DIAGNOSTIC AND THERAPEUTIC APPROACHES IN ANAEMIA OF DOGS

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DECLARATION

I hereby declare that this thesis, entitled **"DIAGNOSTIC AND THERAPEUTIC APPROACHES IN ANAEMIA OF DOGS"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Certified that this thesis, entitled **"DIAGNOSTIC AND THERAPEUTIC APPROACHES IN ANAEMIA OF DOGS"** is a record of research work done independently by **Ashwin Jayarajan** under my guidance and supervision and it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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Introduction

1. INTRODUCTION

The obedient and loyal dog is not only a pet but also a vital member of our society on account of its presence in many roles as guide dogs, army dogs, police dogs etc. to name a few. Not surprisingly they are also found to be prone to many disease conditions similar to those affecting humans and one such disorder is 'Anaemia'.

Anaemia is not a disease in itself but a sign or indicator of some underlying disease (Rogers, 2000). It is one of the most common clinical signs encountered in many infectious and non-infectious diseases in dogs. Anaemia adversely affects the quality of life of the individual leading to weakness, lethargy, cardiovascular abnormalities etc. Although in some cases the clinical signs are suggestive of the cause of the anaemia, in most cases a precise diagnosis of the aetiology and initiation of suitable therapy is often found to be difficult. Anaemia can be caused by a number of factors such as haemoparasites, gastrointestinal parasites, immune-mediated diseases, neoplasms, genetic abnormalities etc. The treatment of these different types of anaemia depends upon the identification of the underlying cause. Lack of proper diagnosis about the underlying causes prevents prompt and early treatment of the condition. Routine examination of animal and blood will also not reveal the cause of the anaemia in most of the cases.

Weiss (2008) mentioned that bone marrow aspiration is an important tool for the further diagnosis and classification of non-regenerative anaemias and is indicated in all such cases.

According to (O'hara and Richardson, 2008) blood transfusion is one of the best supportive treatments in cases of anaemia regardless of the cause as it is a rich source of erythrocytes. However, due to stringent rules, many a times blood collection bags are not available readily and hence blood transfusion is not performed routinely in veterinary practice. So evolving an easy and cost effective method of blood transfusion is necessary in veterinary field.

Hence the study entitled Diagnostic and therapeutic approaches in anaemia of dogs has been taken up with the following objectives;

- To study the etiological factors of anaemia in dogs.
- To standardize bone marrow aspiration technique for diagnosis of anaemia in dogs.
- To suggest suitable therapeutic measures including a convenient and easy method of immediate blood transfusion in anaemia of dogs.

Review of Literature

2. REVIEW OF LITERATURE

2.1. ANAEMIA IN DOGS

Anaemia was an important haematological abnormality which was frequently encountered in dogs. It was always associated with an underlying disease which must be identified to allow appropriate case management (Squires, 1993).

Aird (2000) mentioned that anaemia was the decreased ability of blood to supply tissues with adequate oxygen for proper metabolic function and said that the response to treatment of anaemia was transient unless the underlying disease process was identified.

Anaemia was defined as a decrease in the red blood cell (RBC) mass as expressed by a reduction in number of circulating RBCs, haematocrit and haemoglobin and the severity of signs depended on the rapidity of onset, the degree and cause of anaemia and the extent of physical activity (Giger, 2000).

Neiger *et al.* (2002) proposed that a corrected reticulocyte count above 1 per cent was used to indicate regenerative anaemia and a corrected count below 1 per cent was used to indicate non-regenerative anaemia. They also mentioned that the MCV could be a useful tool for assessing anaemia in dogs and that an increase of 1 fl in the MCV denoted the presence of regenerative anaemia.

Anaemia, an absolute decrease in red blood cell mass was a laboratory finding, not a diagnosis and by using a systematic approach to the signalment, history, physical examination and laboratory testing, definitive diagnosis could be made in most cases (Cotter, 2003).

McLellan *et al.* (2003) was of the opinion that anaemia was a condition in which the haemoglobin concentration was below the normal range. He also stated that anaemia was a common complication of critical illness.

Weiss and Tvedten (2004) expressed the severity of anaemia depending on the packed cell volume in dogs as follows: Mild: 30-37 per cent; Moderate: 20-29 per cent; Severe: 13-19 per cent and Very severe < 13 per cent.

Benjamin (2007) had mentioned the reference range for serum biochemical parameters in normal dogs as; Total protein: 5.4-7.1 g/ dL, albumin: 2.3-3.2 g/ dL, globulin: 2.7-4.4 g/ dL, total bilirubin: 0.07-0.61 mg/ dL, direct bilirubin: 0.06-0.12 mg/ dL and creatinine: 1-2 mg/ dL.

Zygner *et al.* (2007) stated that a low red blood cell count, haematocrit and the concentration of haemoglobin defined anaemia in dogs.

Sasanelli *et al.* (2009) had recorded the normal canine haematological values as; TEC: 5.5-8.5 X 10^6/ cu.mm, VPRC: 37-55 per cent, haemoglobin: 12-18 g/ dL, MCV: 60-77 fl, MCH: 19.5-24.5 pg, MCHC: 32-36 g/ dL, TLC: 6-17 X 10^3/ cu.mm, neutrophils: 3-11.5 X 10^3/ cu.mm, lymphocytes: 1-4.8 X 10^3/ cu.mm, monocytes: 0.1-1.3 X 10^3/ cu.mm, eosinophils: 0.1-1.2 X 10^3/ cu.mm, basophils: 0-0.1 X 10^3/ cu.mm and platelet count: 200-500 X 10^3/ cu.mm.

2.1.1 Classification and Causes of Anaemia

A key requirement when attempting to categorise anaemia was to determine whether the anaemia was regenerative or nonregenerative. An increased reticulocyte count was the hallmark of regenerative anaemia whereas nonregenerative anaemia was characterised by a sustained absence of reticulocytosis (Squires, 1993).

Aird (2000) proposed that blood loss anaemia could be caused due to coagulopathies, epistaxis, gastrointestinal haemorrhage, platelet disorders, splenic rupture, trauma etc. and haemolytic anaemia by RBC fragmentation, immune-mediated disease, infections, intrinsic RBC defects, toxicities etc.

Giger (2000) classified anaemia on the basis of pathophysiologic mechanisms, bone marrow response and RBC indices.

Regenerative anaemias resulted from excessive blood loss (haemorrhage) or RBC destruction (haemolysis) leading to reticulocytosis. Nonregenerative anaemias were characterised by decreased or ineffective production of RBCs by the bone marrow and might be caused by anaemia of chronic diseases, iron deficiency, bone marrow disorders, drug induced haematologic dyscrasias, certain infections etc. (Weiss and Tvedten, 2004).

Benjamin (2007) opined that anaemia could be classified according to the cause as occurring due to acute haemorrhage, increased erythrocyte destruction or decreased erythrocyte production. He further mentioned that these causes could be broadly categorised into regenerative or nonregenerative anaemias.

2.1.2 Clinical Examination Findings in Anaemia

Klag *et al.* (1993) reported that the most common clinical signs of haemolytic anaemia were anorexia (90 per cent), lethargy (86 per cent), pallor (76 per cent), weakness (67 per cent) and icterus (60 per cent). Tachycardia, splenomegaly and hepatomegaly were also observed.

According to Squires (1993) the common physical examination findings in anaemic cases were pallor, icterus or petechiation and ecchymosis of the mucous membranes, fever, lymphadenopathy and a low grade 'haemic murmur'. He also mentioned the importance of careful abdominal palpation in anaemic patients.

The clinical signs of anaemia were attributed to a reduction in oxygen carrying capacity of the blood and some of these were a direct result of tissue hypoxia. However the majority were related to compensatory mechanisms that increased the circulating blood volume and reduced the cardiac workload (Aird, 2000).

Rogers (2000) reported that the severity of the symptoms of oxygen deficiency were more often related to the rapidity of onset rather than the degree of anaemia and hence chronic anaemias showed milder signs as compared to acute anaemias.

Kraje (2002) observed that anaemia could be initially masked by dehydration and the typical clinical signs not evident until fluid therapy had been initiated.

Abiramy *et al.* (2003) and Bhikane *et* al. (2006) reported that parenteral iron therapy was very effective in cases of anaemia in dogs and goats respectively.

Cotter (2003) mentioned that a complete physical examination could provide additional valuable informations about the cause of the anaemia. The presence of fever or splenomegaly indicated acute intravascular haemolysis and neoplasia (lymphoma, malignant histiocytosis etc.) respectively.

Jutkowitz (2004) reported that at haematocrit values in the range of 10 to 15 per cent, myocardial ischemia could be noted indicated by signs of S-T segment elevation or depression in electrocardiogram.

Normovolaemic anaemic patients were relatively bright with a normal or only marginally elevated heart and respiratory rates and had pale pink rather than white mucous membranes (Rozanski and De Laforcade, 2004).

Saini *et al.* (2005) in a study conducted in anaemic dogs found that the main symptoms exhibited were pale mucous membranes, depression, anorexia, weakness, tachycardia and tachypnoea.

De Gopegui *et al.* (2007) in a study conducted on forty five cases of canine babesiosis found that clinical signs were due to haemolytic anaemia and included fever, anaemia, tachypnea, tachycardia, splenomegaly, icterus and depression.

2.2 BLOOD LOSS ANAEMIA

2.2.1 Chronic Blood Loss Anaemia

Chronic external blood loss resulted from gastrointestinal parasitism caused by hookworms and rarely whipworms (Giger, 2000).

Hall and Simpson (2000) opined that helminth infestation was common in dogs and cats and *Ancylostoma caninum* was the most important hookworm of dogs and was associated with blood loss and haemorrhagic enteritis.

Ozkanlar *et al.* (2004) was of the opinion that ascarids, especially *Toxocara canis* were the most common gastrointestinal nematodes of dogs worldwide.

Bond *et al.* (2007) reported that *Ctenocephalides felis* was the species of flea found most commonly in dogs.

Tinoco-Gracia *et al.* (2009) mentioned that the dog tick *Rhipicephalus sanguineus* was distributed worldwide and the larval, nymph and adult stages all fed on blood.

Beugnet and Marie (2009) opined that despite regular antiparasitic treatment, fleas remained the most common ectoparasites on dogs.

2.2.1.1 Clinical Signs

Hall and Simpson (2000) stated that infection with *A.caninum* caused a rapid and fatal anaemia or more chronic iron deficiency anaemia.

According to Jones and Cappello (2004) most of the deleterious health effects associated with chronic hookworm infection were attributable to adult worms, which attached to the intestinal mucosa and led to substantial haemorrhage. The cumulative effects of chronic gastrointestinal blood and serum protein loss included iron deficiency, anaemia and retardation of growth.

Rani (2004) mentioned that ectoparasites such as fleas and ticks were considered mainly as disease vectors and were not directly responsible for anaemia. However they were responsible for the development of anaemia if the infections were severe enough.

Infections with intestinal parasites resulted in disease in dogs such as vomiting, diarrhoea, anaemia, anorexia, dermatitis and loss of condition (Little *et al.*, 2009).

2.2.1.2 Clinical Pathology

2.2.1.2.1 Haematological Changes

Giger (2000) mentioned that on a blood smear of dogs having chronic blood loss the hypochromic red blood cells were readily recognised by the increased central pallor and were reflected by a decreased mean corpuscular haemoglobin concentration. Anisocytosis was also a recognisable morphologic change.

Vardhani (2003) concluded in a study conducted in female swiss albino mice infected with multiple doses of *Ancylostoma caninum* larvae that there was a significant rise of both circulating and intestinal eosinophils in all infected animals when compared with controls.

Rani (2004) recorded a severe anaemia and decreased serum protein values in a calf with severe ectoparasite infestation.

Saini *et al.* (2005) reported that hookworm infection was responsible for causing microcytic hypochromic anaemia.

2.2.1.2.2 Changes in Serum Biochemistry

According to Weiss and Tvedten (2004) a low or low-normal plasma protein level in regenerative anaemia was frequently associated with external blood loss and was a useful diagnostic feature.

In a study conducted by Paltrinieri *et al.* (2009) it was concluded that microcytosis was not a hallmark of hyposideremia in dogs except when MCV values were very low.

2.2.1.3 Diagnosis

Baker and Thomsett (1990) mentioned that when flea faeces were placed on moist paper a brown halo of digested blood developed quickly and this was used for diagnosing flea infestation in the absence of visible fleas on the body.

Minnaar *et al.* (2002) mentioned the use of adhesive tape swabs for diagnosis of cestode eggs and/ or segments on the peri-anal skin or hair. The peri-anal area of each dog was dabbed with the adhesive side of ordinary clear stationary 'sticky tape' which was then flattened with the adhesive side down on a clean microscope slide and examined under a light microscope using 10 x magnification in the laboratory.

Bond *et al.* (2007) reported that the signs of active flea infestation were the presence of flea dirt or adult fleas on the body and skin lesions compatible with FAD

In the study carried out by Yacob *et al.* (2007) the faecal samples were taken directly from the rectum of the dogs using gloved finger and were then subjected to qualitative (Flotation) technique and quantitative examination using McMaster egg counting technique. Identification of eggs was made by measuring each nematode egg and by observing their characters.

Tinoco-Gracia *et al.* (2009) postulated that finding > 30 ticks on each dog was considered to be a severe tick infestation.

2.2.1.4 Treatment and Control

More potent killers of adult fleas such as fipronil was found to be more than 99 per cent effective for atleast three weeks after treatment and more than 97 per cent effective upto 36 days after treatment (Hutchinson *et al.*, 1998).

Dryden and Ridley (1999) postulated that Pyrantel pamoate 5mg/ kg was effective against ascarids upto 88.3 per cent and 94.2 per cent in some studies. Fenbendazole 50 mg/ kg for three days was found to be 95.8 per cent effective.

Campbell (2000) recommended manual removal, use of pyrethrins, pyrethroids, fipronil and amitraz as treatment in cases of tick infestation.

Hall and Simpson (2000) recommended treatment of roundworm ascarids with ivermectin plus pyrantel pamoate, hookworms with pyrantel pamoate and tapeworms with praziquantel along with adequate flea control.

Cadiergues *et al.* (2001) reported that the use of fipronil @ 6.7 mg/ kg completely eradicated the fleas from dogs with 100 per cent efficacy for upto 5 weeks.

Chhabra and Khahra (2003) reported that ivermectin @ 200 mcg/ kg body weight was effective against *Rhipicephalus sanguineus* ticks but was ineffective against flea infestation.

Ozkanlar *et al.* (2004) used selamectin against ascardiosis (*Toxocara canis*) infested puppies and reported that success rates were 100 per cent in two doses and 92.8 per cent in one dose of 6 mg/ kg applied topically.

Rani (2004) suggested the use of amitraz as an external application for control of tick infestation.

Pullola *et al.* (2006) reported that 64.9 per cent of all dewormings of dogs in Finland were carried out using benzimidazoles or Febantel-pyrantel-praziquantel compound. However he also pointed out that unnecessary use of broad spectrum anthelmintics was not a sustainable strategy to control parasites since anthelmintic resistance was a major concern.

Bond *et al.* (2007) recommended monthly treatment of dogs against flea infestation.

Kopp *et al.* (2008) opined that pyrantel was highly effective against hookworms with an efficacy of 91-100 per cent against *Ancylostoma caninum.*

Routine monthly control products that included efficacy against intestinal nematodes were expected to reduce although not eliminate parasitism in pet dogs (Little *et al.*, 2009)

Tinoco-Gracia *et al.* (2009) postulated that fumigation campaigns to kill ticks, regular treatment of dogs with acaricides and public education related to tick prevention strategies were necessary to eradicate tick infestation in dogs.

2.2.2 Haemolytic Anaemia

Squires (1993) mentioned that the commonest cause of non-traumatic severe anaemia in dogs was immune-mediated haemolytic anaemia.

Haemolytic anaemia was the primary manifestation of several rickettsial and protozoal diseases in domestic animals (Gaunt, 2000).

Erythrocyte destruction took place either extra- or intravascularly. Extravascular haemolysis was the predominant form and referred to erythrophagocytosis by macrophages of the spleen, liver and bone marrow. Intravascular haemolysis took place less commonly as a consequence of membrane permeability changes or cellular fragmentation (Giger, 2000).

Haemolytic anaemia was usually diagnosed by finding markedly regenerative anaemia without hypoproteinemia or other evidence of blood loss. Erythrophagocytosis seen in blood smears or cytologic smears of bone marrow, spleen, liver or lymph nodes was infrequent but a strong indication of haemolysis (Weiss and Tvedten, 2004).

Benjamin (2007) reported that haemolytic anaemia had many causes such as RBC parasites (*Babesia canis*, *Haemobartonella* etc.), antibody mediated destruction, chemical agents, intrinsic inherited erythrocyte abnormalities etc.

2.2.2.1 Haemolytic Anaemia Due to Infectious Agents

2.2.2.1.1 Ehrlichiosis

The parasite *Ehrlichia canis*, was an obligate intracytoplasmic rickettsia which localized in the reticuloendothelial cells of the liver, spleen and lymph nodes where it replicated primarily in mononuclear macrophages and lymphocytes (Waner *et al.*, 1996).

Different species of obligate intracellular bacteria belonging to the genus *Ehrlichia* had been shown to infect specific haematopoietic cell lineages in several animal species and in man (Egenvall *et al.*, 1997).

Harrus *et al.* (1997a) concluded that severe anaemia, severe leucopenia, pancytopenia and a tendency to bleed (like epistaxis) especially in German shepherd dogs were important indicators of poor survival.

Combined infections of *Ehrlichia platys* and *Ehrlichia canis* were common and *Ehrlichia canis* infections contributed to the pathogenesis of *Ehrlichia platys* (Harrus *et al.*, 1997b).

Ehrlichia canis was a potentially fatal tick borne disease of dogs and its main vector was *Rhipicephalus sanguineus* (Suto *et al.*, 2001).

Ehrlichiosis was a tick-borne disease of dogs caused by organisms belonging to genera *Ehrlichia* and *Anaplasma*. *Ehrlichia canis* had a worldwide distribution, but the main clinical manifestations varied geographically (Dagnone *et al.*, 2003).

Mylonakis *et al.* (2009) postulated that *Ehrlichia canis* was the primary cause of canine monocytic ehrlichiosis (CME).

2.2.2.1.1.1 Clinical Signs

Initial thrombocytopenia followed by pyrexia and anorexia was common in canine ehrlichiosis (Waner *et al.*, 1996).

Lymphadenomegaly was usually noticed during the acute stage of the disease, but was rarely seen in the chronic state. Respiratory distress secondary to pulmonary haemorrhage, inflammation and necrosis was also prevalent in cases of ehrlichiosis (Waner *et al.*, 1996).

According to Egenvall *et al.* (1997) a study on granulocytic ehrlichiosis revealed clinical signs like depression, pyrexia and reluctance to move.

The clinical signs reported in a study of experimental ehrlichiosis were anaemia, depression, lymphadenopathy, splenomegaly, inappetence and pyrexia (Harrus *et al.*, 1998).

E. canis infection resulted in a wide variety of clinical signs of which depression, lethargy, weight loss, anorexia, pyrexia, signs of anaemia, lymphadenopathy, splenomegaly and tendency for haemorrhage were most common (Suto *et al.*, 2001).

Reine (2004) described the clinical signs like depression, lethargy, mild weight loss, and anorexia in dogs with ehrlichiosis.

2.2.2.1.1.2 Clinical Pathology

2.2.2.1.1.2.1 Haematological Changes

According to Waner *et al.* (1995) the haematological changes observed in cases of canine ehrlichiosis included mild transient leucopenia, mild non-regenerative anaemia and thrombocytopenia which were attributed to accelerated platelet destruction.

A transient mild decrease in leucocytes (6.7 ± 0.2 X 10^3/ cu.mm), mild non-regenerative anaemia (PCV 31.8 ± 1.2 per cent) and platelet count (30.2 ± 10.6 X 10^3/ cu.mm) in infected dogs were reported by Waner *et al.* (1996).

The main abnormal haematological findings included thrombocytopenia, a low haematocrit and monocytosis in canine ehrlichiosis (Harrus *et al.*, 1997a).

Harrus *et al.* (1998) found a significant decrease in the haematocrit, red blood cell count, haemoglobin concentration, leucocyte count and platelet count in dogs affected with ehrlichiosis as compared to control animals.

Suto *et al.* (2001) mentioned that the principal haematological abnormalities in cases of ehrlichiosis included thrombocytopenia, mild anaemia and mild leucopoenia.

Dagnone *et al.* (2003) opined that ehrlichiosis was an important differential diagnosis in dogs with anaemia and thrombocytopenia.

In the acute phase of *Ehrlichia canis* infection, thrombocytopenia was caused by increased platelet consumption as well as sequestration (Botsch *et al.*, 2009)

2.2.2.1.1.2.2 Changes in Serum Biochemistry

The hypergammaglobulinaemia observed in chronic ehrlichiosis suggested that autoantibodies were important in the pathogenesis of this infection (Waner *et al.*, 1995).

Serum protein changes included hyperglobulinaemia, hypoalbuminaemia, hyperproteinaemia and increased serum alanine aminotransferase and serum creatinine in dogs affected with ehrlichiosis (Harrus *et al.*, 1997a).

The main abnormal biochemical finding included low albumin concentrations which was induced by a peripheral loss to oedematous inflammatory fluids as a result of vasculitis (Harrus *et al.*, 1997b).

According to Suto *et al.* (2001) dogs with ehrlichiosis showed a high concentration of serum total protein and hypergammaglobulinaemia.

2.2.2.1.1.3 Diagnosis

According to Troy and Forrester (1990) serologic testing was the most useful and reliable method to diagnose ehrlichiosis and the immunofluorescence test was most reliable in cases of *E.canis* infections.

The disease was diagnosed by clinical and haematological signs, demonstration of morulae in peripheral monocytes and detection of serum antibodies to *E. canis* by indirect immunofluorescent antibody (IFA) technique (Waner *et al.*, 1996).

Morulae detected in the cytoplasm of macrophages by Giemsa staining and serological examination including indirect immunofluorescence (IF) to detect antibodies were most useful methods for diagnosing canine ehrlichiosis (Suto *et al.*, 2001).

2.2.2.1.1.4 Treatment and Control

Dogs affected with ehrlichiosis were treated with doxycycline orally @ 5 mg/ kg once daily for 21 days (Harrus *et al.*, 1997b).

Suto *et al.* (2001) recommended the use of tetracycline as the first choice among antibiotics for treating *E. canis* infection of dogs.

Hegarty *et al.* (2009) mentioned the treatment of *E. canis* infections in dogs using doxycycline or other tetracycline derivatives.

