THERAPEUTIC MANAGEMENT OF ANAEMIA ASSOCIATED HYPERGLOBULINEMIA WITH SPECIAL REFERENCE TO ERYTHROCYTE SURVIVAL IN DOGS

T H E S I S

Submitted

In partial fulfillment of the requirements for the Degree of

DOCTOR OF PHILOSOPHY

IN

VETERINARY CLINICAL MEDICINE, ETHICS AND JURISPRUDENCE

BY

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(INDIA)

2024

Appendix - B

DECLARATION OF STUDENT

I hereby declare that the experimental research work and interpretation of the thesis entitled **"THERAPEUTIC MANAGEMENT OF ANAEMIA ASSOCIATED HYPERGLOBULINEMIA WITH SPECIAL REFERENCE TO ERYTHROCYTE SURVIVAL IN DOGS"** or part thereof has not been submitted for any other degree or diploma of any University, nor the data have been derived from any thesis/publication of any University or scientific organization. The sources of materials used and all assistance received during the course of the investigation have been duly acknowledged.

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Appendix – C (i)

DECLARATION OF ADVISORY COMMITTEE

Mr. **HASE PANKAJ BHANUDAS** has satisfactorily prosecuted his course of research for a period of not less than two semesters and that the thesis entitled "**THERAPEUTIC MANAGEMENT OF ANAEMIA ASSOCIATED HYPERGLOBULINEMIA WITH SPECIAL REFERENCE TO ERYTHROCYTE SURVIVAL IN DOGS**" submitted by him is the result of original research work and is of sufficiently high standards to warrant its presentation to the examination in the subject of **VETERINARY CLINICAL MEDICINE, ETHICS AND JURISPRUDENCE** for the award of **DOCTOR OF PHILOSOPHY** degree by the Maharashtra Animal and Fishery Sciences University, Nagpur.

We also certify that the thesis or part thereof has not been previously submitted by him for a degree from any other University.

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Appendix – C (ii)

CERTIFICATE

This is to certify that the thesis entitled **"THERAPEUTIC MANAGEMENT OF ANAEMIA ASSOCIATED HYPERGLOBULINEMIA WITH SPECIAL REFERENCE TO ERYTHROCYTE SURVIVAL IN DOGS"** submitted by **Mr. HASE PANKAJ BHANUDAS** to the Maharashtra Animal and Fishery Sciences University in partial fulfillment of the requirement for the degree of **DOCTOR OF PHILOSOPHY** in the subject of **VETERINARY CLINICAL MEDICINE, ETHICS AND JURISPRUDENCE** has been approved by the Student's Advisory Committee after oral examination in collaboration with the External Examiner.

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Dedicated to my
Family Members...

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"We must find time to stop and thank the people who make a difference in our lives." - John F. Kennedy.

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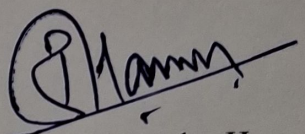
Place: Mumbai



Pankaj Bhanudas Hase

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LIST OF ABBREVIATIONS

%	Percentage
&	And
/	Per
/mm^3	Per cubic millimeter
±	Plus, or minus
ALT	Alanine transaminase
AST	Aspartate transaminase
B	Basophils
BUN	Blood Urea Nitrogen
B.V. Sc. and AH	Bachelor of Veterinary Science and Animal Husbandry
CD	Critical difference
CRD	Complete Randomized Design
dL	Deciliter
DLC	Differential Leukocyte Count
Dist.	District
D + R	Donor + Recipient
E	Eosinophils
E. coli	*Escherichia coli*
EDTA	Ethylene Diamine Tetra Acetic Acid
Ery/ul	Erythrocytes per microliter
et al.	And others
etc.	Et cetera
FCT	Fibrous connective tissue
Fig.	Figure
Fl	Femtoliters
g/dl	Gram per deciliter
GSD	German shepherd
Hb	Hemoglobin
HR	Heart rate
i.e.,	That is
IVD	*In-vitro* Donor
IVR	*In-vitro* Recipient
IVDR	*In-vitro* Donor + Recipient
KFT	Kidney Function Tests
L	Lymphocytes
LFT	Liver Function Tests
M	Monocytes
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
Mg/dl	Milligram per deciliter
MM	Mucus Membrane
M. V. Sc.	Master of Veterinary Science
N	Neutrophils
ND	Non-descript

NS	Non-significant
PCV	Packed cell volume
Pg	Picogram
RBC	Red Blood Cells
RR	Respiration rate
RT	Rectal temperature
S	Significant
SE	Standard error
Spp.	Species
Sr. No.	Serial number
TEC	Total Erythrocyte Count
TLC	Total Leucocyte Count
TP	Total Protein
U/cumm	Units per cubic millimeter
ug/L	Microgram per liter
Viz.	Namely
VCC	Veterinary Clinical Complex
WBC	White Blood Cells
µl	Microliter

