs	GSD	MN	S	1	1.39	4.00	13.00	93.50	28.70	30.80	117.00	87.00	13.92	57.85	1.31	6.96	0.44	0.00	6.55
				MO	2	2.87	8.00	26.50	92.00	27.80	30.10	207.00	35.10	0.52	28.78	2.11	1.93	0.00	0.00	1.75
					3	5.43	13.50	43.50	80.00	24.80	31.00	485.00	11.80	10.03	0.00	0.85	0.47	0.47	0.00	0.00
8	1.5yrs	Cross	FS	S	1	0.83	3.30	10.50	127.00	39.70	31.40	16.00	89.20	8.50	70.50	0.89	1.78	0.00	0.00	6.24
				S	2	0.51	2.60	9.30	182.00	50.00	27.90	10.00	70.20	2.80	54.05	0.70	4.56	0.00	0.00	8.07
				S	3	1.19	4.10	13.30	112.00	34.40	30.80	5.00	33.20	2.98	25.89	0.66	1.66	0.00	0.00	0.99
				S	4	1.44	4.40	14.80	103.00	30.50	29.70	11.00	36.40	1.82	26.57	0.73	5.46	1.36	0.00	1.45
				S	5	1.97	6.00	19.20	97.00	30.40	31.40	62.00	24.00	0.24	21.36	0.24	1.68	0.12	0.00	0.36
				MO	6	2.35	7.10	22.90	97.00	30.20	31.00	47.00	31.30	0.31	28.64	0.46	1.56	0.00	0.00	0.31
				MO	7	3.00	8.40	27.50	92.00	28.00	30.50	75.00	37.80	0.00	35.91	0.56	1.32	0.00	0.00	0.00
				MO	8	3.19	9.20	29.10	91.00	28.80	31.60	239.00	8.50	0.00	50.57	0.26	2.95	0.00	0.00	0.00
				MO	9	3.02	8.70	27.50	91.00	28.80	31.60	270.00	45.30	0.45	42.35	0.00	2.49	0.00	0.00	0.00
				MO	10	3.13	8.70	27.50	88.00	27.70	31.60	408.00	46.70	0.46	43.66	0.23	2.33	0.00	0.00	0.00
				MO	11	3.21	9.30	27.60	86.00	28.90	33.60	288.00	24.00	0.00	22.68	0.00	1.32	0.00	0.00	0.00
				MI	12	3.81	10.70	32.20	8.50	28.00	33.20	30.20	26.50	0.00	25.84	0.40	0.27	0.00	0.00	0.00
					13	4.69	13.00	37.80	81.00	27.70	34.30	431.00	23.70	0.00	21.92	0.12	1.66	0.00	0.00	0.00
					14	4.73	13.00	37.50	79.00	27.40	34.60	257.00	16.80	0.08	15.29	0.84	0.5	0.08	0.00	0.00
*Other refers to normoblasts and other nucleated cells																				

Units: RBCx10 ^{12}/l, Hb g/dl, PCV%, MCV fl, MCH pg, MCHC g/dl, Plt. 10 ^{9}/l (zero indicates platelet aggregates), WBC and differentials x 10 ^{9}/l, BN=band neutrophils
PC= Prospective Case, MI=mild, MO=moderate, S=severe.

Appendix D: Haematological data of anaemic dogs (PCV<37%) studied as Prospective Cases at the University of Glasgow Veterinary School in 1995.