Sasanelli *et al.* (2009) confirmed the use of Doxycycline @ 10 mg/ kg/ day orally for the treatment of ehrlichiosis in dogs.

2.2.2.1.2 Mycoplasmosis

Eberle and Kirchhoff (1976) recorded that dogs were first exposed to mycoplasmas when passing through the birth canal.

Reine (2004) found that haemoplasmosis could cause anaemia in dogs that were immunosuppressed.

Several species of *Mycoplasma* were pathogenic and *M. haemocanis* was associated with canine anaemia (Chalker, 2005).

The attachment of the organism lead to an indentation of the RBC surface which indicated direct damage of the RBC membrane. Immunological mechanisms including the production of autoantibodies were also involved in RBC destruction (Willi *et al.*, 2007).

Novacco *et al.* (2009) stated that in dogs, *Mycoplasma haemocanis* were small cell wall-less bacteria that infected the red blood cells and that kennel-kept dogs were significantly more frequently infected with haemoplasmas than dogs living in private homes and infested with ticks, fleas, or both.

2.2.2.1.2.1 Clinical Signs

The acute form of the disease was characterised by a rapidly developing anaemia, most often in immune-compromised or splenectomised dogs with symptoms such as lethargy, weight loss, fever and anorexia (Chalker, 2005).

The typical clinical signs included pallor, lethargy, weakness, tachycardia, tachypnoea, splenomegaly, lymphadenopathy and occasionally icterus (Willi *et al.*, 2007).

Novacco *et al.* (2009) described a severe haemolytic anaemia in immunocompromised or splenectomised infected dogs with clinical signs of fever, pale mucous membranes, enlarged mandibular and popliteal lymph nodes and severe infestation with ticks and fleas.

2.2.2.1.2.2 Clinical Pathology

2.2.2.1.2.2.1 Haematological Changes

Haematologic findings in canine haemoplasmosis included reticulocytosis, increased polychromasia, anisocytosis, circulating nucleated erythrocytes and frequent.Howell-Jolly bodies (Harvey, 1990).

In severe cases of mycoplasmosis, acute haemolytic anaemia occured (Chalker, 2005).

The typical haematological abnormalities attributable to haemoloysis included regenerative, macrocytic and normochromic anaemia with increased reticulocyte counts, anisocytosis, polychromasia, Howell – Jolly bodies and sometimes a marked increase in nucleated RBC's (Willi *et al.*, 2007).

2.2.2.1.2.2.2 Changes in Serum Biochemistry

Breitschwerdt (2000) reported non specific biochemical changes in cases of mycoplasmosis.

Blood biochemistry revealed hyperbilirubinaemia and hyperproteinaemia and increased liver enzyme blood levels due to hypoxic damage (Willi *et al.*, 2007).

2.2.2.1.2.3 Diagnosis

Harvey (1990) mentioned that the most useful criterion for diagnosis of canine haemobartonellosis was the tendency of the organism to form chains across the erythrocyte surface.

Reine (2004) concluded that the diagnosis of haemoplasmosis typically relied on visualization of the organism in red blood cells.

Diagnosis of haemoplasma infections mainly relied upon cytological identification of the organisms on Romanowsky-stained blood smears. They

usually appeared as coccoid forms although rod and ring forms had also been observed (Willi *et al.*, 2007).

2.2.2.1.2.4 Treatment and Control

Treatment with doxycycline was recommended for acute infection with *M. haemocanis* (Messick, 2003).

Treatment with antibiotics such as doxycycline or tetracycline was effective against most canine mycoplasmas (Chalker, 2005).

Doxycycline @ 10 mg/ kg daily PO, was the preferred antibiotic to treat haemoplasma infections followed by enrofloxacin @ 5 mg/ kg daily PO for 2 weeks (Willi *et al.*, 2007).

2.2.2.1.3 Babesiosis

Infection with *Babesia* was associated with anaemia of complex aetiology (Schetters *et al.*, 1997).

Chandoga *et al.* (2002) mentioned that babesiosis was a cosmopolitan disease, restricted only by the availability of suitable tick vectors.

Babesia gibsoni was an intra-erythrocytic, tick-transmitted protozoan that caused clinical babesiosis in dogs characterized by remittent fever, thrombocytopenia, severe anaemia and sometimes death (Fukumoto *et al.*, 2005).

Babesia gibsoni was a haemoprotozoan parasite reported to cause clinically significant haemolytic anaemia in dogs (Inokuma *et al.*, 2005).

Canine babesiosis was a common tick borne haemoprotozoan disease of domestic dogs in which the destruction of the parasitized erythrocytes was the common consequence leading to anaemia (Chaudhuri *et al.*, 2008).

2.2.2.1.3.1 Clinical Signs

Recovery from babesiosis was associated with a dramatic reduction of spleen size with accompanying restoration of PCV values. Blocking of the microcirculation in spleen appeared to be the main cause of splenomegaly with associated anaemia with retention of erythrocytes in the spleen in babesiosis (Schetters *et al.*, 1997).

The clinical signs of canine babesiosis included fever, anaemia, pale mucous membranes, icterus, haemoglobinuria, lymph node enlargement, splenomegaly, tachycardia, tachypnoea, shallow breathing, weak pulse and rarely convulsions (Chandoga *et al.*, 2002).

Fukumoto *et al.* (2005) mentioned the presence of transplacental transmission of *B.gibsoni* which caused fatal congenital babesiosis in dogs.

Inokuma *et al.* (2005) mentioned that the clinical and laboratory findings of *B. gibsoni* and *B. canis* were similar, but *B. gibsoni* infection was more difficult to treat than *B. canis* and relapses occurred weeks or months after apparent recovery from *B. gibsoni* infection.

De Gopegui *et al.* (2007) classified canine babesiosis clinically as either uncomplicated or complicated. In uncomplicated babesiosis, clinical signs were due to haemolytic anaemia which included fever, anaemia, tachypnoea, tachycardia, splenomegaly, icterus and depression. Complicated babesiosis was characterized by additional organ involvement such as renal failure, hepatopathy, respiratory distress, myocardial lesions and central nervous system signs.

Dogs suffering from babesiosis showed clinical signs of anorexia, pyrexia, depression, pale mucous membranes, weight loss, weakness, vomiting, hepatomegaly and/ or splenomegaly (Chaudhuri *et al.*, 2008).

The classical presentation of canine babesiosis was a febrile syndrome with apparent anaemia (Schetters *et al.*, 2009).

2.2.2.1.3.2 Clinical Pathology

2.2.2.1.3.2.1 Haematological Changes

Conrad *et al.* (1991) reported haemolytic anaemia in *B. gibsoni* infected dogs, which was attributed to intravascular haemolysis.

PCV values in *B.canis* infections were influenced by changes in the plasma volume, retention of erythrocytes in the spleen and erythrocyte destruction which was due to parasite proliferation and removal in the spleen during the period of erythrocyte retention (Schetters *et al.*, 1997).

Chandoga *et al.* (2002) confirmed haematological changes like erythropenia, leucopenia, eosinopenia, thrombocytopenia, low haematocrit, poikilocytosis and a shift of neutrophils to the left.

Inokuma *et al.* (2005) demonstrated marked polychromasia, anisocytosis and nucleated erythrocytes in blood smears of *B.gibsoni* infected dogs.

De Gopegui *et al.* (2007) opined that in the absence of thrombocytopenia, babesiosis was an unlikely diagnosis.

Matijatko *et al.* (2007) reported that severe thrombocytopenia and haemolytic anaemia were well known haematological abnormalities in babesiosis and that these values showed a gradual increase during treatment.

An increase of MCHC above the normal in cases of babesiosis suggested anaemia caused by intravascular haemolysis and decrease of MCHC below the reference value was associated with polychromasia and probably with extravascular haemolysis (Zygner *et al.*, 2007).

Anaemic changes with lowered haemoglobin and PCV concentrations were reported in *B gibsoni* infections by Chaudhuri *et al.* (2008)

The possible mechanisms of low platelet counts in babesiosis were local and systemic DIC, immune-mediated destruction and sequestration of platelets in the spleen (Botsch *et al.*, 2009).

Schetters *et al.* (2009) mentioned a decrease in the haematocrit, total white blood cell count and platelet count in cases of canine babesiosis.

2.2.2.1.3.2.2 Changes in Serum Biochemistry

Chandoga *et al.* (2002) reported changes of hypoalbuminemia, increased activity of hepatic enzymes and hyperbilirubinemia in cases of canine babesiosis

The low levels of iron, zinc and copper observed in babesiosis seemed to have an additional role in the genesis of anaemia (Chaudhuri *et al.*, 2008).

Schetters *et al.* (2009) mentioned a clear trend of decreasing values of serum creatinine in infected dogs which was restored to normal after treatment.

2.2.2.1.3.3 Diagnosis

Breitschwerdt (1990) recommended the use of immunofluorescence assay (titres > 1: 40) for the diagnosis of both *B.canis* and *B.gibsoni.*

Specific diagnosis of canine babesiosis depended on detection of the parasites in the red blood cells of stained blood smears (Chandoga *et al.*, 2002).

Dvir *et al.* (2004) observed electrocardiographic changes of low R amplitude, prolonged QRS, S-T depression and coving, large T wave and notched R waves in dogs affected with babesiosis. The arrhythmias recorded in the same study were sinoatrial blocks or sinus arrest and ventricular premature complexes.

Usually the diagnosis of canine *B. gibsoni* infection was performed by microscopic examination of Giemsa-stained thin blood smear films in which a single peripheral RBC conatined only one to two parasites. PCR was also employed for the diagnosis (Fukumoto *et al.*, 2005).

Inokuma *et al.* (2005) demonstrated the presence of small bodies of *B.gibsoni* in a fine needle aspiration of the spleen.

Diagnosis of canine babesiosis was often made by piroplasm identification in stained peripheral blood smears (De Gopegui *et al.*, 2007).

Microscopic examination of Giemsa stained peripheral blood smears confirmed *Babesia gibsoni* infection in the erythrocytes (Chaudhuri *et al.*, 2008).

2.2.2.1.3.4 Treatment and Control

Breitschwerdt (1990) mentioned that no drug had been completely effective in clearing *B.gibsoni* parasites from erythrocytes.

Chandoga *et al.* (2002) recommended the treatment of canine babesiosis by the administration of Diminazene (Berenil) at a dose of 3 mg/ kg body weight.

Inokuma *et al.* (2005) reported positive reactions to diminazene aceturate in cases of *B.gibsoni* infections.

Animals suffering from babesiosis received two intramuscular injections of imidocarb dipropionate solution on two consecutive days (Schetters *et al.*, 2009).

2.2.2.1.4 Microfilariosis

Radhika (1997) identified microfilaria of *Dirofilaria repens* as the causative agent of microfilariosis in Thrissur district.

Kramer *et al.* (2005) reported that *Dirofilaria immitis* harboured intracellular bacteria of the genus *Wolbachia* which played an important role in the pathogenesis of the filarial infection.

Sabu *et al.* (2005) conducted a study on dirofilariasis in dogs and identified *Dirofilaria repens* as the sole cause of canine microfilariosis in Kerala.

2.2.2.1.4.1 Clinical Signs

Tarello (2003) reported clinical signs of multifocal alopecia, erythema on head, neck and hind limbs in cases of *Dirofilaria repens* infection.

Jabina and Ajithkumar (2005) observed off-feed, fever, congested mucous membrane, vomiting and oedema of hind limbs and scrotum as major symptoms of *Dirofilaria repens* microfilaraemic dogs.

2.2.2.1.4.2 Clinical Pathology

2.2.2.1.4.2.1 Haematological Changes

Kumar (1980) attributed the cause of decreased erythrocyte count to the liver damage in microfilaraemic dogs.

Sharma and Pachauri (1982) noticed a non significant reduction in haemoglobin, total erythrocyte count and volume of packed red cells (PCV) in dogs infected with *Dirofilaria repens.*

Chakrabarthi and Choudhury (1983) found that microfilaria infected dogs had decreased erythrocyte count as well as haemoglobin content and increased eosinophil count.

Anaemia in canine filariosis was due to haemolysis as a result of destructive motility of microfilariae as suggested by Kitagawa *et al.* (1989).

Ananda and D'souza (2006) observed a non significant reduction in haemoglobin (12.61 ± 0.14 g/ dL), total erythrocyte count (7.28 ± 0.21 millions/ mm^3) and volume of packed red cells (PCV) and leucocytosis (15.86 ± 0.42 thousands/ mm^3), eosinophilia, lymphocytosis, neutropenia and thrombocytopenia (191.20 ± 15.14 thousands/ mm3) in dogs infected with *Dirofilaria repens.*

2.2.2.1.4.2.2 Changes in Serum Biochemistry

Tomodo (1962) studied the serum protein changes in canine filariosis and its correlation with liver dysfunction and noted that Dirofilaria infected dogs showed increased plasma globulin and decreased albumin levels.

Snyder *et al.* (1967) found that the total serum proteins and the globulin fractions were significantly higher in dogs with microfilaremia when compared to clinically normal dogs.

Keenan *et al.* (1978) attributed the cause of increased Aspartate amino transferase (AST) and Alanine amino transferase (ALT) values to liver damage in canine filariosis.

2.2.2.1.4.3 Diagnosis

Valsala and Bhaskaran (1974) diagnosed microfilariosis by examination of blood films and stained the smear with Wright's-Giemsa method to detect and identify microfilaria.

Saseendranath *et al.* (1986) studied the incidence of canine microfilariosis in Trichur district by wet film examination of peripheral blood.

Courtney and Zeng (2001) reported that the sensitivity of the concentration test and direct smear evaluation for diagnosis of microfilariae was similar.

Gringoli *et al.* (2001) recommended the modified Knott's techinique to count and identify the microfilariae.

Suprabha and Devada (2003) stated that microfilariae of *Dirofilaria repens* were sheathless, with a tapering head and long pointed tail.

2.2.2.1.4.4 Treatment and Control

Courtney and Zeng (2001) suggested a monthly prophylactic treatment against microfilariae with ivermectin @ 6 – 12 mcg/ kg.

Venco *et al.* (2004) recommended the use of ivermectin @ 6 mcg/ kg for prolonged periods of time as an adulticide therapy.

Soulsby (2005) reported that an oral administration of ivermectin @ 0.05-0.1 mg/ kg body weight was effective against microfilaremia.

Rishniw *et al.* (2006) reported the efficacy of ivermectin 50 mcg/ kg PO or SQ against canine microfilariae.

Ambily (2009) stated that ivermectin administered @ 200 mcg/ kg body weight subcutaneously once weekly for 2 weeks was effective against microfilariosis caused by *Dirofilaria repens*.

According to Simon *et al.* (2009) combined ivermectin and doxycycline treatment had microfilaricidal activity in infected dogs due to activity against endosymbiont *Wolbachia*.

2.2.2.1.5 Trypanosomosis

According to De La Rue (2000), anaemia was one of the most consistent findings in animal *Trypanosomosis*.

Proposed mechanisms of anaemia in trypanososmosis included hemolysis as a result of erythrophagocytosis, hemodilution and depression of erythropoiesis (Aquino *et al.*, 2001).

Herrera *et al.* (2004) mentioned that anaemia was the main outcome of trypanosomosis.

Surra in dogs was characterized by high mortality and morbidity and anaemia was a consistent finding in infected dogs. The anaemia occurred due to

erythrophagocytosis or metabolic products and toxins liberated from the parasites (Gunaseelan *et al.*, 2009).

2.2.2.1.5.1 Clinical signs

Gross examination of all *Trypanosoma* infected dogs revealed lymphadenopathy and splenomegaly (Silva *et al.*, 1995).

De La Rue *et al.* (2000) mentioned clinical signs of intermittent fever, anaemia, urticaria, oedema of the legs, progressive weakness, loss of condition and inappetence in cases of *Trypanosoma evansi* infection in dogs.

Nabity *et al.* (2006) reported clinical signs of anorexia, lethargy, weight loss, fever, generalized lymphadenopathy and pitting oedema of the distal extremities in a dog with trypanosomosis..

Gunaseelan *et al.* (2009) observed clinical signs of pale conjunctival mucous membranes, increased temperature (101.8 – 107.4 ^{o}F), tachycardia (85 – 104/ min) and tachypnoea (15 – 28/ min) in a case of canine trypanosomosis.

2.2.2.1.5.2 Clinical Pathology

2.2.2.1.5.2.1 Haematological Changes

Erythrocyte abnormalities, trypanosome adhesion to red cells and erythrophagocytosis were important causes of anaemia in trypanosomosis.due to *T. evansi* in dogs (Silva *et al.*, 1995).

De La Rue *et al.* (2000) recorded erythrocyte counts of 5.6 ± 1.5 million/ mm^3 , reticulocyte percentage of 0.3 ± 0.4, leucocyte counts of 8.5 ± 6.4 thousands/ mm^3, neutrophils 72.1 ± 9.6 percent, band neutrophils 0.2 ± 0.8 per cent, eosinophils 7.1 ±7.1 per cent, basophils 0.0 ±0.0, lymphocytes 15.6 ±9.7 per cent and monocytes 5.3 ± 3.9 per cent in acute infection of dogs with *Trypanosoma evansi*.

Infected animals showed progressive decrease in red blood cell count and haemoglobin concentration, leading to anaemia. Leucopenia and neutropenia were also observed in canine trypanosomosis. (Aquino *et al.*, 2001).

Gunaseelan *et al.* (2009) recorded microcytic hypochromic anaemic changes (Hb – 9 g/ dL, RBC – 4.32 million/ mm^3, PCV – 28 per cent) in *Trypanosoma evansi* infected dog.

2.2.2.1.5.2.2 Changes in Serum Biochemistry

A significant increase in serum protein level, as a consequence of globulin rise, and a parallel decrease in albumin concentration were observed in infected dogs. The fall in albumin level was secondary to hyperglobulinemia as a compensatory mechanism for the maintenance of normal blood viscosity which was increased by high globulin levels (Aquino *et al.*, 2001).

Nabity *et al.* (2006) recorded a moderate hypoalbuminemia (1.5 g/ dL) in canine trypanosomosis.. This along with increased vascular permeability due to inflammation and decreased lymphatic drainage contributed to oedema development.

Gunaseelan *et al.* (2009) recorded hypoalbuminemia and hypoglycaemia in a case of canine trypanosomosis which were due to hepatic changes. They also postulated that trypanosomes utilized large amounts of glucose.

2.2.2.1.5.3 Diagnosis

De La Rue *et al.* (2000) mentioned the presence of erythrocyte changes such as microspherocytes and acanthocytes and haemolysis due to the direct traumatic effect of *Trypanosoma.*

Herrera *et al.* (2004) was of the opinion that diagnosis of *T.evansi* was mainly based on wet film examination, blood smear examination or the

microhaematocrit test. Other techniques like PCR and immunofluorescent test were also used.

Nabity *et al.* (2006) suggested the diagnosis of Trypanosome organisms by buffy coat examination.

The diagnosis of *Trypanosoma evansi* was done on the basis of observing the organisms on the blood smear stained with Leishman-Giemsa stain (Gunaseelan *et al.*, 2009).

2.2.2.1.5.4 Treatment and Control

According to Soulsby (2005), iso-metamidium chloride @ 0.5 to 1 mg/ kg body weight by deep intramuscular injection was effective against trypanosomosis. and diminazene aceturate was less active against *T.evansi* in dogs.

Barr (1990) and Lappin (2000) mentioned that nifurtimox was successful in treating canine trypanosomosis. and improved survivial occurred in dogs treated concurrently with anti-inflammatory doses of glucocorticoids.

Dakshinkar and Bhojne (2001) reported recurrence of infection after treatment with diminazene aceturate and attributed it to a resistant strain of *Trypanosome.* However Saravanan *et al.* (2005) reported that this medicine was very effective in eliminating the trypomastigotes in dogs affected with trypanosomosis..

2.2.2.2 Immune – mediated Haemolytic Anaemia

Klag *et al.* (1993) postulated that IMHA was one of the most common immunohaematologic disorders in dogs and was classified as primary (idiopathic) or secondary when it was secondary to various other conditions.

Autoimmune haemolytic anaemia was characterised by the binding of autoantibodies to erythrocyte membrane antigens leading to a decreased red blood cell life-span (Corato *et al.*, 1997).

According to Mathes *et al.* (2006) most cases of IMHA were idiopathic and life -threatening in dogs and death occurred due to severe anaemia. They also reported that recent vaccination triggered the disease onset in some animals.

Goggs *et al.* (2008) defined Evans' syndrome as concurrent or sequential IMHA and IMT without a known underlying cause and suggested vaccination as a trigger for the development of IMHA.

Weiss (2008) concluded that Immune – mediated haemolytic anaemia (IMHA) was thought to result from antibody mediated destruction of erythrocytes in the blood.

Evans' syndrome was an uncommon, life-threatening haematological disease of dogs, characterized by the co-occurrence of immune-mediated haemolytic anaemia (IMHA) and immune-mediated thrombocytopenia (IMT) and carried a poor short-term prognosis, with a mortality rate of upto 80 per cent (Bianco and Hardy, 2009).

Botsch *et al.* (2009) reported that 25 per cent of dogs with AIHA had been vaccinated within one month of the onset of disease and suggested an association between AIHA and vaccination.

2.2.2.2.1 Clinical Signs

The most common clinical signs at admission were anorexia, lethargy, pallor, weakness, icterus, splenomegaly and/ or hepatomegaly (Klag *et al.*, 1993).

The animals with Immune-mediated haemolytic anaemia showed an acute onset of signs such as haemolytic and regenerative anaemia, icterus, anorexia or haematuria at presentation (Inokuma *et al.*, 2005).

Goggs *et al.* (2008) mentioned the clinical signs of lethargy, inappetence, pallor and hepatosplenomegaly in cases of IMHA with concurrent IMT.

2.2.2.2.2 Clinical Pathology

2.2.2.2.2.1 Haematological Changes

In a study conducted by Klag *et al.* (1993) affected dogs were anaemic (PCV 8 to 28 per cent) with a mean PCV of 15.2 ± 5.1 per cent and severely thrombocytopenic (12 000 to 1 34 000/ mm^3).

Goggs *et al.* (2008) reported an overestimation of MCV values by the automated measurement of microagglutinated red cells.

Bianco and Hardy (2009) detected severe thrombocytopenia, severe anaemia and spherocytosis and hyperbilirubinemia in a case of Evans' syndrome.

Botsch *et al.* (2009) mentioned that dogs with Evans' syndrome had lower platelet counts ($P < 0.001$), a higher prevalence of anemia ($P < 0.001$) and monocytosis ($P < 0.001$).