Introduction

CHAPTER- I

INTRODUCTION

The dog, technically referred to as *Canis familiaris*, is recognised as the oldest domesticated animal and the initial species to undergo domestication (David *et.al.*, 1974). The dog has fulfilled several roles, including that of a guard dog, a hunting companion, a companion, and even a guide. Before the 18th century, dogs mostly had utilitarian roles in human society. During this period, the expression 'man's best friend' gained frequent usage. Of late, with the advancement in the diagnostics and therapeutics in canine medicine, dog owners are now more particular and keener on general health and the adoption of modern treatment approaches. “Anaemia” is such a disease condition that most dog owners come across as an individual identity or sequelae of the other disorder.

Anaemia is a manifestation of many diseases or the result of multifactorial causes. It is a medical condition characterized by a deficiency of red blood cells or hemoglobin in the blood, resulting in a reduced capacity to transport oxygen to body tissues. Haemoglobin, an essential protein found in erythrocytes, has a vital function in the binding and transportation of oxygen from the lungs to other tissues in the body. Shah *et al.* (2011) found that the major causes of anemia were renal dysfunction (22%), *E. canis* infection (9.17%), Iron deficiency (8.2%), *B. canis* (8.1%), parvovirus infection (6.42%), hepatic dysfunction (6.4%), leptospirosis (4.5%), pyometra (3.6%) and hepatozoonosis (2.75). Protozoa are one of the most important causes of haemolytic anemia in the dog (Irwin, 2010).

Anaemia presents with diverse clinical manifestations that indicate the diminished ability of the blood to transport oxygen. Typical symptoms include exhaustion, debility, and paleness. Dogs suffering from anaemia may encounter breathlessness even with slight physical effort, and their heart rate may rise to compensate for the reduced oxygen supply. The presence of paleness in the skin, mucosal membranes is frequently observed, highlighting the reduced amounts of haemoglobin. (Davidow, 2013).

Immune-mediated hemolytic anaemia (IMHA) is a significant cause of illness and death in dogs. It is characterised by the improper destruction of red blood cells (RBCs) due to immune system activity. The self-attack can be initiated by infections, inflammatory illnesses, neoplasia, certain poisons, and medications. The diagnosis of IMHA can be readily established by seeing the presence of anaemia accompanied by agglutination, spherocytosis, or a positive Coomb's test.

Acquired Immune Haemolytic Anaemia (AIHA) is a condition that has been recognized for a long time and was originally documented in 1954 by Halliwell (1978), and Dodds (1991). Autoimmune Hemolytic Anaemia, is a severe immunohaematological condition that occurs when red blood cells are destroyed as a result of Type II hypersensitivity, after the binding of immunoglobulins to the cell membrane (Jain *et al.*, 1986). The illness has been documented in cats, horses, and cows, although it is more prevalent in dogs (Bennett *et al.,* 1981). The major clinical signs associated with AIHA are lethargy, depression, pale mucus membranes, discolored urine, icterus, petechial hemorrhages on the skin, splenomegaly, hemoglobinemia and hemoglobinuria (Mills *et al.*, 1985; Miller *et al.,* 2009)

Anemia can be classified into two main types based on the capacity of the body and bone marrow to react to the anemia, namely regenerative and non-regenerative anemia. The type of anemia can be identified based on multiple factors, mainly the number of reticulocytes in the blood. Reticulocytes, identified as immature red blood cells (RBCs), originate in the bone marrow from orthochromatic normoblasts through a process called nuclear exclusion. The severity of anaemia can be classified based on the hematocrit or packed cell volume percent as mild (30-37 %), moderate (20-29 %), severe (13-19 %) and very severe (<13 %) (Douglas and Wardrop, 2010).

The Reticulocyte Production Index (RPI) is a numerical value that provides information about the rate of reticulocyte production in the bone marrow relative to the degree of anemia. It is a calculated index that aids in assessing the bone marrow's response to anemia and helps distinguish between different causes

of anemia. The Reticulocyte Production Index helps clinicians to assess the appropriateness of the bone marrow response to anemia and aids in determining the underlying cause of anemia. It is a valuable tool in the field of hematology for understanding the dynamics of red blood cell production in response to various conditions affecting the blood. In cases of anemia, a higher count of peripheral blood reticulocytes, termed reticulocytosis, is observed in patients with a functioning bone marrow. Conversely, anemic patients with dysfunctional bone marrow exhibit decreased reticulocyte numbers, resulting in reticulocytopenia. (Riley *et al.,* 2001). It usually occurs in disorders such as hemorrhagic gastroenteritis, trauma, parasite gastroenteritis, immune-mediated hemolytic anaemia, hemoprotozoan diseases, septicemia, chronic renal disease, and liver diseases.