PC.	Age(Yrs)	Breed	Sex	Severity	Sample	RBC	Hb	PCV	MCV	MCH	MCHC	Plt.	WBC	BN	Neutro	Lympho	Mono	Eo	Baso	Others*
9	4yrs	New	M	MO	1	3.19	7.10	22.00	69.00	22.20	32.20	272.00	7.00	0.00	5.88	0.70	0.28	0.00	0.00	0.00
		Foundland		MO	2	3.09	7.40	21.30	69.00	23.90	34.70	20.00	1.40	0.00	0.95	0.41	0.04	0.00	0.00	0.00
10	3.5yrs	GSD	F	MI	1	4.64	11.10	30.30	65.00	23.90	36.60	9.00	1.20	0.00	0.24	0.91	0.05	0.00	0.00	0.00
				MI	2	4.78	11.50	32.20	67.00	24.00	35.70	7.00	1.80	0.11	0.65	1.01	0.03	0.00	0.00	0.00
				MO	3	3.87	9.20	25.40	66.00	23.70	36.20	9.00	1.60	0.06	0.58	0.94	0.03	0.00	0.00	0.00
				MO	4	4.18	10.20	27.90	67.00	24.40	36.50	17.00	1.80	0.11	0.91	0.72	0.06	0.00	0.00	0.00
				MO	5	3.72	8.50	25.10	67.00	22.80	33.80	8.00	3.10	0.06	1.12	1.70	0.19	0.00	0.00	0.03
				MO	6	4.26	9.90	28.20	66.00	23.20	35.10	16.00	4.20	0.00	2.12	1.81	0.23	0.02	0.00	0.02
				MI	7	4.66	11.40	31.60	68.00	24.40	36.00	18.00	4.40	0.00	2.09	2.02	0.18	0.00	0.00	0.11
				MI	8	5.15	12.50	35.60	69.00	24.20	35.10	53.00	5.40	0.00	2.97	1.81	0.19	0.27	0.00	0.16
					9	5.62	13.50	38.70	69.00	24.00	34.80	108.00	4.80	0.00	2.78	1.78	0.05	0.19	0.00	0.00
					10	6.64	15.70	44.90	68.00	23.60	34.90	158.00	6.30	0.00	3.72	2.08	0.13	0.38	0.00	0.00
11	5yrs	Rottweiler	FS	MO	1	2.40	6.40	21.00	89.00	27.00	31.00	0.00	5.70	0.17	0.00	1.50	0.40	0.20	0.00	0.00
				S	2	1.90	5.80	17.00	88.00	30.00	34.00	0.00	6.40	0.15	0.00	0.06	0.00	0.00	0.00	0.00
				S	3	2.25	5.10	17.40	77.00	22.60	29.30	89.00	6.20	0.12	3.81	1.86	0.19	0.08	0.00	0.08
				S	4	1.89	5.00	15.10	80.00	26.40	33.10	230.00	7.60	0.15	4.98	2.01	0.30	0.12	0.00	0.00
				S	5	1.89	4.70	14.80	78.00	24.80	31.70	0.00	8.30	0.25	4.89	2.78	0.25	0.00	0.00	0.00
				S	6	3.19	7.10	22.00	69.00	22.20	32.20	272.00	7.00	0.00	5.90	0.70	0.28	0.07	0.00	0.07
12	11yrs	GSD	FS		1	6.03	13.70	40.20	67.00	22.80	34.20	88.00	14.10	0.00	11.70	1.06	1.13	0.07	0.00	0.14
				MO	2	4.18	8.80	28.70	69.00	21.00	30.60	98.00	20.10	0.10	17.48	1.21	0.80	0.10	0.00	0.40
13	10yrs	Labrador	MN	MI	1	4.38	10.00	30.60	70.00	22.80	32.60	93.00	13.70	0	11.44	0.82	0.75	0.55	0	0.14

Units: RBCx10 ^{12}/l, Hb g/dl, PCV%, MCV fl, MCH pg, MCHC g/dl, Plt. 10 ^{9}/l (zero indicates platelet aggregates), WBC and differentials x 10 ^{9}/l, BN=band neutrophils
PC= Prospective Case, MI=mild, MO=moderate, S=severe.

Appendix D: Haematological data of anaemic dogs (PCV<37%) studied as Prospective Cases at the University of Glasgow Veterinary School in 1995.