Breuhl *et al.* (2009) reported spherocytes as 3+ if 51 to 150 spherocytes were noted per 1000x field, 2+ if 11 to 50 were noted per 1000x field, and 1+ if 1 to 10 were noted per 1000x field.

2.2.2.2.2.2 Changes in Serum Biochemistry

Klag *et al.* (1993) reported serum bilirubin concentrations ranging from 0.2 to 11.5 mg/ dL, with a mean of 10.4 mg/ dL and a median of 2.2 mg/ dL in dogs affected with IMHA.

Shaw and Harrell (2008) reported elevated liver enzyme activities and serum bilirubin concentrations in cases of IMHA in dogs.

Breuhl *et al.* (2009) recorded the presence of hyperbilirubinemia and hypoalbuminemia in cases of IMHA in dogs.

2.2.2.2.3 Diagnosis

Klag *et al.* (1993) opined that cases of autoagglutination were thought to represent a severe form of IMHA and that the degree of reticulocytosis, PCV and serum bilirubin concentrations appeared to be useful prognostic indicators.

Clinical diagnosis of IMHA included presence of regenerative anaemia, spherocytosis, in-saline agglutination plus the absence of intercurrent diseases such as renal disease or cancer (Mathes *et al.*, 2006).

The diagnosis of IMHA was based on the presence of anaemia (haematocrit less than 37 per cent) with a positive in-saline agglutination test and moderate to marked spherocytosis on a blood smear (Goggs *et al.*, 2008).

Weiss (2008) mentioned the criteria for the diagnosis of IMHA in dogs as a haematocrit < 30 per cent, a positive direct Coombs test or autoagglutination of erythrocytes or the presence of > 30 per cent spherocytes.

Bianco and Hardy (2009) diagnosed a case of primary Evans' syndrome on the basis of finding a severe anaemia and thrombocytopenia, presence of spherocytosis on blood smear, exclusion of other causes of anaemia and thrombocytopenia and response to immunomodulatory therapy.

2.2.2.2.4 Treatment and Control

Corato *et al.* (1997) recommended treatment of IMHA with immunosuppressive therapy using prednisolone @ 2 mg/ kg daily.

The target haematocrit after transfusion in a haemolytic anaemia patient was typically in the low 20's (Rozanski and De Laforcade , 2004).

The two primary forms of treatment of IMHA were splenectomy or high-dose corticosteroid therapy (Mathes *et al.*, 2006).

Bianco and Hardy (2009) suggested that human intravenous immunoglobulin and leflunomide were beneficial as alternative immunomodulatory therapy for dogs with Evans' syndrome, where glucocorticoids were contraindicated.

Breuhl *et al.* (2009) recommended the administration of unfractionated heparin at an initial dosage of 300 IU/ kg *q* 6 hours so as to prevent the thromboembolic events.

2.2.3 Acute Blood Loss Anaemias

In dogs, traumatic haemorrhage frequently resulted from intra-abdominal (liver/ spleen) damage (Rozanski and De Laforcade, 2004).

Aronsohn *et al.* (2009) recorded the presence of ventricular arrhythmias in cases of haemoabdomen and mentioned that any dog presented with hemoabdomen had a life-threatening disease process.

2.2.3.1 Clinical Signs

Dogs with hypovolaemic anaemia were very weak, and had rapid and faint pulses, and their mucous membranes appeared whiter than their haematocrit suggested (Rozanski and De Laforcade, 2004).

Clinical signs associated with acute hemoperitoneum were related to hypovolemic shock and included weakness or collapse, pale mucous membranes, delayed capillary refill time, tachypnea, dyspnea, and tachycardia (Aronsohn *et al.*, 2009).

2.2.3.2 Clinical Pathology

2.2.3.2.1 Haematological Changes

Lanevschi and Wardrop (2001) mentioned that in acute haemorrhage, packed cell volume (PCV) was a poor indicator of the degree of blood loss and the PCV dropped gradually over the 72 hours following the initiating incident, as extravascular fluid was redistributed and entered the intravascular space.

Murphy and Warman (2007) opined that in cases of haemoabdomen the peripheral PCV remained normal until there was redistribution of fluid into the vascular space.

2.2.3.2.2 Changes in Serum Biochemistry

Murphy and Warman (2007) reported a decreased serum protein value in cases of haemoabdomen.

2.2.3.3 Diagnosis

Murphy and Warman (2007) suggested that if the PCV of the abdominal fluid was similar to peripheral blood that indicated a recent haemorrhage.

Diagnostic tests for acute haemoperitoneum included complete blood count (CBC), serum biochemical and plasma clotting profiles, abdominocentesis, thoracic and abdominal radiography, blood pressure measurements, electrocardiogram (ECG), and possibly abdominal ultrasonography (Aronsohn *et al.*, 2009).

2.2.3.4 Treatment and Control

Lanevschi and Wardrop (2001) proposed that whole blood was indicated in a patient that required several blood components or had acutely lost more than 50 % of its total blood volume, in order to replace oxygen-carrying capacity, oncotic activity and to expand the blood volume.

Jutkowitz *et al.* (2002) suggested that acute blood loss such as in cases of haemoabdomen was one of the most common indications for transfusion.

Hypovolaemic patients required aggressive volume resuscitation as well as blood transfusion and medical management of traumatic haemoabdomen included the application of a tight abdominal wrap including the entire abdomen (Rozanski and De Laforcade , 2004).

Treatments of acute haemoperitoneum included management of hypovolemic shock, blood replacement therapy, external abdominal counter-pressure, and surgical intervention (Aronsohn *et al.*, 2009).

2.3 ANAEMIA OF CHRONIC DISEASE

Finora (2003) opined that the most signifiacant and commonly seen paraneoplastic syndromes were anaemia, hypercalcemia, hypoglycaemia and cancer cachexia.

Hohenhaus (2003) mentioned that anaemia in cancer patients resulted from haemorrhage, haemolysis and bone marrow failure.

Lymphoma (malignant lymphoma, lymphosarcoma) was a common neoplastic condition in the dog and was one of the more treatable cancers in small animal medicine (Dobson, 2004).

Lucas *et al.* (2004) mentioned that lymphosarcoma was the most common hematopoietic neoplasm of dogs, and represented 83 per cent of all hematological malignancies.

Dobson *et al.* (2006) reported that neoplasia of the haematopoietic system was common in dogs and lymphoma occurred more frequently than leukaemia.

Kawarai *et al.* (2006) reported that canine hepatocellular carcinoma (cHCC) was uncommon and accounted for less than 1 per cent of all canine tumours, and had a poor prognosis.

2.3.1 Clinical Signs

Vail (2000) observed a generalised lymphadenopathy in cases of canine lymphoma.

Anaemia of chronic disease was a common neoplastic syndrome seen in the veterinary patient (Finora, 2003).

Dobson (2004) reported that the clinical stage of the tumour appeared to have some bearing on outcome. He stated that dogs which showed clinical signs or complications carried a poorer prognosis than those which did not.

Most animals with leukaemia presented with vague, non specific clinical signs such as lethargy, weakness, inappetence, anaemia and weight loss which were attributed to the consequences of the disease process. The signs of acute leukaemia were more severe both in terms of degree and speed of onset (Dobson *et al.*, 2006).

According to Kawarai *et al.* (2006) the clinical signs of cHCC were nonspecific such as anorexia, weakness, and vomiting and weight loss. Ultrasonographic signs included hepatomegaly.

Presley *et al.* (2006) reported that mucous membranes of dogs having acute lymphoblastic leukaemia (ALL) often appeared pale at examination.

2.3.2 Clinical Pathology

2.3.2.1 Haematological Changes

Vail (2000) mentioned that finding a predominance of immature lymphoid cells on fine-needle aspirate cytology was suggestive of lymphoma in dogs and reported a normocytic, normochromic and nonregenerative anaemia.

As the primary disease process progressed, there was a decrease in iron metabolism and storage. Along with this an associated decrease in red blood cell life span and decreased response of the marrow to a low red blood cell count contributed to the anaemia which was of a normochromic, normocytic type (Finora, 2003).

Leukaemia was characterized by the presence of excessive numbers of abnormal neoplastic cells in both the peripheral blood and bone marrow and routine haematological assessment of a patient provided the first indication of leukaemia (Dobson *et al.*, 2006).

Kawarai *et al.* (2006) mentioned the presence of slight leucocytosis in cases of cHCC.

Presley *et al.* (2006) reported that anaemia was the most common abnormality occurring in cases of acute lymphoblastic leukaemia and was of a normocytic, normochromic and regenerative type. Severe leucocytosis with lymphocytosis, neutropenia and thrombocytopenia was generally observed.

2.3.2.2 Changes in Serum Biochemistry

Vail (2000) and Finora (2003) mentioned that cancer was the most common cause of hypercalcemia in animals with lymphoma and that a finding of hypercalcemia was a negative prognostic indicator.

Serum biochemistry reflected paraneoplastic complications such as hypercalcemia in cases of lymphoid leukaemia (Dobson *et al.*, 2006).

Kawarai *et al.* (2006) reported that the serum biochemical tests in cHCC revealed high levels of alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP) and high serum alpha-fetoprotein (AFP).

2.3.3 Diagnosis

The diagnosis of acute leukaemia was based on finding more than 30 per cent blast cells in the bone marrow, usually accompanied by similar cells in the peripheral blood (Dobson *et al.*, 2006).

Kawarai *et al.* (2006) opined that in dogs, re-expression of AFP was known to occur in some hepatic tumours and that serum AFP values were increased in most cHCC patients. Therefore for early diagnosis of cHCC, AFP was used as a tumour marker.

Weiss (2008) had defined a hypercellular bone marrow when more than 75 per cent of the haemopoietic space in unit particles or core biopsy specimens consisted of haemopoietic cells.

2.3.4 Treatment and Control

Hohenhaus (2003) opined that both solid tumours and haematopoietic tumours were associated with anaemia and needed transfusion of red blood cells.

According to Dobson (2004) the more complex protocols containing doxorubicin provided a better response in cases of canine lymphoma.

Jeffreys *et al.* (2005) reported that combination chemotherapy protocols using cyclophosphamide, vincristine, prednisone were superior to single-agent protocols for the treatment of canine lymphoma.

Dobson *et al.* (2006) advocated the use of standard lymphoma protocols especially containing doxorubicin for animals with acute lymphoblastic leukaemia.

Kaiser *et al.* (2007) proposed that the one common treatment regimen used for canine lymphosarcoma was the 'University of Wisconsin-Madison' (UW-M) 2-year chemotherapy protocol.

2.4 ANAEMIA SECONDARY TO RENAL DISEASE

The causes of acute renal disease included toxins, ischemia, infectious agents etc. (Cowgill and Elliott, 2000).

The anaemia observed in cases of acute renal failure was caused by gastrointestinal ulceration and haemorrhage or rarely, haemolytic uraemic syndrome (Kraje, 2002).

Erickson and Rubin (2007) suggested that anaemia in chronic renal failure was caused due to a shortened red blood cell life span, nutritional abnormalities, erythropoietin inhibitor substances in the plasma, blood loss, myelofibrosis in which erythropoietin deficiency was the major cause.

2.4.1 Clinical Signs

Kraje (2002) reported signs of vomiting, diarrhoea, halitosis, oral ulceration, anorexia, tachypnoea, dehydration, depression and hypothermia in cases of acute renal failure and long-standing weight loss, polyuria, polydipsia, vomiting and diarrhoea in cases of chronic renal failure.

Polzin (2007) observed the clinical signs of impaired appetite, lethargy, weakness, anaemia, vomiting, nausea, gastrointestinal haemorrhage, diarrhoea and haemorrhagic colitis.

Erickson and Rubin (2007) mentioned anaemia as a typical clinical sign in cases of chronic renal failure.

2.4.2 Clinical Pathology

2.4.2.1 Haematological Changes

Polzin *et al.* (2000) and Erickson and Rubin (2007) mentioned the laboratory finding of a progressive hypoproliferative anaemia mainly due to erythropoietin deficiency in cases of chronic renal failure.

The complete blood count revealed anaemia or a normal haematocrit and leucocytosis in cases of acute renal failure (Kraje, 2002).

2.4.2.2 Changes in Serum Biochemistry

Cotter (2000) recorded decreased concentrations of serum iron in chronic renal failure patients.

Typical serum chemistry profile included increased blood urea nitrogen and creatinine concentration and hyperphosphatemia in acute renal failure (Kraje, 2002).

Polzin (2007) observed a hyperphosphatemia, hypokalemia or hyperkalemia in cases of chronic renal failure.

2.4.3 Diagnosis

Cotter (2000) mentioned that the presence or absence of anaemia was a clue to identify whether renal failure was acute or chronic.

Kraje (2002) mentioned that diagnosis of acute renal failure was done on the basis of clinical signs, history, urinalysis, haematology, serum biochemistry and imaging techniques such as ultrasonography.

2.4.4 Treatment and Control

Pechereau *et al.* (1997) reported that recombinant human erythropoietin (rHuEPO) had been investigated extensively in the treatment of anaemia associated with chronic renal failure @ 22-66 units/ lb subcutaneously three times weekly.

Cotter (2000) recommended iron supplementation in chronic renal failure patients until the haematocrit was stabilised, due to the increased demands on the marrow for erythropoiesis.

Kraje (2002) recommended the treatment of acute renal failure by correcting fluid deficits and metabolic abnormalities, improving renal perfusion and combating infectious diseases if any.

Polzin (2007) mentioned that treating anaemia was indicated in a patient with chronic kidney disease when the haematocrit declined below 20 per cent and the patient had clinical signs of anaemia. He further opined that administering erythropoietin or darbepoietin alfa was the only effective means of correcting the anaemia of chronic renal disease.

2.5 BLOOD TRANSFUSION IN DOGS

Lewisohn (1916) was of the opinion that the citrate method of blood transfusion was a generally adopted method of blood transfusion because of the simple technique and perfect safety of its application.

Jutkowitz *et al.* (2002) noted that transfusions consisted of whole blood or blood components, such as packed RBC (PRBC) or fresh-frozen plasma (FFP).

Saini *et al.* (2002) mentioned that the first successful direct transfusion from one dog to another was performed in 1666 by Richard Lower.

Rozanski and De Laforcade (2004) were of the opinion that transfusion medicine was a vital part of veterinary emergency and critical care medicine and the indications included anaemia and coagulopathy.

Spinella and Halcomb (2009) had defined fresh whole blood as either whole blood at room temperature for less than 24 hours or refrigerated at 4 °C for less than 48 hours.

2.5.1 Indications

According to Jutkowitz *et al.* (2002) acute blood loss, haemolysis, coagulopathy and bone marrow failure were the most common indications for blood transfusion.

According to Saini *et al.* (2002) when the blood haemoglobin (Hb) level fell below 6 g/ dL, blood transfusion was indicated to maintain circulating Hb concentration at a level which provided adequate tissue oxygenation. They also recommended a blood transfusion when the PCV was 12 per cent or less.

Jutkowitz (2004) proposed that animals with acute anaemia with resulting haemoglobin values of less than 7 g/ dL (haematocrits of less than 21 per cent) frequently required transfusions.

Transfusion was considered when clinical signs attributable to anaemia such as tachypnea, tachycardia, weakness were present (Rozanski and De Laforcade, 2004).

2.5.2 Blood Grouping and Cross Matching

Knottenbelt and Mackin (1998a) and Feldman (1999) mentioned that eleven different blood group systems with eight types had been identified in dogs and the most clinically important types were the dog erythrocyte antigen (DEA) 1.1, 1.2, and 7. Out of these, DEA 1.1 was considered the most significant.

According to Lanevschi and Wardrop (2001) the crossmatching test did not identify the blood group but, instead, detected serological incompatibility between a donor and the patient and reasons for testing included the need to avoid acute haemolytic reactions during or following transfusion, an assurance of an optimal lifespan of the transfused RBCs, the prevention of incompatible blood transfusions in the future, and the prevention of neonatal isoerythrolysis.

Saini *et al.* (2002) concluded that since blood typing was not feasible in general veterinary practice, crossmatching was the most practical approach to reduce the risk of transfusion in previously sensitized patients. They also found that in dogs, the first transfusion was relatively safe because of lack of naturally

occurring alloantibodies and were unlikely to experience an acute incompatibility reaction after first transfusion.

According to Hohenhaus (2003) a cross match or blood type was not typically performed before a first transfusion because dogs had a low incidence of naturally occurring alloantibodies but a cross match was done if more than 4 days elapsed between transfusions.

2.5.3 Collection and Storage Of Blood

It was very important that a large size needle should be used to collect blood from the donor because citrate and blood mixed only after the blood had left the needle and the use of a needle with a narrow lumen did not permit a free flow and the blood clotted before it mixed with the citrate solution.(Lewisohn, 1916)

Henry (1922) suggested the flushing of the collection tube and blood collection bottle with citrate solution to avoid the chances of preliminary clotting.

Lanevschi and Wardrop (2001) mentioned that citrate was an anticoagulant that did not contribute to cell preservation during long-term storage and whole blood collected using citrate should be used within 24 hours.

Saini *et al.* (2002) proposed that Acid-citrate-dextrose (ACD) and citrate-phosphate-dextrose-adenine (CPDA) were the most important anticoagulant preservatives which were commonly used and CPDA was preferred to ACD because it offered enhanced storage life. They also found that if blood was collected in glass bottles it deactivated platelets, factor XII and factor VIII.

Reine (2004) recommended a physical examination at each donation because many subtle clinical signs of infectious disease (i.e. fever, anaemia, joint swelling) were noticed and mentioned that screening of all dogs for infectious diseases and ectoparasites should be done.

2.5.4 Administration of Blood

Knottenbelt and Mackin (1998b) mentioned the blood volume to be transfused in mL in dogs to be equal to

$$\frac{90 \text{ X weight (kg) X (required PCV} - \text{recipient PCV)}}{\text{PCV of donated blood}}$$

Saini *et al.* (2002) stated that in dogs the estimated dose of whole blood was 10 – 20 mL/ kg body weight and that 20 mL/ kg body weight of whole blood increased the PCV by 10 per cent. The initial transfusion was slow to monitor adverse effects and after the first 30 minutes, approximately 10 mL/ kg/ hour was administered safely.

Hohenhaus (2003) suggested that the blood was delivered at a rate fast enough to complete the transfusion within 4 hours to reduce the potential for bacterial overgrowth.

Jutkowitz (2004) opined that blood was not administered with any fluid other than 0.9 per cent sodium chloride and blood was given at an initial rate of 0.25 mL/ kg for the first 30 minutes and then increased to 10 to 20 mL/ kg/ hour.

2.5.5 Hazards of Blood Transfusion

Knottenbelt and Mackin (1998b) advised the treatment of anaphylaxis after blood transfusion with corticosteroids, antihistamines and adrenaline in severe cases.

Lanevschi and Wardrop (2001) documented 2 types of adverse reaction, an immediate reaction that occurred during or within 1 to 2 hours following transfusion or a delayed reaction that occurred days, months or years later.

Jutkowitz *et al.* (2002) recorded transfusion reactions such as transient fever, vomiting, facial swelling and delayed haemolysis which were mild and self limiting in all cases.

Transfusion reactions could be immune mediated or non-immune mediated and immune mediated reactions could be haemolytic or non-haemolytic (Saini *et al.*, 2002).

Hohenhaus (2003) expressed that immediately before the transfusion, heart rate, respiratory rate and body temperature was recorded and thereafter in every 15 and 30 minutes during the transfusion to detect changes that indicated a transfusion reaction.

The anticoagulant citrate acted by binding calcium and toxicity led to significant hypocalcemia resulting in tremors or hypotension. In such cases infusion of calcium gluconate was warranted @ 30 mg/ kg intravenously over 2 – 5 minutes (Rozanski and De Laforcade, 2004).

Materials and Methods

3. MATERIALS AND METHODS

The study was conducted at Department of Clinical Medicine, College of Veterinary and Animal Sciences, Mannuthy, over a period of three semesters from January 2009 to March 2010. Dogs presented at the College Veterinary Hospital, Mannuthy and University Veterinary Hospital, Kokkalai with clinical signs suggestive of anaemia were selected and used for the study.

3.1 SELECTION OF ANIMALS

Twenty two dogs, which had clinical signs suggestive of anaemia, were chosen for the study.

The cases of anaemia were classified as

Group I - Chronic blood loss anaemia

Group II - Haemolytic anaemia

Group III - Acute blood loss anaemia

Group IV - Anaemia associated with chronic diseases

Group V - Anaemia secondary to renal disease

All the parameters under the study were carried out in six apparently healthy dogs to establish normal values.

3.2 OUTLINE OF STUDY

3.2.1 Clinical Examination

Anamnesis, signalment and detailed clinical examination of the patients were conducted as described by Radostits (2000).

3.2.2 Examination of Clinical Materials

Relevant clinical materials like peripheral wet film, blood smear, faecal sample, bone marrow aspirate, splenic aspirate were collected and detailed examinations were done to elucidate the cause of anaemia.

3.2.2.1 Screening of Blood Smears

Thin smears were prepared by collecting one drop of peripheral blood from the ear tips of the animals on clean grease free glass slides and stained using Giemsa stain. Smears were examined under the oil immersion objective of a light microscope to detect the presence of blood parasites.

3.2.2.2 Screening of Faecal Samples

Faecal samples were thoroughly emulsified with water using a mortar and pestle, then sieved and the filtrate was collected and centrifuged at 3000 rpm for five minutes. The sediment was examined under the low power objective of a light microscope for the presence of ova of parasites.

3.2.2.3 Screening of Splenic Aspiration Smears

In selected cases with splenomegaly, the area over the spleen was prepared aseptically. Using a sterile 21 gauge needle and syringe, splenic aspirates were taken and smeared onto grease free clean glass slides to make thin smears. These were stained using Giemsa stain and observed under the oil immersion objective of a light microscope to detect the presence of haemoparasites.

3.2.2.4 Examination of Bone Marrow Aspirates

Bone marrow aspiration was performed in selected cases as per the method described by McGuire (1991). Bone marrow was aspirated under local anaesthesia from the dorsal crest of wing of ilium by the use of Jamshidi needle. The aspirated bone marrow was then subjected to cytology to elucidate the possible cause and prognosis of anaemia.

3.2.2.5 Electrocardiography

Dogs were subjected to detailed electrocardiographic examination using BPL – CARDIART – 6108^{R} machine with 25 mm/ s paper speed and one sensitivity to evaluate the cardiac changes which might be a cause or an effect of anaemia.

3.2.2.6 Ultrasonography

Dogs were subjected to detailed abdominal ultrasound scanning using DC-2000-VET machine to identify the presence of any kind of organ abnormalities.