Coagulopathies or coagulation disorders in dogs refer to disorders or abnormalities in the blood's ability to coagulate or clot properly and go hand in hand with anemia, The coagulation process is vital for stopping bleeding and promoting wound healing. Various factors contribute to the proper functioning of blood coagulation, including platelets, clotting factors, and the blood vessel wall. Coagulopathies may be caused by a variety of reasons including Vitamin K deficiency, autoimmune disease, liver diseases as well as inherited disorders. Coagulopathies may be diagnosed by tests including Prothrombin time test and Partial thromboplastic time test. Clotting time can also be checked using capillary tube test.

Blood lactate level is one of the prognostic indicators in Anemic dogs (Stevenson, 2007). Under aerobic conditions, the intermediate product of glycogenolysis, pyruvic acid, follows an aerobic glycolysis pathway and eventually participates in the Citric-acid cycle or "Krebs cycle" that provides substrates (16 H+) for oxidative phosphorylation. This oxidative phosphorylation provides a large amount of energy for the cells. Under anaerobic conditions, pyruvic acid follows a different route, the anaerobic glycolysis pathway, and the end-product of this complex cascade of reactions results in the accumulation of lactate. The normal resting blood lactate level in dogs is between 1.8 and 22.5 mg/dl or <2.5 mmol/L. In dogs, the measurement of blood lactate holds

significant clinical importance, serving as a key indicator in assessing the physiological status and overall health, particularly in critical or emergency situations.

Red cell distribution width can provide insight into early iron shortage prior to other diagnostic tests. It conveys the concept of variation in the size of red blood cells, which is the initial morphological alteration observed in cases of iron deficient anaemia. Vitamin B12 or folate deficiencies result in a macrocytic anaemia, characterised by the presence of big red blood cells. In about two-thirds of cases, the red cell distribution width (RDW) is increased. Nevertheless, iron deficiency anaemia is characterised by a diverse range of red blood cell sizes, resulting in an elevated RDW in nearly every instance. Both iron and B12 shortages typically result in a combination of big and tiny cells, leading to an increased RDW. High RDW, characterised by red blood cells of uneven sizes, is referred to as anisocytosis.

Serum protein electrophoresis (SPE) shows the distribution of protein fractions, helping clinicians to characterize pathologic processes. (Gori *et al.*, 2022). Protein electrophoresis holds significant importance in various scientific and clinical applications due to its ability to separate, analyze, and characterize proteins based on their electrical charge and size. The electrophoretogram splits the protein fraction of serum or plasma into its constituent components including albumin and globulins. Globulins can be divided into three fractions based on their electrophoretic mobility. Most of the α and β globulins are synthesized by the liver, whereas γ globulins are produced by lymphocytes and plasma cells in lymphoid tissue. α globulins consist of α-1 and α-2 globulins, and β globulins consist of β-1 and β-2 globulins. The third fraction known as γ globulins consists of the immunoglobulins: IgM, IgA, and IgG. It helps in clinical monitoring of a patient, identification of proteins based on size and charge, and as an important diagnostic tool in veterinary medicine.

Hyperglobulinemia refers to an elevated level of globulins in the blood. Globulins are a group of proteins, including antibodies, that play a crucial role in the immune system. A rise in globulin levels can be indicative of various

conditions, such as chronic inflammation, certain infections, autoimmune diseases, or neoplasia. High blood globulin levels and skewed A: G ratios have been noted in CKD, Canine Ehrlichiosis, *Babesia canis,* and pyometra-origin anemic dogs. (Harrus *et al.,* 1999). Several glomerular diseases can result in hypoalbuminemia with an increased serum globulin concentration. A dramatic increase in α-2 globulins is often seen in canine nephrotic syndrome due to VLDL α-2 macroglobulins. Urine protein concentrations and urine protein/creatinine ratios should both be increased with glomerular disease. By SPE analyses decreased levels of albumin and elevated levels of α-2 globulin and β-globulin were noted in dogs with pyometra (Yoon *et al.*, 2021).

Gammopathies are conditions in which serum immunoglobulin levels are greatly increased. They can be either polyclonal (Increases in all major immunoglobulin classes) or monoclonal (Increases in a single homogenous immunoglobulin).

The therapeutic management of anaemia is indeed an important factor that must be taken into consideration as it includes a multifactorial approach, including diet modifications, identifying and treating the cause of anemia, hematinics, iron and vitamin supplementation, erythropoietin stimulating agents, bone marrow stimulants and blood transfusions.