PC.	Age(Yrs)	Breed	Sex	Severity	Sample	RBC	Hb	PCV	MCV	MCH	MCHC	Plt.	WBC	BN	Neutro	Lympho	Mono	Eo	Baso	Others*
14	6.5yrs	Boxer	FS	MI	1	4.96	12.00	36.90	74.00	24.10	32.50	102.00	6.70	0.00	3.38	2.65	0.30	0.00	0.00	0.37
				MO	2	2.84	6.90	21.70	76.00	24.20	31.70	46.00	8.60	0.00	1.07	1.81	0.09	0.04	0.00	5.59
				MI	3	5.03	12.30	36.50	73.00	24.40	33.60	53.00	3.30	0.00	2.44	0.4	0.23	0.00	0.00	0.23
				MI	4	4.96	11.50	35.30	71.00	23.10	32.50	40.00	4.00	0.00	3.10	0.66	0.16	0.00	0.00	0.08
				MI	5	4.72	11.20	33.40	71.00	23.70	33.50	18.00	4.00	0.00	2.78	0.20	0.08	0.02	0.00	0.92
				MO	6	3.55	8.40	26.90	76.00	23.60	31.20	14.00	5.50	0.00	2.56	0.47	0.08	0.17	0.00	0.00
				MO	7	3.51	8.20	25.10	72.00	23.30	32.60	50.00	6.60	0.13	4.32	0.66	0.09	0.00	0.00	1.39
				MO	8	3.71	8.80	26.30	71.00	23.70	33.40	66.00	6.00	0.09	3.72	1.02	0.15	0.03	0.00	0.99
				MO	9	3.76	9.00	27.20	72.00	23.90	33.00	82.00	3.30	0.05	0.33	1.15	0.18	0.02	0.00	1.57
				MO	10	3.96	9.40	29.40	74.00	23.70	31.60	91.00	4.70	0.00	1.97	1.13	0.14	0.00	0.00	1.32
				MI	11	4.12	10.20	30.00	73.00	24.70	34.00	132.00	7.80	0.00	2.57	3.43	0.00	0.00	0.00	1.79
				MO	12	4.12	10.20	29.70	72.00	24.70	34.30	179.00	5.50	0.03	1.98	2.58	0.05	0.03	0.00	0.82
				MI	13	4.46	10.70	31.60	71.00	23.90	33.80	190.00	5.50	0.00	2.69	2.14	0.27	0.00	0.00	0.38
				MI	14	4.92	12.00	34.40	70.00	24.30	34.80	275.00	5.80	0.00	3.02	2.61	0.12	0.06	0.00	0.00
				MI	15	5.07	12.20	35.00	69.00	24.00	34.80	260.00	4.40	0.00	2.49	1.67	0.07	0.00	0.00	0.18
				MI	16	4.92	11.70	34.10	69.00	23.70	34.30	274.00	4.30	0.00	3.25	0.75	0.13	0.11	0.00	0.06
				MI	17	5.07	11.70	35.00	69.00	23.00	33.40	284.00	7.10	0.07	4.9	1.42	0.46	0.07	0.18	
				MI	18	5.05	12.90	34.80	69.00	25.50	37.00	263.00	3.60	0.00	2.65	0.76	0.19	0.04	0.05	0.00
				MI	19	5.16	12.10	35.30	68.00	23.40	34.20	248.00	8.40	0.04	7.22	1.01	0.08	0.04	0.00	0.00
15	8yrs	Golden	MN	MI	1	4.99	11.10	35.30	71.00	22.20	31.40	221.00	5.20	0.00	3.51	1.59	0.08	0.03	0.00	0.00
		Retriever		MO	2	3.32	7.90	25.40	77.00	23.70	31.10	588.00	13.70	0.14	12.33	0.41	0.82	0.00	0.00	0.00
				MO	3	3.96	9.00	27.50	69.00	22.70	32.70	695.00	8.60	0.00	7.99	0.39	0.21	0.00	0.00	0.00
				MO	4	3.98	0.00	27.90	70.00	0.00	0.00	646.00	0.36	0.16	0.00	0.00	0.00	0.00	0.00	0.00
				MI	5	4.80	11.30	33.70	70.00	23.50	33.50	391.00	6.80	0.07	4.93	0.99	0.61	0.07	0.00	0.20

Units: RBCx10 ^{12}/l, Hb g/dl, PCV%, MCV fl, MCH pg, MCHC g/dl, Plt. 10 ^{9}/l (zero indicates platelet aggregates), WBC and differentials x 10 ^{9}/l, BN=band neutrophils
PC= Prospective Case, MI=mild, MO=moderate, S=severe.

Appendix E: Biochemistry data of the Prospective Cases studied at the University of Glasgow, Veterinary School during 1995.