3.3 CLINICAL PATHOLOGY

3.3.1 Collection of Clinical Materials

Blood was collected in sterile vacuttainers (Kollect K_3 EDTA^{R}) by puncturing the cephalic or saphenous vein. Potassium ethylene di-amino tetra acetic acid was used as anticoagulant. Serum was separated by using the Kollect Plain vacuttainer tubes.

Blood smear for differential leucocyte count (DLC) was prepared on a clean and dry glass slide with a drop of blood collected from the ear tip and stained with Wright-Giemsa stain.

Blood for evaluation of autoagglutination and clotting time was taken from the ear tip. A drop of blood was taken on a clean glass slide and equal quantity of normal saline added to it and spread and evaluated for the presence of macroscopic agglutination. A coverslip was placed on the slide and also observed for microagglutination.

The clinical materials were collected both on the first day and seventh day for evaluating anaemia and assessing clinical response to therapy.

3.3.2 Examination of Clinical Materials

3.3.2.1 Evaluation of Haematological Parameters

Parameters viz; Volume of packed red cells (VPRC), haemoglobin (Hb), total erythrocyte count (TEC), platelet count, total leucocyte count (TLC), differential leucocyte count (DLC), reticulocyte count, mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were estimated by using the BV-4100 FULLY AUTO HAEMATOLOGY ANALYSER^{R}.

3.3.2.2 Serum Biochemistry

Total serum protein, albumin, globulin, AG ratio, creatinine, total bilirubin and direct bilirubin were estimated by spectrophotometry in MERCK MICROLAB – 200^{R}.

Serum total protein was estimated by direct Biuret method described by Gomall *et al.* (1949) whereas serum albumin was estimated by Bromocresol green methodology described by Doumasa *et al.* (1971). Total and direct bilirubin were estimated by modified DMSO method described by Walter and Gerard (1980) and serum creatinine was estimated by modified Jaffe's method as described by Allen *et al.* (1982).

3.4 TREATMENT OF ANAEMIC CASES

After detailed investigations, necessary steps were adopted in the therapeutic regimen. Suitable therapy was adopted depending on the etiological factors. The drugs indicated in the present study were:

3.4.1 Group I – Dogs with Haemoparasites

1. Oxytetracycline – Intravenous administration of oxytetracycline @ 10 mg/ kg body weight once a day for a period of five days followed by

2. Doxycycline – Oral administration of doxycycline @ 10 mg/ kg body weight once a day for a period of ten days was done in cases of ehrlichiosis, mycoplasmosis and some cases of microfilariosis.

3. Diminazene aceturate – Deep intramuscular administration of diminazene aceturate was indicated in cases of babesiosis. Clinical cure was observed after a single injection given @ 3.5 - 5 mg/ kg body weight.

4. Isometamidium chloride – Deep intramuscular injection of isometamidium chloride was done in cases of trypanosomosis @ 0.5 mg/ kg body weight once.

5. Ivermectin – Ivermectin was given @ 50 or 100 mcg/ kg body weight orally or subcutaneously in cases of microfilariosis.

3.4.2 Group II – Dogs with Gastrointestinal Parasites

1. Fenbendazole – Fenbendazole was given orally @ 50 mg/ kg body weight for three consecutive days in cases positive for ancylostomosis.

3.4.3 Group III – Dogs with Ectoparasites

1. Ivermectin – Ivermectin was administered @ 200 mcg/ kg body weight subcutaneously in cases of tick and flea infestation.

3.5 BLOOD TRANSFUSION

Whole blood was administered in severely anaemic cases in all the above groups. The decision to perform blood transfusion was based on the clinical condition of the animal as well as the haematological values.

3.5.1 Cross matching

Both major and minor cross matching of donor and recipient blood was performed prior to every blood transfusion according to the method described by Michell *et al.* (1989).

3.5.2 Blood Transfusion

The quantity of blood to be transfused was calculated using the formula

Blood volume to be transfused (in mL) =

$$90 \times \text{Weight (kg)} \times \frac{\text{(Required PCV} - \text{Recipient PCV)}}{\text{PCV of donated blood}}$$

(Knottenbelt and Mackin, 1998b) (where required PCV was taken as 30 per cent) Alternately, blood was transfused @ 10 mL/ kg body weight of recipient.

The required quantity of whole blood was collected from the healthy donors by jugular venipuncture with the help of a 16G sterile needle. The blood was collected in new plastic sachets of normal saline using 3.8 per cent sodium citrate as anticoagulant. 3.8 per cent sodium citrate was used at the rate of 1 mL per 9 mL of whole blood. The collected blood was then transfused using sterile blood transfusion sets. The blood was administered at an initial rate of 0.25 mL/ kg for the first 30 minutes and then increased to 10 to 20 mL/ kg/ hour. The signs of transfusion reactions if any were monitored and managed accordingly.

3.6 INJECTABLE HAEMATINIC IRON DEXTRAN

Injectable iron dextran (Imferon^{R}) 1 mL IM every alternate day was administered in all the groups to evaluate the efficacy of iron dextran in the treatment of anaemia.

3.7 STATISTICAL ANALYSIS

Data obtained were analysed statistically as per Snedecor and Cochran (1980). The means of all the groups were compared with that of the apparently normal animals using analysis of variance (ANOVA).

Results

4. RESULTS

4.1 INCIDENCE OF ANAEMIA

In the present study 22 dogs showing clinical signs of anaemia were subjected to detailed clinical examination and all parameters under study such as signalment, history, physical examination, screening of clinical materials for the different aetiologies, haematology, serum biochemistry and treatment response were carried out. The data obtained were analyzed statistically as mentioned in Statistical Methods.

The clinical cases of anaemia were grouped into five.

Group I (n = 3) - Chronic blood loss anaemia

Group II (n = 12) - Haemolytic anaemia

Group III (n = 1) - Acute blood loss anaemia

Group IV (n = 5) - Anaemia associated with chronic diseases

Group V (n = 1) - Anaemia secondary to renal disease

4.1.1 Group I – Chronic Blood Loss Anaemia

Chronic blood loss anaemia was caused due to ecto- and endo- parasitism. Single case of tick infestation caused by *Rhipicephalus sanguineus*, flea infestation caused by *Ctenocephalides felis* and hookworm infestation caused by *Ancylostoma caninum* were recorded. The above cases were diagnosed by finding the presence of the ectoparasites on the body of the dogs and ova of *Ancylostoma caninum* on faecal sample examination. Chronic blood loss was caused by continuous sucking of blood by these parasites. Tick infestation, flea infestation and hookworm infestation therefore constituted 33.33 per cent each of this type of anaemia whereas chronic blood loss anaemia itself was responsible for 13.63 per cent of all the cases of anaemia in this study.

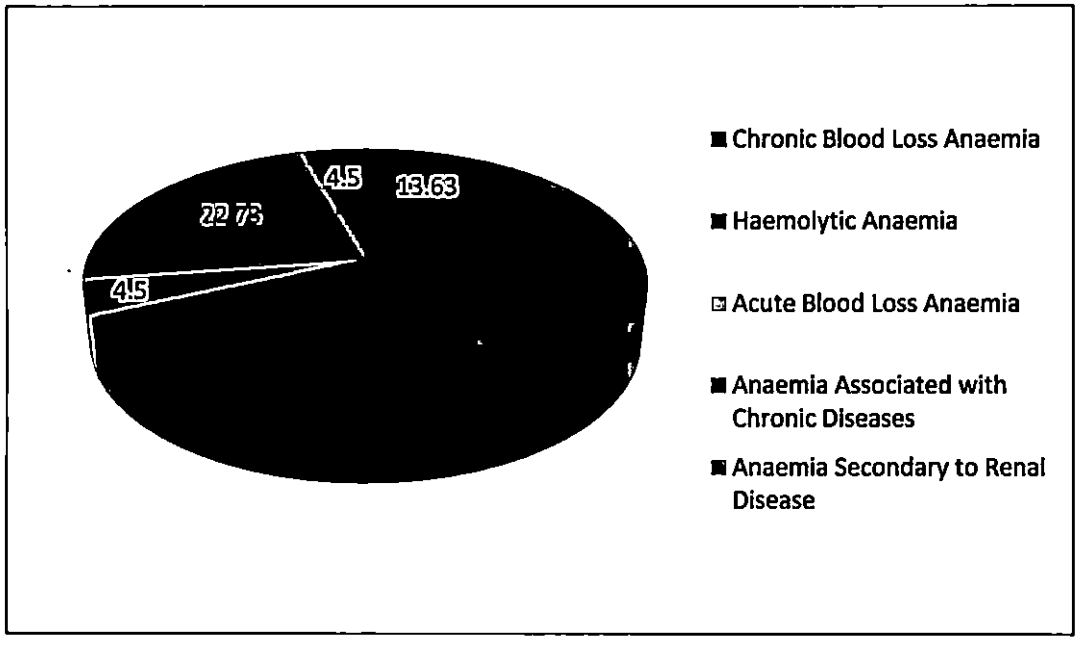


Fig. 1 Classification of anaemic cases

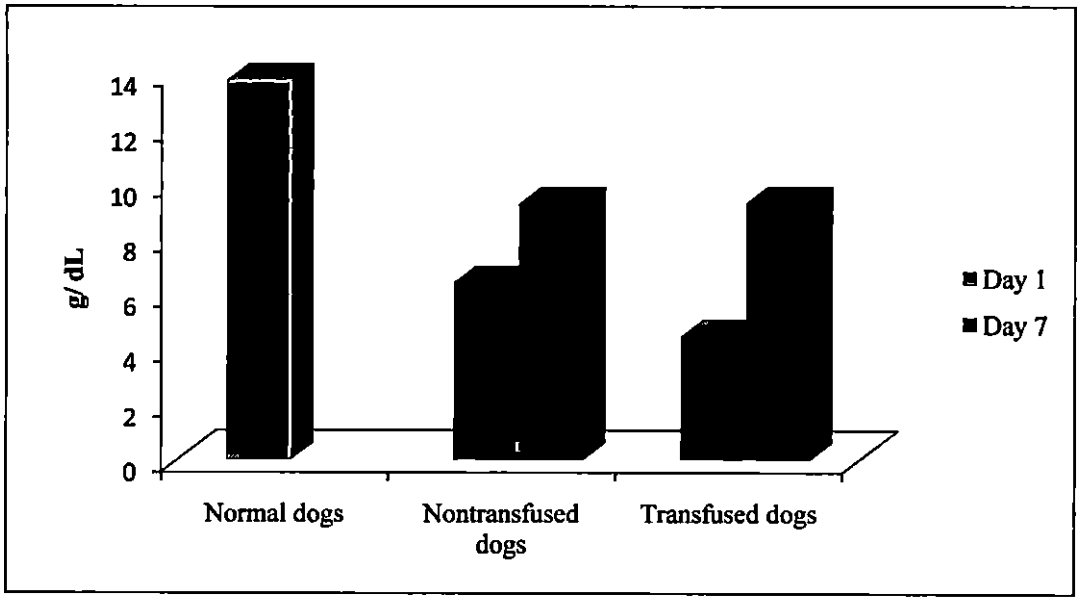


Fig. 2: Comparison of haemoglobin concentration on day 1 and day 7 in dogs transfused and not transfused with blood

4.1.2 Group II – Haemolytic Anaemia

Haemolytic anaemia was caused due to infectious agents and immune – mediated haemolytic disease. The various haemoparasites observed in the current study were *Ehrlichia*, *Dirofilaria*, *Trypanosoma*, *Babesia* and *Mycoplasma*. A total of 12 cases were recorded which included 4 cases of ehrlichiosis, 3 cases of microfilariosis, 2 cases of trypanosomosis and one case each of babesiosis and mycoplasmosis. Thus ehrlichiosis was found to be the predominant infection (33.33 per cent) causing haemolytic anaemia followed by microfilariosis (25 per cent), trypanosomosis (16.66 per cent), babesiosis and mycoplasmosis (8 per cent). A single case of Evans' syndrome was recorded which constituted 8 per cent of the cases. Altogether, haemolytic anaemia was responsible for 54.54 per cent of the anaemia cases.

4.1.3 Group III – Acute Blood Loss Anaemia

A single case of acute blood loss anaemia was recorded. The acute blood loss was caused by traumatic haemoabdomen. Acute blood loss anaemia contributed to 4.5 per cent of the total anaemic cases.

4.1.4 Group IV – Anaemia Associated with Chronic Diseases

Five cases of anaemia associated with chronic diseases were encountered. Out of these, 3 were neoplastic conditions. They were acute lymphoblastic leukaemia, lymphosarcoma and hepatocellular carcinoma. Two cases of liver dysfunction were also observed. The neoplasms and liver dysfunction were responsible for 13.63 per cent and 9.09 per cent of total anaemic cases encountered in the study. This group was responsible for 22.73 per cent of total anaemic cases.

4.1.5 Group V – Anaemia Secondary to Renal Disease

A single case of anaemia secondary to renal disease was recorded. The case was one of chronic renal failure. This group hence represented only 4.5 per

cent of the total anaemic cases studied. The percentage distribution of anaemic cases is shown in Fig. 1.

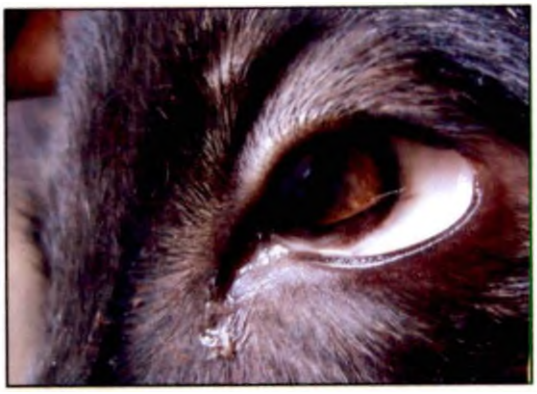
4.2 CLINICAL MANIFESTATIONS

In the majority of cases the striking features observed were pallor of visible mucous membranes (Plate 1a: A), tachycardia, rapid, strong and bounding pulses and exaggerated respiration with tachypnoea. Anorexia, lethargy, exercise intolerance and weakness were reported in most of the cases. Abdominal aortal thudding and heart sounds on auscultation of the trachea were also recorded in majority of the cases and were indicative of the severity of the anaemia. The clinical manifestations observed varied with the aetiology.

The cases of chronic blood loss anaemia showed relatively reduced intensity of the above mentioned signs. The case of tick infestation showed severe anaemic signs of pale mucous membranes, lethargy, anorexia, repiratory distress and presence of ticks on the body (Plate 1b: B). The case of flea infestation showed presence of flea faeces and additional signs of hindlimb weakness and a reluctance to get up (Plate 1b: C). However the mucous membranes in the present case were congested and the haematological values were also not indicative of anaemia. The animal was found to be dehydrated. However abdominal aortal thudding could be auscultated. The case of endoparasitism showed the presence of gastrointestinal signs such as diarrhoea with mucus and blood in faeces.

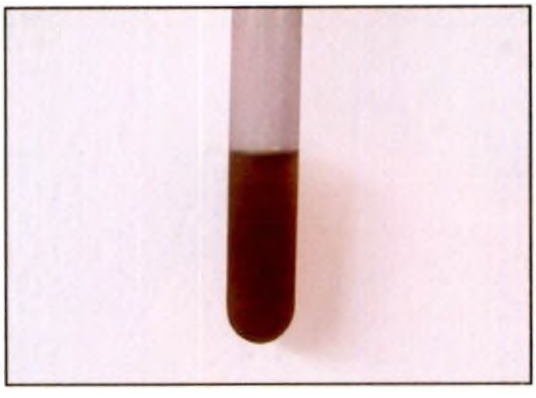
The important clinical signs noted in haemolytic anaemia were pale and/ or icteric mucous membranes, fever, enlargement of superficial lymph nodes, splenomegaly and anorexia. Haemoglobinuria was noted in the case of Evans' syndrome (Plate 1a: C). Unilateral expistaxis was observed in a case of trypanosomosis. Limb and scrotal oedema was observed in single cases of microfilariosis, trypanosomosis and ehrlichiosis (Plate 1a: D). A case of chronic ehrlichiosis also showed the presence of wheezing and respiratory exaggeration

Plate 1a: Clinical manifestations



A. Pale mucous membrane

B. Icteric sclera



C. Haemoglobinuria

D. Scrotal oedema

on thoracic auscultation. Severe splenomegaly was observed in cases of mycoplasmosis, babesiosis, trypanosomosis and acute lymphoblastic leukaemia.

The case of acute blood loss anaemia was presented in a recumbent stage and showed cyanosis of conjunctival mucous membranes along with exaggerated respiratory efforts. The pulse was weak and rapid and the capillary refill time was prolonged.

The cases of neoplasms were presented with typical clinical signs in addition to those of anaemia. Severe splenomegaly and severe enlargement of all palpable lymph nodes (Plate 1b: A) were observed in case of acute lymphoblastic leukaemia and lymphosarcoma respectively. The case of canine hepatocellular carcinoma was presented with non specific clinical signs. Icterus of mucous membranes and sclera (Plate 1a: B) and hepatomegaly were observed in cases of liver dysfunction along with anorexia and weakness.

The case of renal failure was presented with the signs of pale mucous membranes, tachycardia, weakness and anorexia. Signs suggestive of chronic renal failure such as vomiting, melena, polyuria and polydypsia along with a wooden, stilted gait were also observed.

4.3 CLINICAL PATHOLOGY

4.3.1 Haematology

The comparison of the haemogram values and platelet counts between the normal and the diseased groups before and after treatment are presented (Table 1 and 2). The comparison of the leukogram between the normal and the diseased groups before and after treatment are presented in Table 3 and 4.

4.3.1.1 Volume of Packed Red Cells (VPRC)

There was statistically significant variation in the mean values of VPRC of Group I (26.90 ±11.79 per cent), Group II (14.63 ±1.78 per cent) and Group V (18.00 ±5.00 per cent) with that of the normal group (40.00 ±0.50 per cent) before

treatment. The values recorded in the groups III and V which consisted of single cases were 14.70 percent and 8.70 percent respectively. After treatment the mean values for VPRC in Group I and Group II increased to 34.83 ± 7.76 per cent and 23.63 ± 2.10 per cent respectively which was less when compared to the normal group. The VPRC in Group V increased to 21.30 per cent. There were statistically significant differences in the VPRC before and after treatment in all treated animals. Statistically significant difference in the VPRC was also found after treatment between those animals transfused with blood and those that were not (Fig. 3 and Table 7).

4.3.1.2 Haemoglobin (Hb)

The mean Hb concentration of the normal and diseased groups before and after treatment varied significantly. The mean value for haemoglobin concentration of the normal group was 13.77 ±0.16 g/ dL. The mean values observed in the diseased groups before treatment were 9.23 ±3.65 g/ dL (Group I), 5.16 ±0.66 g/ dL (Group II) and 7.26 ±1.57 g/ dL (Group IV). The Hb values in Groups III and V were 4.40 g/ dL and 3.60 g/ dL respectively. After treatment the mean Hb values increased but did not reach the normal range and were recorded as 12.70 ±2.40 g/ dL (Group I) and 8.90 ±0.74 g/ dL(Group II) . The Hb concentration in Group V was 3.94 g/ dL. There was statistically siginficant difference in the Hb values before and after treatment in all the surviving animals. Statistically significant difference in the haemoglobin concentration was also found after treatment between those animals transfused with blood and those that were not (Fig. 2 and Table 7).