Blood transfusion is a medical procedure that involves the transfer of blood or blood components from one individual (the donor) to another (the recipient). The primary goal of blood transfusion is to replace lost blood components or to provide specific blood constituents to address a medical condition. Blood transfusion is considered a highly suitable supportive treatment for anemia in patients (O'Hara and Richardson, (2008) irrespective of its underlying cause, owing to its rich erythrocyte content. While the history of successful blood transfusions in animals can be traced back to 1665, these procedures have become more commonplace in the last five to six decades (Davidow, 2013). The decision to administer an appropriate blood product is contingent upon accurately quantifying the predominant deficit. When critical levels of hematocrit (HCT) are reached, immediate stabilization of the patient is

imperative to facilitate further diagnosis. Transfusing blood from a compatible donor serves as an excellent solution, ensuring prompt and noticeable stabilization of the patient. Advancements in the preparation and storage of blood products have significantly enhanced the effectiveness of transfusions, with whole blood, erythrocyte concentrate, and plasma emerging as the most frequently used products for transfusion purposes (Freireich, 2011).

Blood transfusion is a procedure that involves a donor, a recipient and the cross-matching of blood to check the compatibility of the blood before transfusion. The recipient must be monitored for acute or delayed transfusion reactions such as hives, respiratory distress, icterus, etc. Despite this, the paramount concern in human and veterinary blood banking and transfusion therapy is to guarantee the safety of blood products. Blood Transfusion responses can be caused by several factors, and their clinical presentations range from immediate to delayed, immune-related or non-immune-related, and including the destruction of red blood cells or not.

Red blood Cell dynamics evolved that the typical lifespan of red blood cells (RBCs) is around 120 days. Every day, around 1% of the erythrocytes in a healthy dog's bloodstream are eliminated owing to senescence, whereas the bone marrow continuously generates new blood corpuscles. In contrast to humans, dogs possess movable storage of red blood cells in their spleen and have the ability to promptly replenish one-third of the given blood. The remaining blood cells will undergo regeneration within a few days. Every day, 200 billion red blood cells (RBCs) are produced, which necessitates the acquisition of almost 2×310^{15} iron atoms every second to sustain sufficient erythropoiesis. These statistics correspond to the production of 20 mL of blood every day, which contains 6 g of haemoglobin and 20 mg of iron.

The “RBC storage lesion” refers to cumulative biochemical and biomechanical changes that occur in RBCs during storage *In-vitro* that decrease their function and survival *In-vivo*. Based on the existing data, it is recommended to focus on two main areas to enhance the quality of red blood cell (RBC) blood bank products. Firstly, efforts should be made to prevent the significant loss of

transfused RBCs that occurs shortly after the transfusion. Secondly, measures should be taken to minimize the interaction between the transfused RBCs and the patient's immune system. Addressing these concerns is crucial for minimizing the frequency and severity of adverse effects associated with transfusions.

Therefore, it is crucial for blood bank research to not only evaluate the traditional RBC characteristics for quality control during storage, but also to find the factors that can predict the survival, function, and behaviour of RBCs in the patient following transfusion. The quality of blood products and the occurrence of hemolysis, both in *in vitro* and *in vivo* can be significantly affected by the circumstances in which the products are stored and the duration of storage.

This study was conducted to find out the keeping quality of canine blood in CPDA to ascertain its suitability for blood transfusion. Preservation of red cells implies maintenance of normal functional capacity of such an extent that after transfusion to the body, they will stay in circulation and perform their functions. *In vitro* studies are useful as screening procedures in evaluating the stored blood.

The Department of Veterinary Medicine, Mumbai Veterinary College has established the country’s first Animal Blood and Blood Component Bank and Referral Coagulopathy Laboratory with the help of ICAR under the Revolving Fund. Therefore, present study entitled “Therapeutic Management of Anaemia Associated Hyperglobulinemia with Special Reference to Erythrocyte Survival in Dogs” was planned with the following objectives.

1. Clinical and laboratory assessment of anaemic dogs.
2. To analyse the serum protein pattern in hyperglobulinemic dogs.
3. To evaluate the therapeutic efficacy of anaemic dogs.
4. To study the survival pattern of erythrocytes (in-*vitro*).

Review of Literature

CHAPTER- II

REVIEW OF LITERATURE

Anemia is commonly encountered in veterinary practice. It is not clinically evident until the level of haemoglobin falls below 8 g/dl in dog (Ettinger *et al.* 2005). Anemia is defined as the decreased ability of blood to supply tissues with adequate oxygen for proper metabolic functions (Hoffbrand and Pettit, 1993). It is characterized by reduction in haemoglobin (Hb), haematocrit (Hct) or total erythrocyte count (TEC) per unit volume of blood in a normally hydrated animal (Aird, 2000). Protein Electrophoresis is a specialized test that analyzes specific groups of proteins in the blood serum and measures how much of each group of proteins is present. Present research work entitled "Therapeutic Management of Anaemia Associated Hyperglobulinemia with special reference to Erythrocyte Survival in Dogs" was undertaken. The literature was reviewed on the following subheadings.