PC.	Sample	Urea mmol/l	Creatinine µmol/l	Na mmol/l	K mmol/l	Cl mmol/l	Ca mmol/l	Ph mmol/l	Mg mmol/l	Cholesterol mmol/l	Bilirubin µmol/l	Alk.Phos. mmol/l	A.L.T. IU/l	A.S.T. IU/l	T. Protein g/l	Albumin g/l	Globulin g/l
1	1	3.00	94.00	14.30	3.70	112.00	2.57	1.24	0.65	5.31	1.00	104.00	23.00	38.00	57.00	25.00	32.00
2	1	10.10	86.00	149.00	3.50	116.00	2.25	0.99	0.95	5.52	16.00	149.00	20.00	18.00	58.00	30.00	28.00
	2	10.10	74.00	138.00	2.80	103.00	2.02	1.00	1.18	5.79	33.00	162.00	23.00	15.00	49.00	26.00	23.00
3	1	4.90	72.00	127.00	2.30	80.00	2.11	1.18	0.00	6.54	0.00	45.00	14.00	21.00	56.00	23.00	33.00
	2	2.30	84.00	143.00	3.70	113.00	2.36	1.25	0.59	5.74	0.00	35.00	26.00	14.00	50.00	22.00	28.00
4	1	6.30	63.00	148.00	4.00	109.00	2.54	2.04	0.70	7.31	3.00	6930.00	180.00	20.00	61.00	32.00	29.00
	2	1.70	89.00	144.00	3.30	108.00	2.28	1.29	0.55	7.60	4.00	2255.00	91.00	13.00	66.00	30.00	36.00
5	1	9.60	65.00	136.00	2.30	104.00	2.42	0.75	2.42	9.15	32.00	417.00	8.00	37.00	65.00	32.00	33.00
6	1	21.00	99.00	147.00	5.30	120.00	2.13	1.58	0.69	4.18	0.00	396.00	289.00	77.00	57.00	25.00	31.00
7	1	11.80	67.00	140.00	3.00	106.00	2.55	1.25	0.99	9.10	16.00	431.00	88.00	111.00	70.00	38.00	32.00
8	1	6.50	40.00	137.00	3.30	112.00	2.10	1.19	0.81	10.48	21.00	889.00	217.00	216.00	77.00	22.00	55.00
	2	3.80	46.00	146.00	3.50	116.00	2.43	1.77	0.95	7.93	2.00	7472.00	1027.00	145.00	59.00	34.00	25.00
	3	3.90	57.00	144.00	3.50	106.00	2.25	1.44	0.76	5.72	5.00	3748.00	324.00	36.00	60.00	33.00	27.00
9	1	5.80	69.00	137.00	3.70	105.00	2.06	0.80	0.76	6.00	3.00	2141.00	92.00	26.00	54.00	29.00	25.00
10	1	4.70	102.00	142.00	3.80	110.00	2.41	1.33	0.58	8.66	2.00	239.00	16.00	13.00	66.00	29.00	37.00
	2	7.90	86.00	143.00	3.70	117.00	2.59	1.60	0.71	8.25	2.00	226.00	17.00	14.00	69.00	30.00	39.00
	3	6.40	82.00	144.00	4.30	114.00	2.51	1.51	0.83	6.48	2.00	174.00	23.00	17.00	61.00	27.00	34.00
11	1	4.20	120.00	148.00	3.80	114.00	2.50	1.54	0.87	5.18	4.00	160.00	19.00	13.00	68.00	37.00	31.00

PC= Prospective Case

Appendix E: Biochemistry data of the Prospective Cases studied at the University of Glasgow, Veterinary School during 1995.