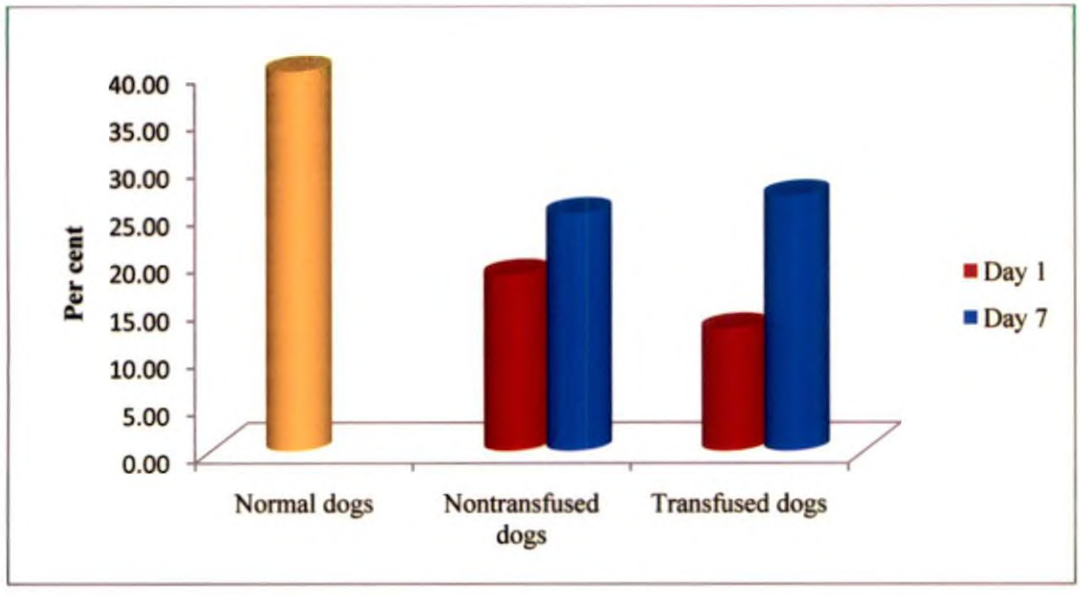


Fig. 3: Comparison of volume of packed red cells on day 1 and day 7 in dogs transfused and not transfused with blood

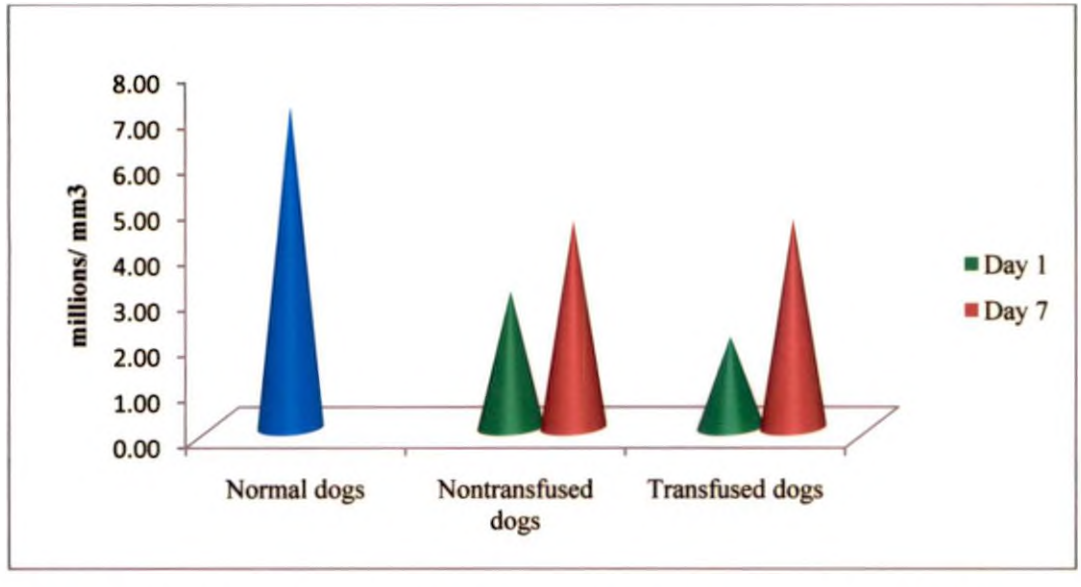


Fig. 4: Comparison of total erythrocyte counts on day 1 and day 7 in dogs transfused and not transfused with blood

Table 1: Comparison of haemogram of apparently normal and clinical cases before treatment (Mean ±S.E)

Parameters	Normal	GroupI	Group II	Group III	Group IV	Group V
VPRC (per cent)	40.00[a] ±0.50	26.90[b] ±11.79	14.63[b] ±1.78	14.70[b]	18.00[b] ± 5.00	8.70[b]
Hb (g/ dL)	13.77[a] ±0.16	9.23[b] ±3.65	5.16[b] ± 0.66	4.40[b]	7.26[b] ± 1.57	3.60[b]
TEC (millions/mm^3)	7.03[a] ±0.10	4.59[b] ±2.13	2.29[b] ±0.30	2.12[b]	2.50[b] ±0.82	1.65[b]
MCV (fl)	61.72 ±0.87	59.29 ±3.10	65.94 ±4.29	69.40	79.62 ±8.14	52.72
MCHC (g/ dL)	32.52 ±0.53	35.50 ±1.66	34.93 ±0.73	29.90	47.06 ±9.91	41.37
MCH (pg)	19.61 ±0.33	21.09 ±1.98	22.80 ±1.35	20.80	39.32 ±11.77	21.81
Platelet (lakhs/mm^3)	3.67[a] ± 0.11	2.34[b] ±1.03	0.95[b] ±0.20	2.10[b]	1.10[b] ±0.50	1.50[b]
Reticulocyte (%)	0.67[a] ±0.19	7.83[b] ±4.28	8.65[b] ±2.81		11.42[b] ±4.49	1.00

Means bearing different superscripts within a row differ significantly ($P \leq 0.05$)

4.3.1.3 Total Erythrocyte Count (TEC)

Statistically significant difference was noted in the mean total erythrocyte count between all the groups and normal animals before and after treatment. The mean value obtained for total erythrocyte count in the normal group was 7.03 ±0.1 millions/ mm^3. The TEC recorded in the various diseased groups were 4.59 ±2.13 millions/ mm^3 (Group I), 2.29 ±0.3 millions/ mm^3 (Group II) and 2.50 ±0.82 millions/ mm^3 (Group IV). Groups III and V recorded a TEC of 2.12 millions/ mm^3 and 1.65 millions/ mm^3 respectively. After treatment the total erythrocyte count increased to 6.31 ±1.81 millions/ mm^3 and 4.18 ±0.34 millions/ mm^3 in group I and II respectively. An erythrocyte count of 3.94 millions/ mm^3 was recorded in anaemia secondary to renal failure after treatment. Statistically significant difference in erythrocyte count before and after treatment was noticed in all the cases. Statistically significant difference in the total erythrocyte count was also found after treatment between those animals transfused with blood and those that were not (Fig. 4 and Table 7).

4.3.1.4 Erythrocyte Indices (MCV, MCH, MCHC)

The MCV in Group I (59.29 ±3.10 fl) and Group II (65.94 ±4.29 fl) were similar to the normal values of 61.72 ±0.87 fl. Group III (69.40 fl) and IV (79.62 ± 8.14 fl) showed an increase in MCV values whereas Group V (52.72 fl) showed a decrease in MCV value. After treatment there was a decrease in MCV values in Group II to 57.39 ±4.45 fl.

The MCHC values before treatment in the Group I (35.50 ±1.66 g/ dL), Group II (34.93 ±0.73 g/ dL) and Group III (29.90 g/ dL) were similar to the normal value of 32.52 ±0.53 g/ dL. An increase in the MCHC values of 47.06 ±9.91 g/ dL and 41.37 g/ dL was observed in groups IV and V. After treatment

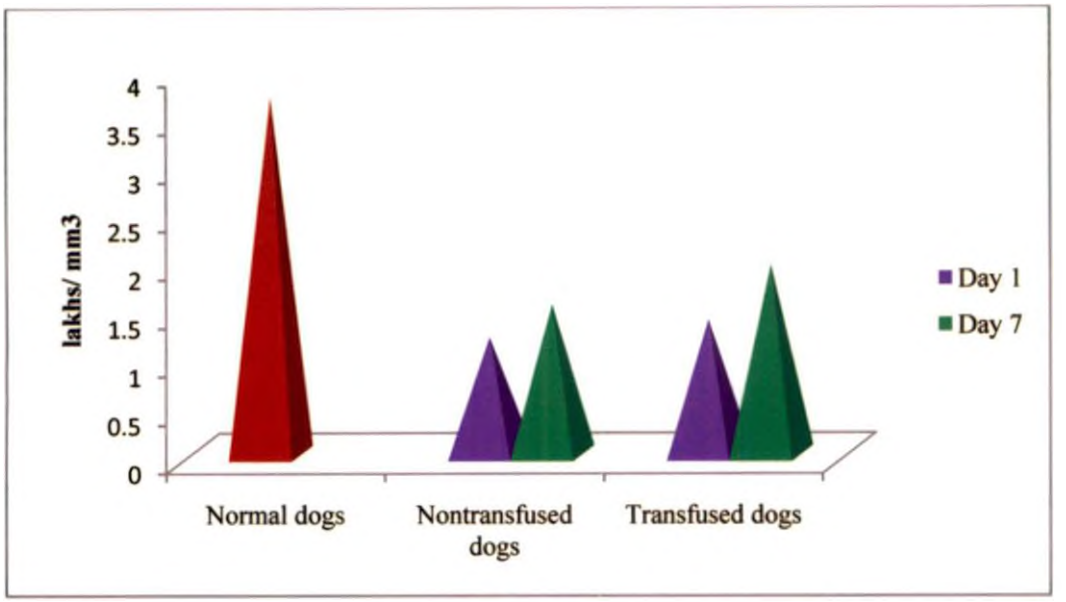


Fig. 5: Comparison of platelet counts on day 1 and day 7 in dogs transfused and not transfused with blood

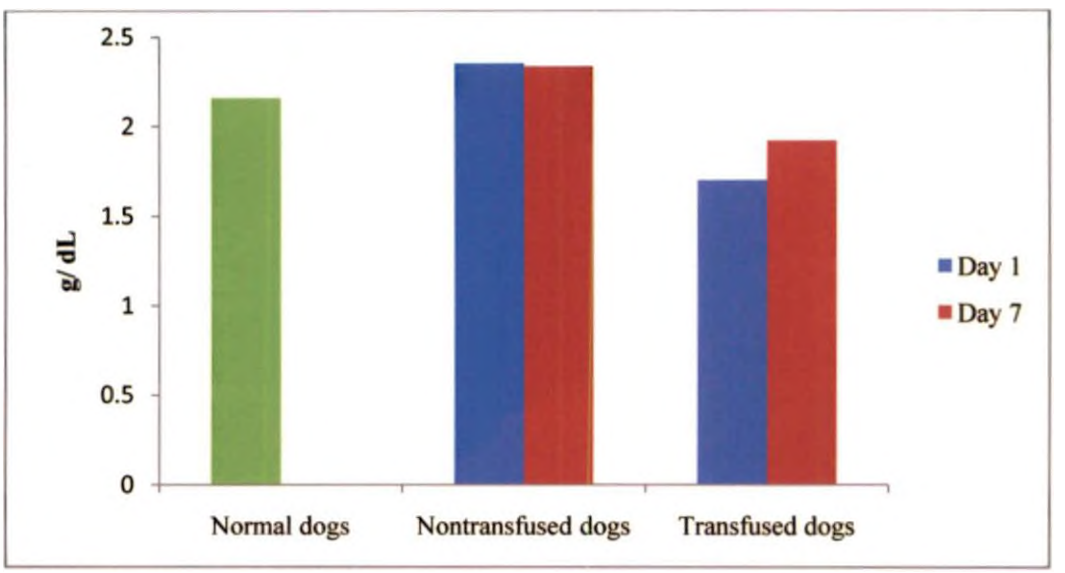


Fig. 6: Comparison of serum albumin concentration on day 1 and day 7 in dogs transfused and not transfused with blood

Table 2: Comparison of haemogram of apparently normal and clinical cases after treatment (Mean ±S.E)

Parameters	Normal	GroupI	Group II	Group V
VPRC (per cent)	40.00 [a] ±0.50	34.83 [b] ±7.76	23.63 [b] ±2.10	21.30 [b]
Hb (g/ dL)	13.77 [a] ±0.16	12.70 [b] ±2.40	8.90 [b] ± 0.74	3.94 [b]
TEC (millions/ mm^3)	7.03 [a] ±0.10	6.31 [b] ±1.81	4.18 [b] ±0.34	3.94 [b]
MCV (fl)	61.72 ±0.87	57.06 ±3.62	57.39 ±4.45	54.31
MCHC (g/ dL)	32.52 ±0.53	27.01 ±3.01	38.03 ±0.54	41.68
MCH (pg)	19.61 ±0.33	21.30 ±2.97	21.66 ±1.39	22.64
Platelet (lakhs/ mm^3)	3.67[a] ±0.11	3.03 ±1.10	1.32 [b] ±0.10	1.68 [b]
Reticulocyte %	0.67 ±0.19	3 ±1.36	4.11 ±1.73	0.90

Means bearing different superscripts within a row differ significantly ($P \leq 0.05$)

the MCHC values of Group I decreased to 27.01 ±3.01 g/ dL whereas that of Group II increased to 38.03 ±0.54 g/ dL. That of Group V remained the same.

The MCH value in Group IV (39.32 ±11.77 pg) was found to be much higher that the normal value of 19.61 ±0.33 pg. The values in all other groups were within the normal limits. After treatment no significant change was observed in these values in any of the groups.

4.3.1.5 Total Leucocyte Count (TLC)

The mean values of TLC recorded in group I (25.45 ±11.50 thousands/ mm^3), Group III (25.30 thousands/ mm^3) and Group IV (36.22 ±13.12 thousands / mm^3) were much higher as compared to normal values (10.15 ±0.61 thousands / mm^3) and showed significant difference. Groups II and V showed only slight variation of 14.23 ±1.74 thousands / mm^3 and 15.80 thousands / mm^3 respectively. After treatment the TLC values reduced slightly to 22.00 ±8.94 thousands / mm^3 and 13.45 ±1.40 thousands/ mm^3 in Groups I and II respectively. The TLC value in Group V was 16.50 thousands/ mm^3. No significant difference was found between the pre and post treatment values of TLC.

4.3.1.6 Differential Count

The mean value of neutrophil count in Group I (70.33 ±13.86 per cent), Group II (69.50 ±3.01 per cent) and Group IV (70.40 ±13.51 per cent) was found to be similar to the normal value of 71.00 ±1.52 . A neutrophilia was recorded in Group III (85 per cent) and Group V (83 per cent) before treatment. After treatment a reduction in these values was noted. Mean value of lymphocyte per cent in Group I, Group II and Group IV were 28.33 ±13.37 per cent, 25.75 ±2.94 per cent and 26.80 ±12.59 per cent respectively. Group III (13 per cent) and Group V (12 per cent) showed low lymphocyte values. The normal value was 25.33 ±1.31 per cent.

Eosinophil percentages did not show significant variation from the normal value of 1.00 ±0.36 per cent and were 1.00 ±0.57 per cent in Group I, 2.08 ±0.48

Table 3: Comparison of leucogram of apparently normal and clinical cases before treatment (Mean ±S.E)

Parameters	Normal	GroupI	Group II	Group III	Group IV	Group V
TLC (thousands/mm^3)	10.15^a ±0.61	25.45^b ±11.50	14.23^a ±1.74	25.30^b	36.22^b ±13.12	15.80^a
Neutrophil (%)	71.00^a ±1.52	70.33^a ±13.86	69.50^a ±3.01	85.00^b	70.40^a ±13.51	83.00^b
Lymphocyte (%)	25.33^a ±1.31	28.33^a ±13.37	25.75^a ±2.94	13.00^b	26.80^a ±12.59	12.00^b
Eosinophil (%)	1.00 ±0.36	1.00 ±0.57	2.08 ±0.48	2.00	1.40 ±1.10	2.00
Monocyte (%)	2.00 ±0.26	0.33 ±0.33	2.67 ±0.75	0.00	1.40 ±0.87	3.00

Means bearing different superscripts within a row differ significantly (P≤0.05)

Table 4: Leucogram of apparently normal and clinical cases after treatment (Mean ±S.E)

Parameters	Normal	GroupI	Group II	Group V
TLC (thousands/ mm^3)	10.15^a ±0.61	22.00^b ±8.94	13.45^a ±1.40	16.50^a
Neutrophil (%)	71.00 ±1.52	68.33 ±10.13	69.17 ±3.32	78.00
Lymphocyte (%)	25.33 ±1.31	30.33 ±10.10	26.75 ±10.73	19.00
Eosinophil (%)	1.00 ±0.36	0.33 ±0.33	2.00 ±0.25	1.00
Monocyte (%)	2.00 ±0.26	1.00 ±0.50	2.08 ±0.48	2.00

Means bearing different superscripts within a row differ significantly (P≤0.05)

per cent in Group II, 2.00 per cent in Group III, 1.40 ±1.10 per cent in Group IV and 2.00 per cent in Group V. Not much change was observed in eosinophil percentages after treatment.

The monocyte percentages also did not show much variation from the normal values (2.00 ±0.26 per cent) before and after treatment. Groups I, II, III, IV and V showed values of 0.33 ±0.33 per cent, 2.67 ±0.75 per cent, 0, 1.4 ±0.87 per cent and 3.00 per cent respectively.

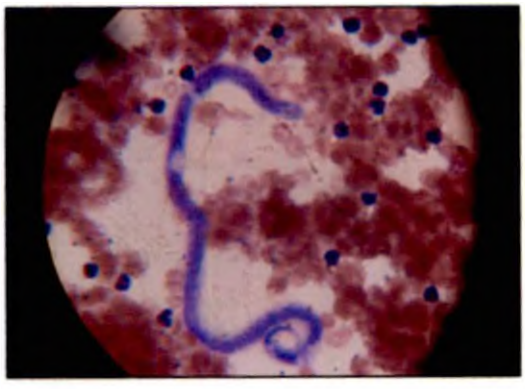
4.3.1.7 Thrombocyte Count

The thrombocyte count in all the groups were found to be significantly reduced from the normal value of 3.67 ±0.11 lakhs/ mm^3. The values recorded were 2.34 ±1.03 lakhs/ mm^3 (Group I), 0.95 ±0.20 lakhs/ mm^3 (Group II), 2.10 lakhs/ mm^3 (Group III), 1.10 ±0.50 lakhs/ mm^3 (Group IV) and 1.50 lakhs/ mm^3 (Group V) before treatment. The lowest thrombocyte counts were recorded in Group II. After treatment the platelet counts increased in all the groups but did not reach the normal values. Statistically significant difference in the thrombocyte count was also found after treatment between those animals transfused with blood and those that were not (Fig. 5 and Table 7).

4.3.1.8 Reticulocyte Percentage

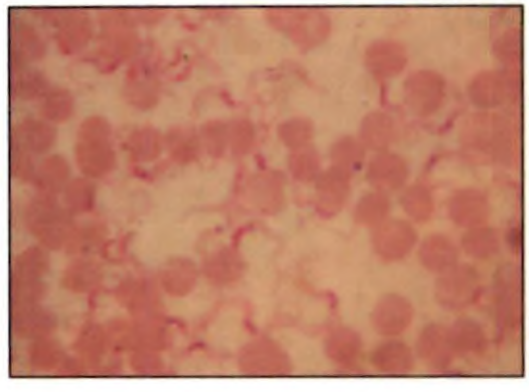
The reticulocyte percentage in the various groups was found to be highly variable with values of 7.83 ±4.28 per cent (Group I), 8.65 ±2.81 per cent (Group II), 11.42 ±4.49 per cent (Group IV) and 1 per cent (Group V). The normal values were found to be 0.67 ±0.19 per cent. After treatment the reticulocyte percentage was found to decrease in all the groups and were 3.00 ±1.36 per cent (Group I), 4.11 ±1.73 per cent (Group II) and 0.90 per cent (Group V). A positive correlation was found between the MCV and the reticulocyte percentage. On the basis of reticulocyte percentages all the cases observed in the present study except one were those of regenerative anaemia. The case of anaemia secondary to renal

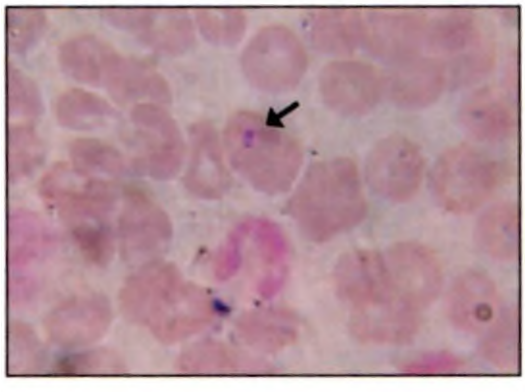
Plate 2a: Screening of blood smears (x1000)



A. *Dirofilaria repens*

B. *Trypanosoma evansi*





C. *Mycoplasma haemocanis*

D. *Ehrlichia canis* morula in buffy coat smear

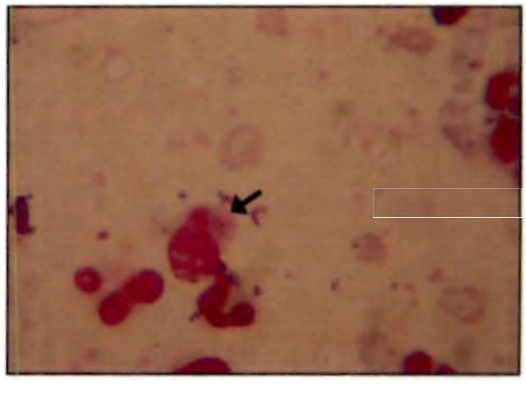
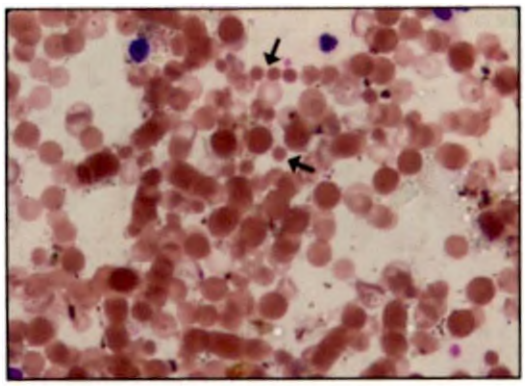
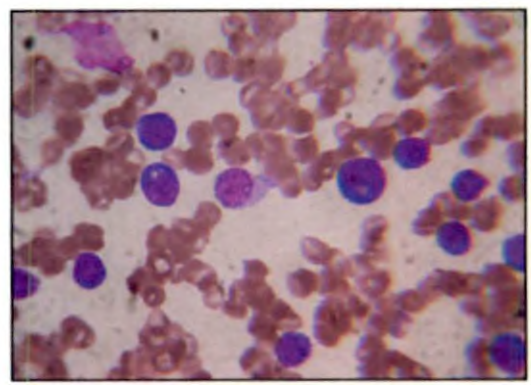


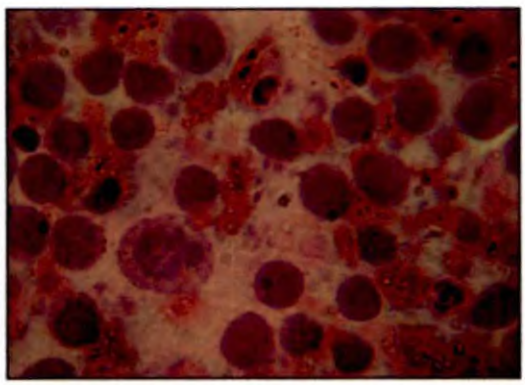
Plate 2b: Screening of blood smears (x1000)



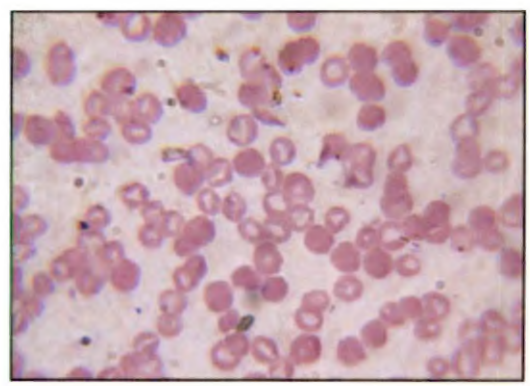
A. Spherocytes



B. Atypical blast cells



C. Pleomorphic round cells with large nuclei in smear of lymph node aspirate



D. Hypochromic RBCs

failure was found to be one of nonregenerative anaemia and recorded the lowest reticulocyte percentage as compared to other groups.

4.3.2 Serum Biochemistry

Table 5 and 6 summarize the comparison of the serum biochemical changes in the normal and the infected groups.

4.3.2.1 Total Serum Protein

Serum total protein values were not significantly different than the normal values (5.85 ±0.22 per cent) in most groups. The values recorded in Groups I, II, III, IV and V were 6.30 ±0.60 g/ dL, 6.38 ±0.23 g/ dL, 3.50 g/ dL, 5.42 ±0.35 g/ dL and 5.10 g/ dL respectively. Group III showed the lowest serum total protein value followed by Group V. After treatment there was an increase in values to 6.53 ±0.56 g/ dL, 6.25 ±0.22 g/ dL and 5.30 g/ dL for groups I, II and IV respectively.

4.3.2.2 Serum Albumin

Serum albumin values showed a decrease in groups I (1.86 ±0.61 g/ dL), III (1.50 g/ dL) and V (1.30 g/ dL). Groups II (2.29 ±0.18 g/ dL) and IV (2.02 ±0.28 g/ dL) showed serum albumin values within the normal limits. The normal value was recorded as 2.16 ±0.12 g/ dL. After treatment the albumin values increased in all the groups with values of 2.03 ±0.43 g/ dL (Group I), 2.31 ±0.17 g/ dL (Group II) and 1.5 g/ dL (Group V). The value of Group V did not reach normal limits even after treatment. Statistically significant difference in the albumin values after treatment were noted between the animals which received a blood transfusion and those that did not (Fig. 6 and Table 7).