2.1 Anaemia in dogs

2.2 Prevalence of Anaemia

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2.6.3 Blood Lactate

2.7 Blood Transfusion in Dogs

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2.9 Plasma protein in anaemia

2.9.1 Albumin

2.9.2 Globulin

2.10 Treatment of Anaemia

2.1 Anaemia in dogs

Jain (1986) defined anemia as the decreased ability of blood to supply tissues with adequate oxygen for proper metabolic function.

Bhalerao (1997) screened forty clinical cases of anaemia for detection of AIHA. Out of forty cases, fourteen cases were positive for direct antiglobulin test with monospecific and polyspecific antiglobulin sera. Direct antiglobulin test was positive in fourteen cases in anaemia with polyspecific antisera to the extent between +1 to +4. The test was positive with monospecific rabbit anticanine serum in twelve cases to the extent +1 to +4.

Moninder (2003) reported a higher prevalence of 68 per cent anemia in state of Punjab and lower incidence i.e., 37.97% recorded in dogs of Palampur valley of Himachal Pradesh.

Furlanello *et al.* (2005) observed anemia in 74% of dogs and classified it as mild (35%), moderate (59%) and severe (6%) during the study undertaken at Italy.

Radostits *et.al*., (2007) defined anemia as a deficiency of circulating RBCs per unit volume of blood.

Vegad and Swamy (2010) defined anemia as a condition in which the body has a decreased number of circulating erythrocytes (RBCs), or decrease in haemoglobin concentration. Further, it can also be defined as reduction in number of the erythrocytes in an animal for that particular age, species, breed, and geographic location.

Singh *et al.* (2012) screened 137 dogs, out of which 65 dogs had Hb value less than 12 g/dl indicating 47.44 % incidence of anemia during the study on incidence of anemia from Jammu region, of India.

Tandel *et al.* (2012) screened 78 dogs, out of which 51 dogs had Hb value less than 12 g/dl, Total erythrocyte count (TEC) less than 5.5 106 /µland Packed cell volume (PCV) less than 35 % indicating overall incidence of anemia as 65.38 % based on physiological as well as hematological parameters in and around Anand Region, Gujarat.

Brar *et al.* (2014) on CBC examination stated that there is decrease in haemoglobin value, packed cell volume and erythrocytes per microliter indicates anemia.

Bhattacharyya (2015) described anemia as a reduction in haemoglobin concentration or RBC number or packed cell volume per unit volume of blood below the normal value specific for the particular species and also suggested that anemia may be due to excessive blood loss or accelerated RBC destruction.

Stokol (2017) stated that the anemia is most common hematologic abnormality in Veterinary practice, occurring in 28% of canine patients during their study on idiopathic pure red cell aplasia and nonregenerative immune-mediated anemia in 43 dogs performed at the Veterinary Medical Teaching Hospital, Cornell University, USA.

Meshram *et al.* (2019) reported that out of 108 samples, 63 (58.33%) revealed various degrees and types of anemia. The anaemic cases were observed as Normocytic Hypochromic 18 (27.27%), Microcytic Hypochromic 39 (59.09%), Microcytic Normochromic 1 (1.515%) and Macrocytic Normochromic 8 (12.12%) during their study on clinicopathological assessment of anemia in dogs at Mumbai Veterinary College, Mumbai.

2.2 Prevalence of anemia

Moninder (2003) reported a higher prevalence of 68 per cent anemia in state of Punjab and lower incidence i.e., 37.97% recorded in dogs of Palampur valley of Himachal Pradesh.

Furlanello *et al.* (2005) observed anemia in 74% of dogs and classified it as mild (35%), moderate (59%) and severe (6%) during the study undertaken at Italy.

Singh *et al.* (2012) screened 137 dogs, out of which 65 dogs were having Hb value less than 12 g/dl indicating 47.44 % incidence of anemia during the study on incidence of anemia from Jammu region, of India.

Tandel *et al.* (2012) screened 78 dogs, out of which 51 dogs were having Hb value less than 12 g/dl, Total erythrocyte count (TEC) less than 5.5 106 /µl and Packed cell volume (PCV) less than 35 % indicating overall incidence of anemia as 65.38 % based on physiological as well as hematological parameters in and around Anand Region, Gujarat.

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2.2.1 Age-wise prevalence of anemia

Chervier *et al.* (2012) revealed that anemia varied significantly with dog age i.e., young dogs were significantly less affected by Cancer Related Anemia as compared to old dogs during the study conducted at the Veterinary Campus Hospital, Lyon, France.

Singh *et al.* (2012) reported the higher incidence of anemia (44.61%) in young pups below 6 months followed by15.38% in 6 months to 1 year, 20.00 % between 1 to 3 years and 12.32 % between 6-to-10-year age groups,

whereas lowest prevalence in dogs of age group 3 to 6 years (7.69 %) in Jammu region, of India.