PC.	Sample	Urea mmol/l	Creatinine µmol/l	Na mmol/l	K mmol/l	Cl mmol/l	Ca mmol/l	Ph mmol/l	Mg mmol/l	Cholesterol mmol/l	Bilirubin µmol/l	Alk.Phos. mmol/l	A.L.T. IU/l	A.S.T. IU/l	T. Protein g/l	Albumin g/l	Globulin g/l
12	1	7.20	110.00	149.00	4.00	108.00	2.58	1.24	0.70	5.39	0.00	86.00	317.00	30.00	56.00	28.00	28.00
	2	7.90	133.00	143.00	3.80	110.00	2.35	1.08	0.73	5.10	0.00	65.00	45.00	49.00	56.00	31.00	25.00
	3	4.80	106.00	143.00	4.20	114.00	2.52	1.35	0.88	6.36	2.00	55.00	70.00	34.00	57.00	31.00	26.00
13	1	6.60	97.00	148.00	4.80	108.00	2.95	1.76	0.63	5.95	8.00	117.00	29.00	39.00	70.00	31.00	39.00
14	1	27.40	274.00	144.00	4.80	105.00	3.54	2.81	0.92	7.05	0.00	289.00	29.00	29.00	59.00	30.00	29.00
	2	17.20	265.00	136.00	4.20	105.00	3.54	1.86	0.67	7.07	4.00	299.00	124.00	36.00	64.00	31.00	33.00
	3	7.40	191.00	146.00	4.80	112.00	1.85	1.23	0.55	6.79	1.00	404.00	38.00	181.00	56.00	28.00	28.00
	4	21.30	245.00	146.00	4.50	120.00	2.71	2.06	0.75	4.81	0.00	182.00	67.00	48.00	47.00	22.00	25.00
	5	17.60	217.00	147.00	4.20	110.00	1.90	1.36	0.63	not done	not done	not done	not done	not done	not done	not done	not done
	6	9.30	177.00	147.00	4.00	113.00	2.00	1.35	0.56	8.15	0.00	349.00	159.00	41.00	59.00	28.00	31.00
	7	9.80	169.00	146.00	3.90	117.00	2.45	1.75	0.56	8.19	0.00	479.00	94.00	17.00	67.00	28.00	39.00
	8	14.60	278.00	142.00	3.90	110.00	2.46	1.55	0.53	6.64	1.00	360.00	32.00	14.00	63.00	28.00	35.00
	9	13.70	217.00	146.00	5.50	115.00	2.41	1.87	0.54	6.73	0.00	248.00	30.00	27.00	61.00	25.00	36.00
	10	14.90	240.00	148.00	4.10	107.00	2.54	1.93	0.73	7.04	0.00	171.00	24.00	25.00	61.00	32.00	29.00
	11	12.50	233.00	149.00	3.70	116.00	2.63	1.52	0.72	6.42	1.00	126.00	20.00	26.00	61.00	32.00	29.00
	12	13.60	217.00	151.00	3.80	114.00	2.59	1.64	0.68	6.45	7.00	113.00	27.00	22.00	60.00	32.00	28.00
	13	16.00	210.00	151.00	3.80	114.00	2.65	1.88	0.70	6.95	0.00	92.00	18.00	23.00	62.00	34.00	28.00
	14	15.10	208.00	151.00	3.70	113.00	2.67	1.65	0.72	9.66	1.00	137.00	70.00	27.00	58.00	30.00	28.00
	15	17.20	205.00	149.00	3.90	112.00	2.80	1.83	0.81	7.38	0.00	99.00	20.00	24.00	63.00	33.00	30.00
	16	15.90	217.00	151.00	3.40	117.00	2.56	1.66	0.74	8.27	0.00	122.00	87.00	40.00	64.00	34.00	30.00
	17	14.00	210.00	151.00	3.70	111.00	2.70	1.76	0.68	8.88	0.00	176.00	51.00	27.00	63.00	34.00	29.00
	18	12.40	210.00	150.00	3.80	119.00	2.56	1.31	0.72	9.01	0.00	135.00	134.00	46.00	61.00	32.00	29.00
15	no biochemistry was done																

PC= Prospective Case

Appendix F: Clinical signs observed in the 15 dogs studied as Prospective Cases at the University of Glasgow, Veterinary School during 1995.

PC.	Condition	Dullness	Lethargy	Pallor	Anorexia	Polydipsia	Pyrexia	Pulse	Vomiting	Haematuria	Malaena	Cardiac murmur
1	Haematoma	+	+	++	+	+	-	NAD	-	++	NAD	+
2	Hypersplenism and DIC	-	-	++	++	-	-	NAD	-	-	NAD	NAD
3	Chronic blood loss	+	+	+++	-	+	-	NAD	-	-	++	NAD
4	Hepatomegaly	+	+	-	++	-	-	NAD	-	+	NAD	NAD
5	AIHA	+	+	++icterus	-	-	-	++	-	-	NAD	+++
6	AIHA	+	-	++	+	+	-	++	-	Haemoglobinuria	+++	NAD
7	AIHA	+	+	++	++	-	-	NAD	-	-	NAD	+
8	AIHA	-	+	+++	+	-	+	NAD	-	+	NAD	NAD
9	Bone marrow hypoplasia	-	-	+	-	-	-	-	-	-	NAD	+
10	Bone marrow hypoplasia	+	+	+	-	-	+	NAD	-	-	++	++
11	Erythroid hypoplasia	-	+	++	-	-	+	++	-	-	NAD	++
12	Haemangiosarcoma	+	+	+	+	-	+	+	-	-	+	NAD
13	Haemangiosarcoma	+	+	-	+	-	-	NAD	+ blood	+	+++	NAD
14	Lymphosarcoma	+	-	+	+	+	-	NAD	+	-	Diarrhoea	NAD
15	Lymphosarcoma	+	-	-	-	+	-	NAD	-	-	NAD	NAD

PC= prospective cases, (+) indicates a mild degree, (++) indicates moderate degree and (+++) a marked degree.
NAD= nothing abnormal diagnosed. Blood in the urine is mentioned,other findings shown in the text.