The globulin concentration in the normal group was 3.69 ±0.16 g/ dL. Group I (4.43 ±0.08 g/ dL) and Group II (4.09 ±0.30 g/ dL) showed increased values as compared to the normal group. The globulin value in group III (2 g/ dL) was significantly lower than that of the normal group. Group IV (3.40 ±0.43 g/ dL) and Group V (3.80 g/ dL) showed values similar to the normal value.

Table 5: Comparison of serum biochemistry of apparently normal and clinical cases before treatment (Mean ±S.E)

Parameters	Normal	Group I	Group II	Group III	Group IV	Group V
T.Protein (g/ dL)	5.85^{a} ±0.22	6.30^{a} ±0.60	6.38^{a} ±0.23	3.50^{b}	5.42^{a} ±0.35	5.10^{a}
Albumin (g/ dL)	2.16^{a} ±0.12	1.86^{a} ±0.61	2.29^{a} ±0.18	1.50^{b}	2.02^{a} ±0.28	1.30^{b}
Globulin (g/ dL)	3.69^{a} ±0.16	4.43^{a} ±0.08	4.09^{a} ±0.30	2.00^{b}	3.40^{a} ±0.43	3.80^{a}
AG ratio	0.60^{a} ±0.04	0.43^{a} ±0.13	0.62^{a} ±0.08	0.75^{a}	0.66^{a} ±0.17	0.30^{b}
Creatinine (mg/ dL)	1.15^{a} ±0.07	1.20^{a} ±0.15	1.18^{a} ±0.22		1.06^{a} ±0.12	10.80^{b}
T.Bilirubin (mg/ dL)	0.25^{a} ±0.10	0.80^{a} ±0.17	1.46^{a} ±0.37		6.36^{b} ±3.15	0.30^{a}
D.Bilirubin (mg/ dL)	0.14^{a}	0.46^{a} ±0.08	0.85^{a} ±0.23		5.06^{b} ±2.50	0.20^{a}
I.Bilirubin (mg/ dL)	0.11^{a}	0.33^{a} ±0.08	0.60^{a} ±0.15		1.30^{b} ±0.60	0.10^{a}

Means bearing different superscripts within a row differ significantly ($P \leq 0.05$)

The AG ratio was recorded as 0.43 ±0.13 (Group I), 0.62 ±0.08 (Group II), 0.75 (Group III), 0.66 ±0.17 (Group IV) and 0.30 (Group V) as compared to normal values of 0.60 ±0.04. Group I and V showed significantly reduced AG ratios. The AG ratio increased in all the groups after treatment to reach values of 0.48 ±0.11 (Group I), 0.65 ±0.09 (Group II) and 0.39 (Group V).

4.3.2.3 Serum Creatinine

The creatinine values of 1.20 ±0.15 mg/ dL, 1.18 ±0.22 mg/ dL, 1.06 ±0.12 mg/ dL and 10.80 mg/ dL were recorded for groups I, II, IV and V respectively. All the groups showed values within the normal limits except for the case of renal failure which showed a high rise in creatinine values. After treatment there was no significant difference in creatinine values and were noted as 1.06 ±0.08 mg/ dL, 1.02 ±0.13 mg/ dL and 10.60 mg/ dL for groups I, II and V respectively. The normal value for serum creatinine was recorded as 1.15 ±0.07 mg/ dL.

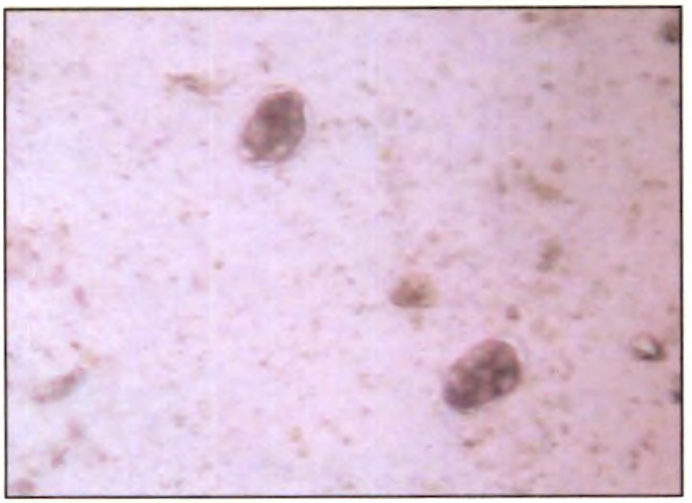
4.3.2.4 Total Serum Bilirubin

The total serum bilirubin value of the normal group was recorded as 0.25 ±0.10 mg/ dL. Groups I, II, IV and V recorded values of 0.80 ±0.17 mg/ dL, 1.46 ±0.37 mg/ dL, 6.36 ±3.15 mg/ dL and 0.30 mg/ dL respectively. Significant difference was observed in Group IV as compared to normal values. The total bilirubin values after treatment did not show any significant difference from the pre-treatment values.

4.3.2.5 Direct Serum Bilirubin

Direct bilirubin values in the various groups were 0.46 ± 0.08 mg/ dL (Group I), 0.85 ±0.23 mg/ dL (Group II), 5.06 ±2.50 mg/ dL (Group IV) and 0.20 mg/ dL (Group V) before treatment. Significant difference was found in Group IV as compared to the normal group. The direct bilirubin values after treatment did not change significantly and did not reach the normal values.

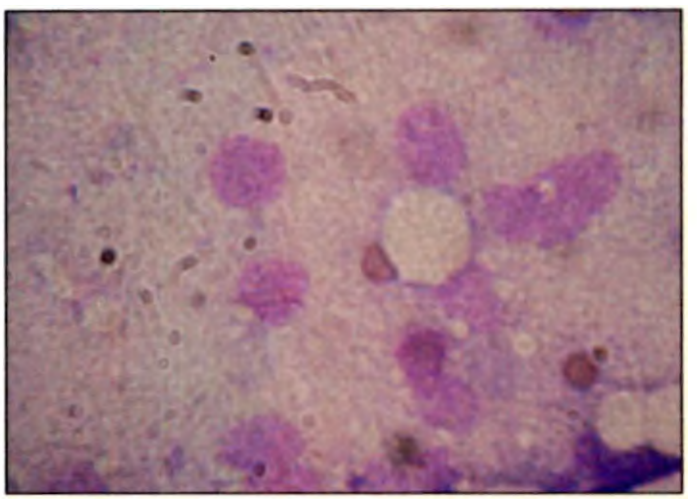
Plate 3: Screening of faecal sample

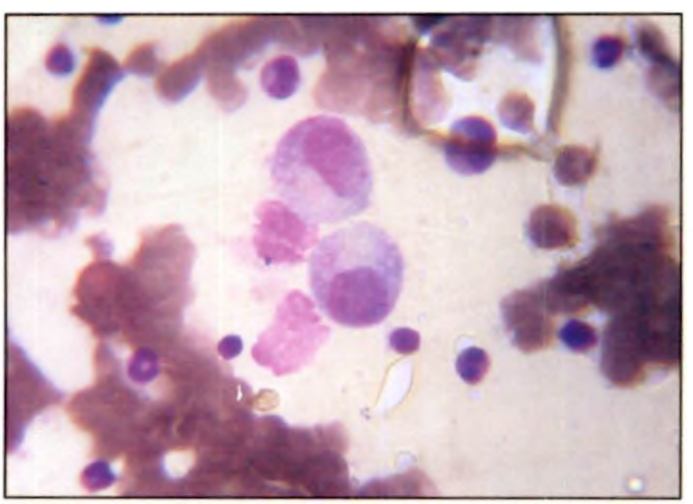


Ova of *Ancylostoma caninum*

Plate 4: Examination of bone marrow aspirates (x1000)

A. Atypical blast cells





B. Pleomorphic round cells with large nuclei

Table 6: Comparison of serum biochemistry of apparently normal and clinical cases after treatment (Mean ±S.E)

Parameters	Normal	Group I	Group II	Group V
T.Protein (g/ dL)	5.85 ±0.22	6.53 ±0.56	6.25 ±0.22	5.30
Albumin (g/ dL)	2.16^{a} ±0.12	2.03^{a} ±0.43	2.31^{a} ±0.17	1.50^{b}
Globulin (g/ dL)	3.69 ±0.16	4.50 ±0.50	3.94 ±0.30	3.80
AG ratio (g/ dL)	0.60^{a} ±0.04	0.48^{a} ±0.11	0.65^{a} ±0.09	0.39^{b}
Creatinine (mg/ dL)	1.15^{a} ±0.07	1.06^{a} ±0.08	1.02^{a} ±0.13	10.60^{b}
T.Bilirubin (mg/ dL)	0.25^{a} ±0.10	0.70^{a} ±0.11	1.38 ± 0.35^{b}	0.30^{a}
D.Bilirubin (mg/ dL)	0.14	0.40 ± 0.05	0.82 ± 0.23	0.20
I.Bilirubin (mg/ dL)	0.11	0.30 ± 0.05	0.55 ± 0.13	0.10

Means bearing different superscripts within a row differ significantly ($P \leq 0.05$)

4.3.2.6 Indirect Serum Bilirubin

The indirect bilirubin values were recorded as 0.33 ±0.08 mg/ dL (Group I), 0.60 ±0.15 mg/ dL (Group II), 1.30 ±0.60 mg/ dL (Group IV) and 0.10 mg/ dL (Group V) before treatment. Post treatment values did not differ much as compared to pretreatment values and did not reach the normal values.

4.3.2.7 Serum Potassium and Phosphorus

Serum potassium and phosphorus levels were estimated in the case of chronic renal failure. A hypokalemia (3.2 mEq/ L) and hyperphosphatemia (12.5 mg/ dL) was recorded as compared to the normal levels of 4.37-5.35 mEq/ L and 0.84-2 mg/ dL.

4.3.2.8 Serum alanine aminotransferase

Serum alanine aminotransferase values were estimated in two cases of liver dysfunction. The values recorded were 1600 IU/ L and 1300 IU/ L. The normal values are reported as 80 IU/ L.

4.3.2.9 Serum Alpha-foetoprotein (AFP)

Serum alpha-foetoprotein values were estimated in the case of hepatocellular carcinoma. High values of 262 ng/ mL as compared to normal values of less than 70 ng/ mL substantiated the diagnosis of hepatocellular carcinoma.

4.4 SCREENING OF BLOOD SMEARS AND LYMPH NODE ASPIRATION SMEARS

Peripheral blood smears and lymph node aspiration smears from infected cases were screened for the presence of haemoparasites and for evaluating the morphology of the blood cells. Haemoparasites such *as Dirofilaria repens*, morulae of *Ehrlichia canis*, *Babesia gibsoni* piroplasms, trypomastigotes of *Trypanosoma evansi* and *Mycoplasma haemocanis* were observed on Giemsa stained blood smears (Plate 2a: A-C).

Table 7: Comparison of haemato-biochemical parameters between animals which were given blood transfusion and animals which were not given blood transfusion (Mean ±S.E)

Parameters	Normal	Non transfused animals		Blood transfused animals	
		Day 1	Day 7	Day 1	Day 7
VPRC (%)	40.00^{a} ±0.50	18.50^{b} ±3.58	25.00^{b} ±3.26	12.86^{b} ±2.61	26.84^{b} ±2.05
Hb (g/ dL)	13.77^{a} ±0.16	6.43^{b} ±1.16	9.29^{b} ±1.04	4.48^{b} ±1.03	9.32^{b} ±1.63
TEC (millions/mm^3)	7.03^{a} ±0.10	2.99^{b} ±0.65	4.55^{b} ±0.64	1.99^{b} ±0.39	4.58^{b} ±0.27
Platelets (lakhs/ mm^3)	3.67^{a} ±0.11	1.19^{b} ±0.28	1.50^{b} ±0.26	1.38^{b} ±0.60	1.94^{b} ±0.78
Albumin (g/ dL)	2.16^{a} ±0.12	2.35^{a} ±0.20	2.33^{a} ±0.18	1.70^{b} ±0.28	1.92^{b} ±0.24

Means bearing different superscripts within a row differ significantly ($P \leq 0.05$)

The buffy coat smear stained with Giemsa stain revealed the presence of *Ehrlichia canis* morulae in the monocytes in one case (Plate 2a: D).

In the case of Evans' syndrome, microagglutination of the red blood cells was observed along with the presence of moderate number of spherocytes and also haemolysis (Plate 2b: A).

In case of acute lymphoblastic leukaemia, the differential count revealed more than 75 per cent of atypical cells which had impression of blast cells in the circulation (Plate 2b: B).

Lymph node aspiration in the case of lymphosarcoma revealed the presence of slightly pleomorphic round or oval cells having larger nuclei suggestive of lymphoma (Plate 2b: C).

Hypochromic RBCs were observed on a few blood smears (Plate 2b: D).

4.5 WET FILM EXAMINATION

Wet film examination was performed in all the cases. 3 cases were positive for microfilariae while one was positive for *Trypanosoma*. In all other cases the wet film examination was negative. The affected cases were treated specifically and the wet film examination performed seven days later was found to be negative.

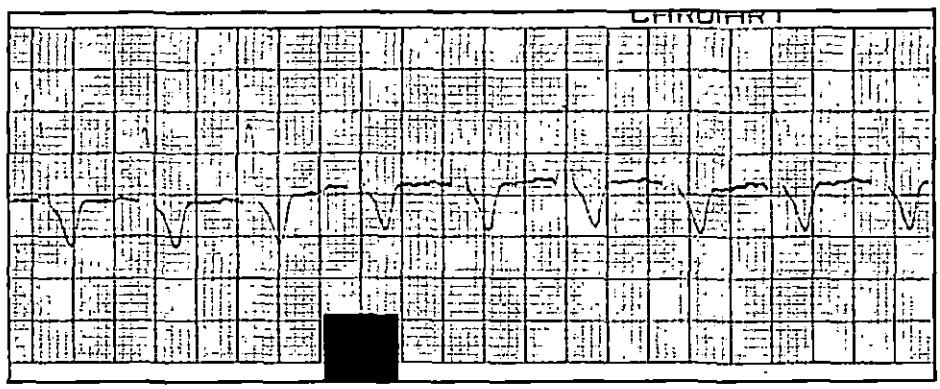
4.6 SCREENING OF FAECAL SAMPLES

The faecal samples of all the anaemic cases were screened for the presence of gastrointestinal parasites. Only one case was found positive for the presence of ova of *Ancylostoma caninum* (Plate 3).

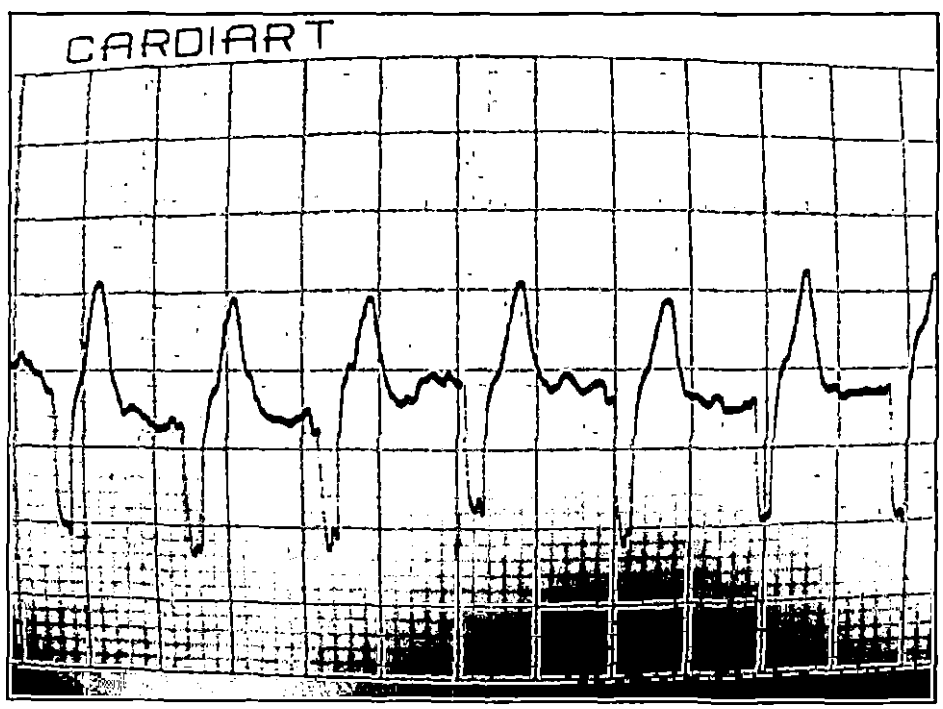
4.7 SCREENING OF SPLENIC ASPIRATION SMEARS

Screening of splenic aspiration smears was done in cases showing severe splenomegaly for the evaluation of haemoparasites. One case of *Babesia gibsoni* was identified on splenic aspiration.

Plate 5: Electrocardiography



A. Prolonged QRS duration and S-T segment coving



B. Ventricular tachycardia

4.8 EXAMINATION OF BONE MARROW ASPIRATES

Bone marrow aspirates were screened in two cases of acute lymphoblastic leukaemia and lymphosarcoma. Bone marrow aspirate smears of the case of acute lymphoblastic leukaemia showed a hyper cellular marrow and revealed more than 30 per cent atypical blast cells which was diagnostic of the condition (Plate 4: A) and that of lymphosarcoma revealed groups of and scattered slightly pleomorphic round or oval cells having larger nuclei. There were also occasional mitotic cells. These abnormal cells formed about 70 per cent of the bone marrow cells. The remaining cells of the myeloid and erythroid series showed normal maturation sequence (Plate 4: B).

4.9 ELECTROCARDIOGRAPHY (ECG)

ECG changes were recorded in most cases. The ECG changes were unremarkable in many. The most commonly found changes were S-T segment depression/ elevation and coving which was observed in 6 cases. Prolongation of QRS complex duration was found in 4 cases and increased amplitude above 3.4 mV was found in 2 cases. An arrhythmia was found only in the case of a dog suffering from haemoabdomen in which Ventricular tachycardia was recorded (Plate 5: A and B).

4.10 ULTRASONOGRAPHY

The most commonly found ultrasound change was splenomegaly (Plate 6: A) which was found in cases of haemolytic anaemia caused by haemoparasites and Evans' syndrome and also in acute lymphoblastic leukaemia.

A highly cellular peritoneal exudate was identified on ultrasound examination of the abdomen in the case of haemoabdomen. Cases of liver dysfunction revealed a mixed echogenicity of liver parenchyma along with hepatomegaly. In case of hepatocelllular carcinoma, nodules were observed in various hepatic lobes on ultrasound scanning (Plate 6: B).

The kidneys in the case of chronic renal failure were devoid of the normal architecture with indistinct corticomedullary distinction. The kidneys seemed to be small and contracted (Plate 6: C).

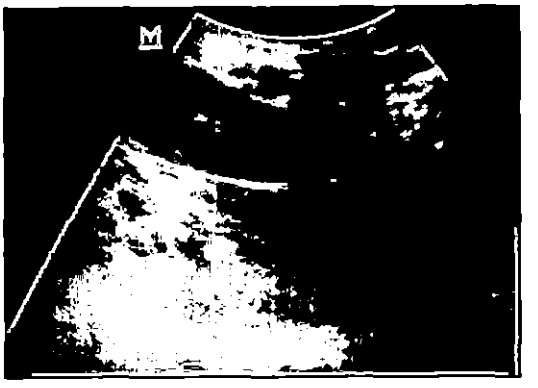
4.11 TREATMENT

Suitable therapy was adopted depending on the aetiological factors. Apart from the specific therapy, supportive therapy was also adopted. The supportive therapy included intravenous dextrose (10 per cent) administration, oral or parenteral haematinics and vitamin supplements. Clinical response in subsequent days of treatment was recorded in all cases. In group II, administration of oxytetracycline evoked desirable clinical response in all cases of ehrlichiosis, mycoplasmosis and some cases of microfilariosis. This was followed by an oral course of doxycycline for ten days.

Isometamidium chloride was administered in cases of trypanosomosis and had a positive response. The single case of *Babesia gibsoni* was treated with Diminazene aceturate with success. The drug was repeated after a week. Low microfilaraemic cases were treated with injectable Ivermectin and heavily positive cases with oral Ivermectin to prevent the risk of thrombosis. In most of the cases fever subsided and food intake improved by the second day itself and the animals became more active. The colour of the mucous membranes remained towards the paler side. Gradual improvement was observed during subsequent days of treatment. Clinical improvement was observed by the seventh day by which time the haematobiochemical profile of the animals showed marked improvement. The mucous membranes were pale pink by the end of this period and the respiratory difficulties also reduced.

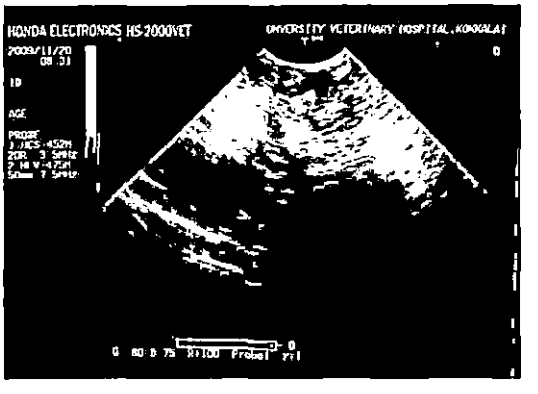
The case of haemoabdomen was treated as an emergency case and immediately transfused with blood as a life saving measure. It had also developed Ventricular tachycardia on an electrocardiogram which indicated a grave prognosis and the dog died on the next day.

Plate 6: Ultrasonography



A. Splenomegaly

B. Nodules in liver



C. Loss of normal renal architecture with indistinct cortico-medullary distinction

Plate 7: Blood transfusion

Group I animals which had tick and flea infestations were administered Ivermectin parenterally. The tick infested dog was additionally treated with a topical application of Cypermethrin. The flea infested case was treated with Fipronil spray. Manual removal of ticks was also performed. Considerable reduction in the number of ticks and fleas on the body of the animal was observed on the seventh day of treatment along with alleviation of the clinical signs. An additional dose of ivermectin was administered on the seventh day.

In the case affected with *Ancylostoma caninum* oral administration of Fenbendazole had the desired effect and the faecal sample examination was negative on the seventh day. The gastrointestinal disturbances lessened with the administration of the anthelmintic. The faeces assumed normal consistency by the third day of treatment. By the seventh day a marked improvement in the condition and haematobiochemical parameters could be observed.