Tandel *et al.* (2012) observed the highest incidence rate of anemia in dogs of adult age group (58.83 %, 30/51 cases), followed by young (21.57 %, 11/51) and senile age group (19.60 %, 10/51) in and Around Anand Region, Gujarat.

Brahmbhatt *et al.* (2015) recorded the occurrence of *Ancylostoma* more frequently in dogs of young age (<1 Year) followed by middle age (1-7 year) and lowest in old age (7 years) and age-wise prevalence was 36.02%, 16.15% & 10.89%, respectively by examining fecal samples of Anand district.

Radakovich *et al.* (2017) concluded that clinically healthy, aging dogs of a variety of breeds, living in differing environments, and eating different diets exhibit changes in CBC and serum biochemical profiles which suggest altered physiology or emerging pathology as dogs age. In such cases most notably, there was evidence for iron deficiency potentially due to gastrointestinal bleeding and inflammation during their study on hematology and biochemistry of ageing, evidence of anemia in older dogs held at Colorado State University's (CSU) Veterinary Teaching Hospital, USA.

Meshram *et al.* (2019) stated that out of total 65 dogs with anemia, the highest incidence was observed in young group (25 cases, 38.46 %) followed by senile group (25 cases, 37.87 %) and adult group (15 cases, 22.72 %) of dog during clinicopathological assessment of anemia in dogs at BVC, Mumbai.

Shah *et al.* (2020) observed anemia at all ages but higher incidence was observed in young pups less than 1 year (33%) and minimum in the age group of more than 10 years (10%) during their etio-pathological study on canine anemia conducted at Small Animal Clinics of the Department of Veterinary Clinical Services Complex, GADVASU, Ludhiana, Punjab, from August 2008 to February 2009.

2.2.2 Breed-wise prevalence of anemia

Vidyabharathi (1990) observed anemia due to Ehrlichiosis mostly in crossbred dogs, followed by Pomeranian, Alsatian, Non-descripts, Doberman and

Labrador, whereas *Hepatozoon canis* infection was mostly encountered in non-descript dogs followed by Pomeranian, Doberman, Labrador and Alsatian and Babesiosis was recorded in crossbreds followed by non-descripts and Alsatian breeds at Madras Veterinary College, Madras.

Stokol *et al.* (2000) reported that Labrador retrievers were significantly over represented with pure red cell aplasia and non-regenerative immune mediated anemia during their study performed at the Veterinary Medical Teaching Hospital, Cornell University.

Singh *et al.* (2012) conducted the study on incidence of anemia in dogs and reported maximum breed-wise incidence in mixed/desi/mongrels i.e., 52.33 per cent, followed by Pomeranian i.e., 10.76 per cent, Labrador and Spitz i.e., 9.23 per cent, German shepherd i.e., 7.69 per cent and followed by other breeds (Saint Bernard, Bull dog, Boxer and Pointer) i.e., 10.76 per cent from Jammu Region, of India.

Tandel *et al.* (2012) observed high incidence of anemia in Labrador (35.29 %; 18/51 cases) followed by German Shepherd and Doberman (13.73 % each, 7/51 cases) during their study of an epidemiological status of Anemia in Dogs in and around Anand Region of Gujarat.

Swann *et al.* (2013) stated that IMHA is prominent problem in a number of popular breeds, including Cocker and Springer Spaniels, Old English Sheepdogs, Bichon Frises, Bearded and Rough-coated Collies, Poodles, and Flat coated Retrievers during American College of Veterinary Internal Medicine consensus statement on the treatment of immune-mediated hemolytic anemia in dogs.

Brahmbhatt *et al.* (2015) recorded the highest prevalence of *Ancylostomosis* in Mongrel (stray dog) (42.85%) followed by German Shepherd (24.67%), Labrador retriever (19.54%), Pomeranian (16.25%), and lowest in a Doberman (14.10%) by examining fecal samples from Anand district, Gujrat.

Liu *et al.* (2015) reported the highest prevalence of anemia in mongrel (40.0%), followed by Maltese (13.1%), Golden Retriever (8.2%), Miniature Schnauzer (6.7%), Miniature Poodle (4.4%) and lowest in Labrador Retriever

(4.2%) breeds of dog during a five-year retrospective survey on causes of canine anemia in Taiwan.

Meshram *et al.* (2019) analysed the haematological data for incidence of anemia in dogs at Mumbai Veterinary College, Mumbai and reported that out of 65 anaemic cases, the incidence of anemia was highest in Mastiff and Chihuahua breed (100%) followed by Cocker spaniel (83.33%), German shepherd and Pomeranian (80.00 %), Labrador (17 cases, 68.00 %), Bull and Dachshund (66.67%), Golden retriever (60.00%) Doberman Pinscher, Beagle, Siberian husky, Great Dane, Rottweiler and Non-descript (50.00 %), Boxer and Pug (33.33%), while it was not observed in Saint Bernard, Lhasa Apso and Shih Tzu breeds of dog in their study at Mumbai Veterinary College, Mumbai.