In the case of Evans' syndrome, the dog was treated with immunosuppressive doses of Prednisolone @ 2 mg/ kg body weight parenterally and orally. This animal too showed a remarkable improvement in clinical signs as well as haematobiochemical values after seven days of treatment. The macroagglutination also disappeared a few days after initiating the immunosuppressive therapy.

The case of chronic renal failure was treated symptomatically with gastrointestinal protectants like ranitidine, anti emetics and fluid therapy. Blood transfusion was performed in this case and improvement in haematobiochemical parameters was observed but no improvement in clinical condition was noted. The animal later developed a urinary tract infection.

All animals of group IV succumbed before the end of seven days. The case of acute lymphoblastic leukaemia was weak and recumbent when presented and died the very next day. In both the cases of hepatocellular carcinoma and lymphoma the owners insisted for euthanasia.

Both cases of liver dysfunction were treated symptomatically and with liver protectants like silymarin and amino acid preparation (Essentiale – L), but succumbed few days after presentation.

Injectable iron dextran was administered in all the cases and on discharge, owners were advised to continue with oral iron supplements.

Thus the treatment regime adopted was found suitable in most cases and uneventful recovery occurred with the haematological and biochemical parameters returning towards the normal range within 7 days. However casualties were recorded in Group IV.

4.12 BLOOD TRANSFUSION

Blood transfusion was performed in seven cases out of which five survived. One of the cases which died was affected with haemoabdomen while the other had liver dysfunction. The cases which survived showed considerable clinical improvement from the next day itself. The clinical signs were also alleviated earlier than those cases which did not receive a blood transfusion. There was significant improvement in haematobiochemical values of the animals receiving a blood transfusion as compared to those animals which did not undergo it (Table 7).

Discussion

5. DISCUSSION

Anaemia is an important haematological abnormality which is frequently encountered in dogs and is responsible for a poor quality of life. Squires (1993) and Aird (2000) mentioned that anaemia was always associated with an underlying disease which must be identified to allow appropriate case management or else the response to treatment would only be transient.

5.1 INCIDENCE OF ANAEMIA

5.1.1 Chronic Blood Loss Anaemia

A 60 days old pup was found to be anaemic due to infestation by *Ancylostoma caninum*. This was the only case of endoparasitism causing severe anaemia. The deleterious health effects associated with chronic hookworm infection was attributable to adult worms, which attached to the intestinal mucosa and caused substantial haemorrhage which was responsible for the anaemia. This was supported by Hall and Simpson (2000) as well as Jones and Cappello (2004). Gastrointestinal parasitism caused by hookworms was a major concern in chronic external blood loss in dogs (Giger, 2000).

A case of anaemia due to infestation by the flea *Ctenocephalides felis* was recorded. It was suggested by Bond *et al.* (2007) that *C.felis* was the species of flea found most commonly in dogs. Fleas were also found to be the most common ectoparasite on dogs and were very difficult to get rid of despite regular antiectoparasitic treatment.

A single case of anaemia due to tick infestation by *Rhipicephalus sanguineus* was recorded in the study. The dog had a severe tick infestation. This was in accordance to the suggestion made by Tinoco-Gracia *et al.* (2009) that finding more than 30 ticks on the body suggested a severe tick infestation. Ectoparasites such as ticks and fleas could be considered as cause of anaemia only

if the infestation was severe. Rani (2004) shared the same opinion in a study conducted on parasitic anaemia in cattle.

5.1.2 Haemolytic Anaemia

In the present study 12 cases of haemolytic anaemia were reported. Eleven were due to haemoparasites while one was due to Evans' syndrome which was concurrent occurrence of immune-mediated haemolytic anaemia and immune-mediated thrombocytopenia.

Gaunt (2000) and Benjamin (2007) also observed *Babesia, Mycoplasma* and *Trypanosoma* as causes of haemolytic anaemia.

A case of immune-mediated haemolytic anaemia was also recorded which according to Squires (1993) was a common cause of non-traumatic severe anaemia in dogs.

5.1.3 Acute Blood Loss Anaemia

In the present study a case of acute blood loss anaemia due to haemoabdomen was identified. The history of the animal revealed a blow on the abdomen by the owner. The most probable cause of the haemoabdomen might be due to traumatic haemorrhage from intra-abdominal organ damage. The occurrence of such a condition was also reported by Rozanski and De Laforcade (2004).

5.1.4 Anaemia Associated with Chronic Diseases

In the present study 5 animals were found to suffer from anaemia due to chronic disease. Out of these, 3 had neoplasms and 2 of the cases had liver dysfunction.

Anaemia was observed as the most commonly occurring paraneoplastic syndrome. In case of neoplasms the erythrocyte count could be decreased due to neoplastic cell proliferation and replacement of erythroid population of marrow Dobson (2004). Another mechanism is that as the primary disease progresses,

there is a decrease in iron metabolism and storage. Along with this an associated decrease in red blood cell life span and decreased response of the marrow to a low red blood cell count contributed to the anaemia. This was similar to the findings reported by Finora (2003) and Hohenhaus (2003).

A case of lymphosarcoma was also recorded to cause severe anaemia and it had been reported by Dobson (2004), Lucas (2004) and Dobson *et al.* (2006) that lymphosarcoma was the most common haematopoietic neoplasm of dogs and that the incidence of acute lymphoblastic leukaemia was less common.

The case of canine hepatocellular carcinoma was diagnosed on the basis of finding hyperechoic nodules in the hepatic lobes on ultrasonography and exploratory surgery. However Kawarai *et al.* (2006) reported a poor prognosis for this condition and the owner requested to euthanize the animal.

5.1.5 Anaemia Secondary to Renal Disease

A single case of anaemia secondary to renal disease was reported. The recorded case was one of chronic renal failure. In chronic renal failure, production of erythropoietin decreases and anaemia ensues. Other causes of the anaemia included decreased life span of red cells, blood loss from gastric ulcers and abnormal platelet function. Anaemia was a typical clinical sign in cases of chronic renal failure and the presence or absence of anaemia could be used as a clue to identify whether the renal failure was acute or chronic. This was in agreement with the suggestion made by Cotter (2000) and Erickson and Rubin (2007).

5.2 CLINICAL MANIFESTATIONS

The major clinical signs observed in the present study were pallor of mucous membranes, tachycardia, rapid, strong and bounding pulses, anorexia, lethargy, exercise intolerance, weakness and exaggerated respiration with tachypnoea. Similar signs were also reported in anaemic cases by Klag *et al.* (1993), Rozanski and De Laforcade (2004) and Saini *et al.* (2005).

In cases of haemolytic anaemia the important clinical signs noted were pale or icteric mucous membranes, fever, enlargement of superficial lymphnodes, splenomegaly and anorexia which were in accordance with the study conducted by De Gopegui *et al.* (2007).

Limb and scrotal oedema was found as a characteristic feature in few cases affected by microfilariosis, trypanosomosis and ehrlichiosis. Similar findings were reported by De La Rue *et al.* (2000), Jabina and Ajithkumar (2005) and Nabity *et al.* (2006).

In case of chronic ehrlichiosis, generalised lymphadenopathy was absent but respiratory distress was present. Waner *et al.* (1996) attributed this sign to the presence of pulmonary inflammation caused due to *E. canis* infection.

The case of Evans' syndrome presented another unique sign of haemoglobinuria which had also been reported by Inokuma *et al.* (2005). Haemoglobinuria occured due to a very high rate of intravascular haemolysis which generally occured due to complement mediated autoantibody reaction (Shaw and Harrell, 2008).

In case of anaemia due to chronic blood loss, the intensity of anaemic signs was relatively reduced. In the case of flea infestation the mucous membranes were congested and not pale. This could be due to simultaneous dehydration along with anaemia (Kraje, 2002). The absence of typical signs of anaemia in case of chronic blood loss had been recorded by Rozanski and De Laforcade (2004). This was because the chronicity and slow development of the anaemia allowed the body to adjust to the hypoxic condition and thus the compensatory processes such as tachycardia, tachypnoea were of reduced intensity. The case of ancylostomiasis was presented with the signs of diarrhoea with mucus and blood in stools as reported by Little *et al.* (2009).

Animal with haemoabdomen was recumbent on presentation with an obvious distension of abdomen. The mucous membranes were cyanotic and the

capillary refill time was prolonged. Cyanosis and prolongation of the capillary refill time signifies a hypoxic and hypovolaemic condition respectively which developed due to acute loss of blood into body cavity and resultant reduction in circulatory volume. The animal also showed rapid and weak pulse and severe respiratory distress. Similar signs were reported by Aronsohn *et al.* (2009).

In case of acute lymphoblastic leukaemia severe splenomegaly was seen. In case of lymphosarcoma, severe enlargement of all palpable lymph nodes was noted. The lymphadenopathy occured due to proliferation of malignant lymphoid cells that affected the lymph nodes. The case of canine hepatocellular carcinoma had the complaint of only non specific signs such as anaemia and weakness. These findings were in confirmation with the studies conducted by Vail (2000) and Kawarai *et al.* (2006).

The cases of liver dysfunction had presented with the symptoms of anorexia, weakness and pale or icteric mucous membranes on clinical examination. Icterus was the most frequent specific physical abnormality in dogs with liver disease and could be first appreciated in sclera. Jaundice was the result of bilirubin accumulation in blood and in extravascular space due to increased production, reduced clearance, or impaired conjugation by the liver and/ or impairment of the bile flow (Rothuizen and Meyer, 2000).

The case of chronic renal failure showed signs like pallor of mucous membranes, tachycardia, weakness, vomiting, melena, polyuria and polydypsia. The dog also had a typical wooden, stilted gait which could be because of hypokalemia induced muscle weakness (Kraje, 2002). Hypokalemia was present in this case. These signs were typical of those of chronic renal failure as mentioned by Erickson and Rubin (2007).

5.3 CLINICAL PATHOLOGY

5.3.1 Haematology

The haematological results values were consistent with anaemia as defined by Benjamin (2007).

5.3.1.1 Volume of Packed Red Cells (VPRC), Haemoglobin (Hb) Concentration and Total Erythrocyte Count (TEC)

VPRC, haemoglobin and total erythrocyte count were the three important parameters to assess the anaemic status in an animal.

The mean values of VPRC, haemoglobin concentration and total erythrocyte counts were significantly decreased in all five groups. In case of group I, it was found that ectoparasties fed on blood and hence heavy infestations could lead to continuous loss of blood volume causing anaemia. The anaemia due to chronic hookworm infestation was caused by adult worms leading to haemorrhage and blood loss. Similar findings were suggested by Jones and Cappello (2004) and Tinoco-Gracia *et al.* (2009).

In case of group II animals the anaemia was seen due to erythrocyte destruction, either extra- or intravascularly. Extravascular haemolysis was the predominant form and refered to erythrophagocytosis by macrophages of the spleen, liver and bone marrow. Intravascular haemolysis took place less commonly as a consequence of membrane permeability changes or cellular fragmentation (Giger, 2000).

In group III, anaemia was due to the blood loss into the peritoneum and resultant hypovolaemia. This was similar to the observations made by Aronsohn *et al.* (2009).

In group IV the cause of the anaemia was attributed to the findings of Finora (2003) who mentioned that as the primary disease progressed, there was a decrease in iron metabolism and storage. Along with this an associated decrease

in red blood cell life span and decreased response of the marrow to a low red blood cell count contributed to the anaemia. Another reason for reduced erythrocyte count was proliferation of the bone marrow by the neoplastic cells leading to decreased normal erythroid population as suggested by Dobson (2004).

In group V, the anaemia associated with renal failure was most probably caused by a shortened red blood cell life span, nutritional abnormalities, erythropoietin inhibitor substances in the plasma, blood loss and myelofibrosis in which erythropoietin deficiency was the major cause (Erickson and Rubin, 2007).

In all cases the values of these parameters improved after treatment but did not reach upto the normal values. This was because the severity of the anaemia in animals warranted a lag period for normalisation. This finding was in agreement with Rani (2004).

5.3.1.2 Erythrocyte Indices (MCV, MCHC, MCH)

An increase in MCV values were found in the group III and IV. Both acute haemorrhage and anaemia due to neoplasia was of a regenerative type according to Presley *et al.* (2006). This was responsible for the increasing values of MCV which indicated regenerative anaemia as stated by Neiger *et al.* (2002). Immature RBCs were larger than normal RBCs and the increased volume was reflected as an increased MCV in the present study (Weiss and Tvedten, 2004)

Most cases of neoplasms caused non-regenerative anaemias due to bone marrow infiltration. However the anaemia in both cases of neoplasms encountered in the present study were of regenerative type. Thus we can conclude that the two cases included in the study were of acute anaemia and therefore showed a regenerative response. Another reason could be that the neoplasia might not be of multicentric type and thus the bone marrow was not affected much and erythropoiesis took place at the normal rate.

Decreased MCV values of group V indicated a non-regenerative anaemia which was expected in cases of anaemia of chronic renal disease. It occurred due

to decreased synthesis of erythropoietin as observed by Polzin *et al.* (2000) and Erickson and Rubin (2007).

Increased MCHC values were observed in groups IV and V which might be due to the presence of intravascular haemolysis (Zygner *et al.*, 2007).

5.3.1.3 Total Leucocyte Count (TLC)

Leucocytosis was observed in the groups I, III, IV and V. In group IV, the leucocytosis was observed in all the cases of neoplasia and the presence of leucocytosis in cases of acute lymphoblastic leukaemia and canine hepatocellular carcinoma had been recorded by Kawarai *et al.* (2006) and Presley *et al.* (2006). This leucocytosis might have developed as part of the inflammatory response (Benjamin, 2007).

The leucocytosis found in case of anaemia due to secondary renal disease had also been reported by Kraje (2002).

5.3.1.4 Differential Count

A neutrophilia and lymphopenia was noted in groups III and V. The lymphopenia might have been compensatory to the increased neutrophil percentage.

5.3.1.6 Reticulocyte Percentage

A significant but variable increase in reticulocyte percentage was observed in all groups except group V where it was low. This increase in reticulocyte percentage above one per cent was a sign of regenerative anaemia. This meant that the bone marrow was responding to the anaemia by increasing the production of erythrocytes causing release of immature RBCs into the circulation. The increase in the reticulocyte percentage had been reported in haemolytic infections by Harvey (1990) and Willi *et al.* (2007). In this study, neoplastic conditions also showed the presence of a regenerative type of anaemia as reported by Presley *et al.* (2006).

A highly regenerative anaemia was observed in case of Evans' syndrome. A marked reticulocytosis was a hallmark of immune-mediated haemolytic anaemia as expressed by Shaw and Harrell (2008).

The case of chronic renal failure showed a low reticulocyte count of 1 per cent. As the renal failure became chronic, production of erythropoietin decreased and anaemia ensued. This was manifested by low reticulocyte counts. This was in agreement with the findings of Cotter (2000).

In the present study a positive correlation was found between MCV and reticulocyte percentage. This was because an increase in reticulocyte percentage meant that RBCs of larger size and volume were liberated in the circulation and this led to an increase in the MCV. It could thus be concluded that the MCV could be a useful tool for assessing the presence of a regenerative or non-regenerative anaemia in dogs (Neiger *et al.*, 2002).

5.3.1.5 Thrombocyte Count

There was significant decrease in thrombocyte counts in all the groups as compared to the normal values.

Group II recorded the lowest counts. Severe thrombocytopenia was observed in cases of haemolytic anaemia. In case of ehrlichiosis, the cause of thrombocytopenia might be due to an increased platelet consumption as well as sequestration. The possible mechanisms of low platelet counts in babesiosis were local and systemic disseminated intravascular coagulation, immune-mediated destruction and sequestration of platelets in the spleen (Botsch *et al.* 2009). Thrombocytopenia in Evans' syndrome was due to the development of autoantibodies as reported by Klag *et al.* (1993).

The thrombocytopenia seen in cases of neoplasms could be attributed to low thrombocyte production or increased destruction of platelets (Dobson, 2004).

5.3.2 Serum Biochemistry

5.3.2.1 Serum Total Protein, Serum Albumin and Serum Globulin

Low serum total protein values were recorded in groups III and V. In case of haemoabdomen, loss of blood into the body cavity led to a reduction in the serum total protein value (Murphy and Warman, 2007).

In case of chronic renal failure, proteinuria was observed due to loss of renal architecture and increased filtration of proteins by the glomerulus. Proteinuria was considered to be a hallmark sign of glomerular injury and dysfunction. This was the cause of hypoproteinemia observed in connection with renal failure in the present study and was in agreement with the findings of Polzin *et al.* (2000).

Reduced feed intake due to anorexia in the affected animals also added to the hypoproteinemia (Rani, 2004).

Reduction in the serum albumin concentration was often reflected as the reduction in serum total protein. Serum albumin values showed a decrease in groups I, III and V. In case of external blood loss, a low or a low normal plasma protein level was observed along with a regenerative anaemia (Weiss and Tvedten, 2004). A decrease in the plasma protein level with an associated reticulocytosis in case of a normally hydrated animal could be a useful diagnostic feature of external blood loss anaemia.

Statistically significant differences in the albumin values before and after treatment were noted in those animals that were given a blood transfusion. Thus it could be inferred that blood transfusion was useful for increasing the albumin levels in those animals with hypoalbuminemia coupled with anaemia.

Group I and group II showed increased serum globulin values as compared to the normal groups whereas group III showed lower values.

Hypergammaglobulinemia was common in most haemoparasitic infections, especially in ehrlichiosis. This was reported by many authors who suggested that autoantibodies might be important in the pathogenesis of this infection (Waner *et al.*, 1995), (Harrus *et al.*, 1997a) and (Suto *et al.*, 2001). Similar changes in serum globulin levels were also found in microfilariosis by Tomodo (1962) and in trypanosomosis by Aquino *et al.* (2001).

5.3.2.2 AG Ratio

A significant reduction in the AG ratio was observed in groups I and V. This corresponded to the low albumin levels encountered in the above groups. AG ratio was below one and no other cause of hypoalbuminemia was there. That indicated chronic liver pathology (Leveille-Webster, 2000). In the present study an AG ratio below 1 was recorded also in cases of renal failure and chronic blood loss anaemia.

5.3.2.3 Serum Creatinine

Serum creatinine values were within the normal range for all the groups except group V which recorded high levels of 10.8 mg/ dL. High creatinine levels were observed in cases of chronic renal failure due to impaired excretion of the nitrogenous waste products by defective kidneys (Kraje, 2002).

5.3.2.4 Serum Bilirubin

Significant alterations in serum total bilirubin values were recorded in group II and IV. Direct bilirubin levels were also greatly increased in group IV and indirect bilirubin levels were increased significantly in groups II and IV.

Higher serum bilirubin values were recorded in haemolytic anaemia. The heme from the lysed erythrocytes was converted into bilirubin by specific enzymes (Benjamin, 2007). This was not being excreted quickly into the bile leading to increased blood bilirubin levels. The hypoxia caused due to the severe haemolysis also lead to liver damage and increased values of bilirubin in the

blood. This was in agreement with the work of many authors like Chandoga *et al.* (2002), Breuhl *et al.* (2009) and Willi *et al.* (2007).

In case of liver dysfunction, bilirubin accumulated in the blood and extravascular space due to increased production, impaired conjugation by the liver or impaired bile flow (Rothuizen and Meyer, 2000).

5.3.2.5 Blood Glucose

Blood glucose was estimated in the cases of trypanosomosis and recorded hypoglycaemic values of 45 mg/ dL and 50 mg/ dL. This hypoglycaemia could be attributed to the fact that trypanosomes utilized large amounts of glucose. Similar observations were made by Gunaseelan *et al.* (2009).

5.3.2.6 Serum Potassium and Phosphorus

Low serum potassium and high serum phosphorus values were recorded in case of chronic renal failure. Low serum potassium levels could be attributed to the increased losses through the urine as well as a decrease in potassium intake due to anorexia. In the present study the animal showed polyuria and anorexia. Chronic renal failure leads to phosphorus retention and ultimately hyperphosphatemia. Polzin *et al.* (2000) reported similar findings in cases of chronic renal failure.

5.3.2.7 Serum Alpha-fetoprotein (AFP)

A high serum alpha-fetoprotein (AFP) value was noted in the case of canine hepatocellular carcinoma. In dogs re-expression of alpha-fetoprotein is known to occur in some hepatic tumours and increased values were seen in most canine hepatocellular carcinoma patients. Therefore for early diagnosis of canine hepatocellular carcinoma, alpha-fetoprotein levels could be used as a tumour marker (Kawarai *et al.*, 2006).

5.3.2.8 Alanine aminotransferase (ALT)

Increased levels of alanine aminotransferase were observed in both cases of liver dysfunction. Large increases were seen with hepatocellular necrosis and inflammation and the magnitude of elevation was proportional to number of injured hepatocytes (Leveille-Webster, 2000).

5.4 SCREENING OF BLOOD SMEARS AND LYMPH NODE ASPIRATION SMEARS

Peripheral blood smears and lymph node aspiration smears from affected cases were screened for the presence of haemoparasites and for evaluating the morphology of the blood cells. The blood smears were stained with Giemsa stain.

The cases of ehrlichiosis were diagnosed by observing the morulae of *Ehrlichia canis* in the cytoplasm of peripheral monocytes and one case of ehrlichiosis was confirmed by staining the buffy coat smear. Buffy coat smear examination was better than blood smear examination in diagnosing ehrlichiosis.

However it was reported that serological examinations by using the indirect immunofluorescent antibody (IFA) technique was the most useful diagnostic method (Waner *et al.*, 1996 and Suto *et al.*, 2001). So moderate anaemic cases should be subjected for these tests to find out ehrlichiosis.

The case of mycoplasmosis was diagnosed by identification of the organism on blood smear. There was tendency for the organisms to form chains across the erythrocyte surface. The usual appearance of the organism was in the coccoid form. This was in agreement with the findings of Harvey (1990), Reine (2004) and Willi *et al.* (2007).

In case of babesiosis, *Babesia gibso*ni organism was detected in which a single peripheral RBC contained only one to two parasites. This confirmed with the work done by Breitschwerdt (1990), Chandoga *et al.* (2002), Fukumoto *et al.* (2005) and Chaudhuri *et al.* (2008).

Microfilariosis caused by *Dirofilaria repens* was diagnosed by identifying the microfilariae on blood smears. The microfilariae of *D. repens* were found to be sheathless, with a tapering head and long pointed tail. It was found that the sensitivity of concentration test and direct smear evaluation for diagnosis was similar. This was in accordance with the findings of many authors such as Valsala and Bhaskaran (1974), Courtney and Zeng (2001) and Suprabha and Devada (2003).