Shah *et al.* (2020) observed maximum anemic cases in Labrador (40.36%) followed by German Shepherd (27.52%), Spitz (11.9%) and Dalmatian (5.5%) among the pure breeds during their etio-pathological study on canine anemia conducted at Small Animal Clinics of the Department of Veterinary Clinical Services Complex, GADVASU, Ludhiana, Punjab, from August 2008 to February 2009.

2.2.3 Sex-wise prevalence of anemia

Stokol *et al.* (2000) recorded that the female dogs were predisposed to develop immune mediated hemolytic anemia. They observed 66% of dogs with Pure Red Cell Anemia and Non- regenerative immune mediated anemia were female, of which 54% were spayed females during their study of idiopathic pure red cell aplasia and non-regenerative immune mediated hemolytic anemia held at Department of Biomedical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, New York.

Tandel *et al.* (2012) reported a higher incidence of anemia in males (52.94 %) as compared to females (47.06 %) during their study of an epidemiological status of Anemia in Dogs in and around Anand Region of Gujarat.

Brahmbhatt *et al.* (2015) recorded highest prevalence of Ancylostomosis in males (29.41%) and the lowest in females (14.61%) by examining fecal samples of dogs in Anand district, Gujrat.

Meshram *et al.* (2019) revealed that the percentage of anemia cases in male was more than the females during their clinicopathological studies in dog at Mumbai Veterinary College, Mumbai.

Shah *et al.* (2020) reported that the majority of anemic cases were falling in moderate to severe type of anemia with higher incidence in males (64.2%) than in females (35.8%) during their study on canine anemia held at GADVASU, Ludhiana, Punjab.

2.2.4 Season wise prevalence of anemia

Vidya Bharathi (1990) recorded high incidence of anemia in dogs during dry season correlating high acarid activity during their study held at Madras Veterinary college, Madras, TN.

Klag *et al.* (1993) reviewed 42 cases of dogs, and observed 40% of total anemic cases diagnosed for IMHA significantly during months of May and June during the study undertaken at Veterinary Hospital of the University of Pennsylvania (VHUP).

Oliveira-Sequeira *et al.* (2002) screened fecal samples from 271 dogs employing sedimentation, simple flotation and centrifugation-flotation methods and reported peak egg count in case of Ancylostomiasis at the beginning of summer during April and May during their prevalence study of intestinal parasites in dogs at Brazil.

Matjila *et al.* (2005) observed more cases of Canine Babesiosis in the spring and autumn in the Netherland.

Ahmad *et al.* (2007) studied babesiosis in dogs and recorded high infection percentage (1.82% to 3.13%) in the year 2004 and slightly low infection percentages i.e., (1.19 % - 2.17%) in the year 2005 during May to September in the Lahore region.

Gadahi *et al.* (2008) collected 300 blood samples and screened for blood parasites, and revealed the highest prevalence of blood-parasites in the month of July (13.30%) followed by June (11%) and May (10%) resulting into anemia in dogs during their prevalence study of Blood parasites in stray and pet Dogs in Hyderabad Area, Sindh.

Kidd *et al.* (2014) studied the seasonal incidence of idiopathic IMHA in dogs as more cases presented in spring and summer at a practice located in San Diego, USA but not at a practice located in Los Angeles, USA. Further, the study noted environmental differences influenced the incidence of IMHA in dogs.

Brahmbhatt *et al.* (2015) in the study of ancylostomiasis from Anand district, reported seasonal prevalence 29.50%, 24.81%, and 15.03% of summer, monsoon, and winter seasons, respectively by fecal sample examination.

2.3 Etiology of Anaemia

Searcy *et al. (*1979) studied that hemolysis can be intrinsic or extrinsic. Intrinsic hemolysis is rare and caused by an inherent metabolic defect in red cells such as red cell pyruvate deficiency in Besenjis

Giger *et al. (*1985) opined that extrinsic hemolysis involves external factors like red cell parasites, drugs or antibodies directed against red cell membrane that renderthe red cell abnormal and more susceptible to phagocytosis resulting inreduction of red cell life span. Based on response of bone marrow in the form of reticulocyte count, anemia is classified into regenerative and non-regenerative.

Kerl and Hohenhaus (1993) reported that anemia can be classified according to its cause into hemorrhagic, haemolytic and bone marrow hypoplasia.