The diagnosis of trypanosomosis was done by observing the organisms on the blood smear which were found to be present intercellularly. Trypanosomosis was diagnosed similarly by Herrera *et al.* (2004) and Gunaseelan *et al.* (2009).

In the case of Evans' syndrome, microagglutination of the RBCs along with the presence of a moderate spherocytosis was observed. The presence of spherocytes was very commonly observed in immune-mediated haemolytic anaemias and took place due to partial phagocytosis of the affected erythrocytes. Microagglutination took place due to the presence of autoantibodies. These findings corresponded to the findings of Mathes *et al.* (2006) and Goggs *et al.* (2008).

In the case of acute lymphoblastic leukaemia, the blood smear evaluation revealed the presence of around 75 per cent abnormal cells which had the appearance of blast cells. Routine evaluation of the blood smear provided the first indication of leukaemia which was also reported by Dobson *et al.* (2006).

Lymphnode aspirations were performed in few cases of anaemia with lymphadenopathy but were not positive for the presence of haemoparasites. The case of lymphosarcoma also presented with severe lymph node enlargement of all palpable lymph nodes. A fine needle aspiration cytology was performed which revealed the presence of slightly pleomorphic round or oval cells which had large nuclei, and suggested the condition as lymphoma. This was similar to the findings recorded by Vail (2000).

Hypochromic red blood cells having increased central pallor were observed in cases of severe tick infestation. Giger (2000) had also mentioned the presence of such RBC changes in dogs with chronic blood loss.

5.5 WET FILM EXAMINATION

Wet film examination was found positive in cases of microfilariosis and one case of trypanosomosis. The diagnosis of microfilariosis and trypanosomosis by wet film examination was also reported by Saseendranath *et al.* (1986) and Herrera *et al.* (2004) respectively.

5.6 SCREENING OF FAECAL SAMPLES

The faecal sample of only one animal, a 60 days old pup, was found to be positive for the presence of gastrointestinal parasites. The ova of *Ancylostoma caninum* were observed. Hall and Simpson (2000) opined that *Ancylostoma caninum* was the most important hookworm of dogs.

5.7 SCREENING OF SPLENIC ASPIRATION SMEARS

Screening of splenic aspiration smears was performed in the cases which showed severe splenomegaly. Out of all the samples screened, only one was positive for the organism *Babesia gibsoni*. In babesiosis there could be retention of erythrocytes in the spleen due to the blocking of microcirculation in the spleen which was also responsible for the splenomegaly (Schetters *et al.*, 1997). Inokuma *et al.* (2005) also demonstrated the presence of small bodies of *B. gibsoni* in fine needle aspirate from the spleen. Therefore in the present study it was concluded that splenic aspirates could also be routinely used for diagnosing cases of babesiosis.

5.8 EXAMINATION OF BONE MARROW ASPIRATES

Bone marrow aspirates were screened in two cases of haemopoietic neoplasms. The results were indicative of acute lymphoblastic leukaemia and lymphosarcoma. Bone marrow aspirate smears of the case of acute lymphoblastic

leukaemia showed a hyper cellular marrow and revealed more than 30 per cent atypical blast cells which was diagnostic of the condition and that of lymphosarcoma revealed groups and scattered slightly pleomorphic round or oval cells having larger nuclei. There were also occasional mitotic cells. These abnormal cells formed about 70 per cent of the bone marrow cells. The remaining cells of the myeloid and erythroid series showed normal maturation sequence. The findings in these two cases were in agreement with Dobson *et al.* (2006) and Weiss (2008).

5.9 ELECTROCARDIOGRAPHY

The most common changes recorded in the electrocardiogram were S-T segment depression, elevation or coving. Prolongation of the QRS duration and increased amplitude of R wave were also found. These changes have been attributed to myocardial hypoxia and compensatory enlargement of left ventricle as mentioned by Dvir *et al.* (2004) and Jutkowitz (2004). Life threatening ventricular tachycardia was observed in the case of haemoabdomen which was similar to the findings of Aronsohn *et al.* (2009). This indicated serious condition of the case and the animal succumbed to death before initiation of antiarrhythmic therapy.

5.10 ULTRASONOGRAPHY

Splenomegaly was the most commonly observed change on ultrasonography. It was seen in case of haemolytic anaemia as well as acute lymphoblastic leukaemia. Splenomegaly was mentioned as a common sign in these diseases by many authors such as Harrus *et al.* (1998), Suto *et al.* (2001) and Willi *et al.* (2007).

The case of haemoabdomen was diagnosed ultrasonologically by finding the presence of a fluid with high cellularity in the peritoneal cavity as recommended by Aronsohn *et al.* (2009).

In case of chronic renal failure, the kidneys were devoid of the normal architecture and had an indistinct cortico-medullary junction. Polzin *et al.* (2000) emphasized that renal ultrasonography should be a part of diagnostic database for patients with chronic renal failure and also to rule out other conditions such as renal neoplasia, renal cystic disease etc.

5.11 TREATMENT

In case of ehrlichiosis and mycoplasmosis oxytetracycline was administered at the rate of 10 mg/ kg body weight intravenously for a period of 5 days. This was followed by oral dosing with doxycycline @ 10 mg/ kg body weight for a period of 10 days. Clinical recovery was observed in all the cases. The treatment regimen followed was as per Chalker (2005) and Sasanelli *et al.* (2009).

Ivermectin @ 0.05 mg/ kg subcutaneously or orally was used against microfilariosis with success as suggested by Soulsby (2005) and Rishniw *et al.* (2006). In some resistant cases combined ivermectin and doxycycline treatment was given as it had microfilaricidal as well as antiendosymbiont (i.e. against *Wolbachia*) activity as suggested by Simon *et al.* (2009).

Diminazene aceturate (Berenil) at the dose rate of 3.5 mg/ kg Body weight was given in the case of *B. gibsoni* infection. Chandoga *et al.* (2002) and Inokuma *et al.* (2005) reported positive responses to diminazene aceturate in cases of *B. gibsoni* infections.

Isometamidium chloride was administered intramuscularly in both the cases of trypanosomosis @ 0.5 mg/ kg Body weight. Recovery was obtained in both cases. Diminazene aceturate was reportedly less active against *Trypanosoma evansi* in dogs (Soulsby, 2005) and so better choice of treatment in canine trypanosomosis was found to be isometamidium chloride.

In case of Evans' syndrome as immunosuppressive agent prednisolone was given @ 2 mg/ kg body weight orally. This was done on the basis of the recommendation of Corato *et al.* (1997).

The cases of ectoparasitism were treated twice with ivermectin @ 200 mcg/ kg body weight subcutaneously at one week interval. The dog suffering from flea infestation was further treated with fipronil spray. Chhabra and Khahra (2003) reported that ivermectin was ineffective against flea infestation. Fipronil on the other hand was found to be a potent killer of fleas (Hutchinson *et al.*, 1998 and Cadiergues *et al.*, 2001).

The case of ancylostomiasis was treated with fenbendazole @ 50 mg/ kg body weight for three days. Dryden and Ridley (1999) reported that fenbendazole was 98.5 per cent effective against *Ancylostoma caninum.*

Injectable iron dextran was administered in all the cases at a dose of 1 mL (50 mg) per animal intramuscularly every alternate day with good results. Similar results were recorded by Abiramy *et al.* (2003) and Bhikane *et al.* (2006).

5.12 BLOOD TRANSFUSION

Blood tansfusion was performed in all the cases which warranted the same on the basis of the clinical condition as well as haematological values of animals as suggested by Rozanski and De Laforcade (2004).

Animals with haemoglobin levels below 6 g/ dL and VPRCs below 12 per cent were selected for blood transfusion. This was in agreement with the work done by Saini *et al.* (2002) and Jutkowitz (2004).

None of the animals that were transfused with blood had a previous history of blood transfusion. None of the cases showed a positive cross matching test with the donor thus reassuring the fact that in dogs the first transfusion was relatively safe because of lack of naturally occurring alloantibodies and were unlikely to experience an acute incompatibility reaction after the first transfusion. Thus it could be concluded that a cross match or blood type was not typically

required before a first transfusion, but it should be done if more than 4 days had elapsed between transfusions (Saini *et al.*, 2002 and Hohenhaus, 2003).

Freshly prepared solution of 3.8 per cent sodium citrate was used as anticoagulant to collect blood. The volume of blood to be collected and transfused was calculated using the formula given by Knottenbelt and Mackin (1998a). The blood was transfused at a slow rate initially to monitor adverse side effects if any and the rate was increased to 10 mL/ kg/ hour after 30 minutes.

Blood transfusion was performed in seven cases out of which five uneventfully survived. One of the cases that succumbed was of haemoabdomen. But the animal was in a poor condition when presented and also had the presence of ventricular tachycardia which indicated a poor prognosis. The other case was one of liver dysfunction which showed a transient recovery but died a few days later. Usually liver diseases expressed clinical signs when 70-80 per cent of liver parenchyma was damaged. This might be the reason for unresponsiveness to blood transfusion (Rothuizen and Meyer, 2000).

Only one case which was administered blood transfusion showed adverse reactions. A facial swelling predominantly over the eyelids occurred. It was countered by treatment with antihistamines. The signs of adverse reactions were in agreement with those mentioned by Jutkowitz *et al.* (2002). The occurrence of oedema during blood transfusion was mainly due to protein present in plasma and not due to antigen antibody reaction. In such cases blood transfusion could be safely resumed after stopping it for a few minutes. These findings were also observed by Michell *et al.* (1989).

Reports suggested that hypocalcemia could be a hazard of blood transfusion when sodium citrate was used as anticoagulant due to the binding of calcium ions (Rozanski and De Laforcade, 2004). However in the present study no such reaction was observed. So it was concluded that the sodium citrate method of immediate blood transfusion was a safe procedure and could be used confidently.

Considerable improvement in the clinical condition was observed from the very next day in most cases transfused with blood and there was significant improvement in the haematobiochemical values as compared to those animals which were not given a blood transfusion. Since CPDA blood collection bags are not freely available due to problems such as transmission of diseases like HIV, using freshly prepared solution of 3.8 per cent sodium citrate is recommended as an alternative method for collection of blood under field conditions.

Summary

6. SUMMARY

Anaemia is one of the important clinical abnormalities frequently encountered in dogs. It results either from an increased rate of destruction or loss of erythrocytes or from a decreased rate of their production. It is always an indicator of some underlying disease. A number of possible causes have been suggested for anaemia and hence an accurate diagnosis of the underlying disease condition is necessary for successful treatment of the condition. In the present study entitled 'Diagnostic and therapeutic approaches in anaemia of dogs', 22 dogs showing clinical signs of anaemia were subjected to detailed screening to elucidate the causes of anaemia. All the animals were subjected to detailed clinical examination and all parameters under study such as signalment, history, physical examination, haematology, serum biochemistry, adoption of suitable therapy including blood transfusion and treatment response were carried out. All the parameters under study were carried out in six apparently healthy dogs to establish the normal values.

Based on the diagnosis the clinical cases of anaemia were divided into 5 groups:

Group I (n=3) – consisted of animals affected with chronic blood loss anaemia. The three cases included in the study consisted of single cases of severe tick infestation caused by *Rhipicephalus sanguineus*, flea infestation caused by *Ctenocephalides felis* and hookworm infestation caused by *Ancylostoma caninum.*

Group II (n=12) – consisted of twelve animals affected with haemolytic anaemia. Eleven cases with haemoparasites were studied. Out of this four cases of ehrlichiosis, three cases of microfilariosis, two cases of trypanosomosis and single cases of babesiosis and mycoplasmosis were included. One unusual case of Evans' syndrome was also recorded.

Group III (n=1) – a single case of acute blood loss anaemia due to traumatic haemoabdomen was recorded.

Group IV (n=5) – five cases of anaemia associated with chronic diseases were recorded. Three of these were single cases of neoplasms and two were of liver dysfunction.

Group V (n=1) – a single case of anaemia secondary to chronic renal failure was recorded in a dog.

Thus haemolytic anaemia was the predominant cause of anaemia with an incidence of 54.54 per cent followed by anaemia associated with chronic diseases (22.72 per cent), chronic blood loss anaemia (13.63 per cent) and acute blood loss anaemia and anaemia secondary to renal disease (4.5 per cent).

Clinical manifestations of anaemia included pallor of visible mucous membranes, tachycardia, rapid, strong and bounding pulse and exaggerated respiration with tachypnoea. Anorexia, lethargy, exercise intolerance and weakness were also recorded. Abdominal aortal thudding and heart sounds on auscultation of the trachea were also recorded in most of the cases and was an indication of the severity of the anaemia. In case of chronic blood loss anaemia, the signs were of reduced intensity and typical pallor of mucous membranes was also not observed in one of the animals which was simultaneously dehydrated. Haemolytic anaemias presented with additional signs of fever, lymphadenopathy, splenomegaly and haemoglobinuria. The case of acute blood loss anaemia showed signs of hypoxia and hypovolaemia such as cyanosis, delayed capillary refill time and exaggerated respiration. The cases of neoplasms showed specific clinical signs such as splenomegaly and severe lymph node enlargement of all palpable lymph nodes. Polyuria, polydypsia, melena and vomiting were the additional clinical signs in case of chronic renal failure.

The haematological studies showed significant decrease in volume of packed red cells, haemoglobin and total erythrocyte counts in all affected cases.

Most cases of anaemia associated with chronic diseases were expected to be non-regenerative. But in the present study cases of neoplasms showed a regenerative response. Group III showed an increase whereas Group V showed a decrease in MCV values. Groups III and IV showed significant increases in total leucocyte counts. Reticulocyte percentage significantly increased in all groups except Group V. Only one case of non-regenerative anaemia was thus recorded in the study. A significant thrombocytopenia was recorded in all the groups. Most severe thrombocytopenia was seen in cases of haemolytic anaemia.

Low serum protein and albumin values were observed in groups III and V. A hyperglobulinemia was recorded in group I and II. A significant reduction in AG ratio was observed in groups I and V due to low albumin levels. Significant alterations in serum bilirubin levels were noted in cases of haemolytic anaemia and liver dysfunction. Blood glucose was lowered in the cases of trypanosomosis. Serum potassium and phosphorus were reduced and elevated respectively in case of chronic renal failure. Increased values of specific tumour marker, serum alpha-fetoprotein indicated a diagnosis of hepatocellular carcinoma. Increased levels of alanine aminotransferase were observed in both cases of liver dysfunction.

The haemoparasitic infections were diagnosed on the basis of identifying the organisms on Giemsa stained blood and buffy coat smears. A case of *Babesia gibsoni* was diagnosed by screening of splenic aspirate. Spherocytes and microagglutination were observed on blood smear evaluation in case of Evans' syndrome, an autoimmune disease causing anaemia. Abnormal blast cells on blood smear gave a diagnosis of acute lymphoblastic leukaemia. Screening of lymphnode aspirate was used to confirm a case of lymphoma.

Wet film examinations were found positive for all cases of microfilariosis and one case of trypanosomosis.

Faecal sample of one case of hookworm infection was found positive for ova of *Ancylostoma caninum.*

Bone marrow aspirates of two cases of neoplasia were performed to confirm the diagnosis of acute lymphoblastic leukaemia and lymphoma.

Electrocardiogram changes were indicative of left ventricular enlargement which might be due to the compensatory dilatation of heart.

Ultrasonography was used to diagnose the presence of splenomegaly, renal failure, hepatic nodules and haemoabdomen. So ultrasonography should be used as an adjunct diagnostic technique in anaemic cases.

Suitable therapy was adopted depending on the aetiological factors. Intravenous administration of oxytetracycline @ 10 mg/ kg body weight for a period of 5 days followed by oral dosing of doxycycline @ 10 mg/ kg body weight once daily for 10 days was done in cases of ehrlichiosis, mycoplasmosis and few cases of microfilariosis. Intramuscular dosing of isometamidium chloride was done @ 0.5 mg/ kg body weight in cases of trypanosomosis. Diminazene aceturate was administered @ 3.5 mg/ kg body weight intramuscularly in case of babesiosis. Ivermectin @ 0.05 mg/ kg body weight subcutaneously or orally was used against microfilariosis. Evans' syndrome was treated with immunosuppressive doses of prednisolone parenterally and orally. Animals with ectoparasitic infestations were administered with ivermectin @ 0.2 mg/ kg body weight and flea infestation was additionally treated with fipronil. In the case of ancylostomiasis fenbendazole @ 50 mg/ kg body weight orally for 3 days was given.

Apart from the specific therapy, supportive therapy was adopted in appropriate cases, which included intravenous fluids, oral and parenteral haematinics and vitamin supplements. Intra muscular injections of iron dextran @ 50 mg/ animal were found to be effective and good clinical response was obtained.

Five out of seven animals which were given blood transfusion showed remarkable clinical improvement. There were significant increases in the volume

of packed red cells, haemoglobin and total erythrocyte counts as well as serum albumin as compared to the animals which did not undergo blood transfusion.

However few deaths were recorded which was considered to be due to the severity of infection as evident from the drastically decreased haematological parameters.

Thus in the present study it was concluded that –

1. Haemolytic anaemia especially caused by haemoparasites could be considered as the most important aetiological factor in development of anaemia in dogs. Among these, cases of ehrlichiosis, microfilariosis, trypanosomosis, babesiosis and mycoplasmosis were observed in the decreasing order of prevalence.

2. Anaemic cases may not always show the typical signs and may be presented with mucous membranes that are congested or normal and not pale. This could occur due to simultaneous dehydration.

3. The MCV was a useful tool for assessing the presence of a regenerative or non-regenerative anaemia in dogs and was positively correlated with reticulocytosis.

4. Blood transfusion using 3.8 per cent sodium citrate was a very useful and lifesaving measure in the treatment of anaemia and hypoalbuminemia and should be used under field conditions.

5. Parenteral iron dextran was an effective adjunct to specific therapy in cases of anaemia in dogs.

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DIAGNOSTIC AND THERAPEUTIC APPROACHES IN ANAEMIA OF DOGS

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ABSTRACT

The study 'Diagnostic and therapeutic approaches in anaemia of dogs' was conducted in 22 dogs with clinical signs suggestive of anaemia.

Haemolytic anaemia recorded the highest incidence rate of 54.54 per cent followed by anaemia associated with chronic disease (22.72 per cent), chronic blood loss anaemia (13.63 per cent) and acute blood loss anaemia and anaemia secondary to renal disease (4.5 per cent).

All the animals were subjected to detailed clinical examination and all parameters under study such as signalment, history, physical examination, haematology, serum biochemistry, adoption of suitable therapy including blood transfusion and treatment response were carried out. The data obtained were analyzed statistically.

Clinical manifestations of anaemia included pallor of visible mucous membranes, tachycardia, rapid, strong and bounding pulses and exaggerated respiration with tachypnoea. Anorexia, lethargy, exercise intolerance and weakness were also reported.

Clinical pathology revealed significant reduction of volume of packed red cells, haemoglobin and total erythrocyte counts. The erythrocyte indices varied depending on the type of anaemia. A high MCV was recorded which correlated with increased reticulocytosis. Leucocyte counts were significantly increased in case of haemolytic anaemia and in anaemia associated with chronic diseases. Thrombocyte counts were decreased in all groups with severe decrease in case of haemolytic anaemia due to haemoparasites.

Serum total protein and serum albumin were decreased in cases of haemoabdomen and chronic renal failure. The cases of haemoparasitic infection were associated with hyperglobulinemia. A complementary decrease in AG ratio

was also observed in affected groups. Serum bilirubin increased in cases of haemolytic anaemia and liver dysfunction.

Oxytetracycline parenterally followed by oral dosing of doxycycline was used in cases of ehrlichiosis, mycoplasmosis and some cases of microfilariosis. Diminazene aceturate was used in case of babesiosis. Cases of trypanosomosis were treated with isometamidium chloride and ivermectin was used to treat microfilariosis as well as ectoparasitism. Flea infestation was additionally treated with fipronil. Fenbendazole was used to treat ancylostomiasis. Apart from the specific therapy, supportive therapy was adopted in appropriate cases, which included intravenous fluids, oral and parenteral haematinics and vitamin supplements. Intra muscular injections of iron dextran were found to be effective and good clinical response was obtained.

Animals which were given blood transfusion showed remarkable clinical improvement. There were significant increases in the volume of packed red cells, haemoglobin and total erythrocyte counts as well as serum albumin as compared to the animals which did not undergo blood transfusion.

However few deaths were recorded which was considered to be due to the severity of infection as evident from the drastically decreased haematological parameters.

Appendix

ANNEXURE – I

PROFORMA

Case No. / Sl No. **Date**

1. Name and Address of the Owner :

2. Details of the Animal

Breed :
Age :
Sex :
Colour :
Vaccination history :
Details :

Deworming history :
Details :
Diet :

3. Clinical History

Date	Diseases encountered in the past	Treatment adopted

1. General Clinical Examination

2. Clinical Observation

a) Clinical Data

1. Temperature :
2. Pulse (rate per minute) :
   (quality) :
3. Respiration (rate per minute) :
4. Mucous membrane
   (pale, discoloured, icteric) :
5. Lymph nodes :
6. Abdominal palpation/ organomegaly :

b) Clinical Signs

(Present / Absent)

1. Lethargy :
2. Exercise intolerance :
3. Weakness :
4. Anorexia :
5. Overt haemorrhage :
6. Dyspnoea :

3. Haemato-biochemical Findings

Sl no	Parameters	Result	
		Day 1	Day7
	Haematology		
1	Hb (gm/dl)		
2	VPRC %		
3	TEC (10^6/ cu.mm)		
4	TLC(10^3/cu.mm)		
5	DLC		
	Neutrophils (%)		
	Lymphocytes (%)		
	Eosnophils (%)		
	Monocytes (%)		
	Basophils (%)		
6	Reticulocyte count		
7	Platelet count		
8	MCV		
9	MCHC		
10	Wet blood film		
11	Haemo-parasites		
	Serum Analysis		
1	Total Protein (g/dl)		
2	Albumin (g/dl)		
3	Globulin (g/dl)		
4	A: G Ratio		

5 6 7 8	Serum Creatinine (mg %) Total Bilirubin (mg %) Direct Bilirubin (mg %) Indirect Bilirubin (mg %)		

3. Faecal sample analysis

i. Ova of any parasitic importance :
ii. Blood :

4. Results of Special Examination

A. Ultrasonography Findings

i Spleen :

ii Kidney :

iii. Liver :

B. Electrocardiographic Findings

5. Diagnosis :

6. Treatment :

7. Response to treatment :

SIGNATURE OF THE CHAIRMAN **SIGNATURE OF THE STUDENT**