Giger (2000) studied that acute blood loss can occur as a result of trauma, surgery, bleeding, gastrointestinal ulcers, renal and bladder neoplasia etc. The causes of chronic blood loss include urinary or gastrointestinal tumors and less frequently of severe flea orhookworm infestation. Initially anemia is regenerative but due to depletion of iron stores, it diverts to microcytic, hyprochomic and non-regenerative nature

Wanner and Harrus (2000) discussed that red cell destruction (hemolysis) and loss are causes of regenerative anaemia. Differentiation of these categories requires evaluation of other parameters including TP, bilirubin, plasma colour and red cell morphology. Non-regenerative anemias are more common and are due to decreased red cell production. These types of anemias are usually normocytic and normochromic or microcytic and hypochromic. Many diseases can result in non-regenerative anemias with most common cause being anemia of chronic disease (ACD) such as Inflammatory processes, chronic infections and disseminated neoplasias lead to decrease in iron availability, erythrocyte survival and response to erythropoietin

Neiger *et al.* (2002) stated that the red cell distribution width (RDW), which provides a quantitative measure of the heterogeneity of red cell population (anisocytosis) in the peripheral blood, the mean corpuscular volume (MCV) and a regression model combining both variables can be used to assess their predictive accuracy in differentiating dogs with regenerative anemia, which had been diagnosed on the basis of corrected reticulocytes count.

Pierre (2002) stated that reticulocytes are immature red cells that contain a reticulum network of RNA, mitochondria and organelles which are visible with supravital stains, such as New methylene blue and Brilliant cresyl blue .

Cowgill *et al. (*2003) opined that reticulocytes are prematurely released from bone marrow in response to elevated erythropoietin levels induced by tissue hypoxia and indicate enhanced bone marrow erythropoesis. In dogs an absolute reticulocytes count of greater than 60000 to 80000/µl or greater than 1 to 1.5 per cent typically indicates regeneration.

Barger (2003) stated that additional causes of non-regenerative anemia include myelofibrosis, myelopthisis, chronic renal disease, infectious diseases that affect red cell maturation (leukaemia virus, *E.canis*), drug toxicities (oestrogen compounds, doxorubicin and vincristine) and when the immune-mediated destruction is directed at erythroid precursors rather than mature red cells.

2.3.1 Anemia due to parasites

2.3.1.1 Babesiosis

Conrad *et al. (*1991) opined that babesiosis is a major cause of haemolytic anemia in dogs *and B. gibsoni* infection is difficult to detect in peripheral blood smear and misdiagnosed as idiopathic or autoimmune haemolytic anemia. The hematological findings of babesiosis reveal regenerative anemia, thrombocytopenia and leucopoenia with neutropenia and degenerative left shift. Serum biochemical values are normal except for lower than normal serum protein concentration.

Irizarry-Rovira *et al.* (2001) studied haematological and biochemical changes in a dog infected with *B. gibsoni* which indicated severe regenerative, normocytic to macrocytic normochromic anemia with poikilocytosis, polychromasia, anisocytosis and marked increase in nucleated RBCs. Increased ALT, ALP and gamma glutamyl transferase (GGT) activities were also present.

Furlanello *et al.* (2005) reported that out of 23 dogs infected with large form of babesia (*B. canis*), 74 per cent dogs presented anemia and among them 70 per cent had haemolytic anemia and 30 per cent had non-haemolytic anemia. The mechanism of hemolysis was not immune-mediated due to absence of spherocytosis and auto-agglutination. In all the cases anemia was normocytic and normochromic. Sixty nine percent of dogs showed leucopenia, 74 per cent neutropenia and 13 per cent showed erythrocytic regeneration. In majority of dogs, mild elevation of AST, ALT, ALP, Creatinine kinase (CK), total bilirubin and lactic acid and decrease of total iron and total iron binding capacity (TIBC) were present.

Jacobson (2006) reported that half of the animals affected with babesiosis were severely anemic (Hct < 15%), 32 per cent were moderately anemic (Hct<15-30%) and 18 per cent non-anemic. On the basis of warm in- saline agglutination and spherocytosis secondary IMHA was observed as complication of babesiosis. Hyperlactemia was present in approximately half and hypoglycaemia in 20 per cent of cases.

Niwetpathomwat *et al.* (2006) studied the clinical haematology and serum biochemistry of dogs infected with babesiosis. The hematological parameters in most of these patients showed microcytic hypochromic anemia and thrombocytopenia. Leucocyte abnormalities were non-specific as either leukocytosis or leucopenia was observed. Serum chemistry values of BUN, creatinine, liver enzymes, total protein and albumin were within the normal range.

Ruiz de Gopegui *et al.* (2007) reported thrombocytopenia, normocytic normochromic anemia, leukocytosis, leucopenia, neutropenia and monocytosis as primary complete blood count abnormalities in canine babesiosis. An increase in BUN was observed in 47 per cent, hyperfibrinogenemia in 74 per cent and discoloured urine due to hemoglobinuria was observed in 33 per cent of dogs. The levels of ALT, ALP, creatinine and total protein were within the normal range, whereas, hyperglycaemia was a common finding.

Zygner *et al.* (2007) evaluated the haematological changes in blood samples from 248 dogs naturally infected with large babesia. The most common disorders observed were thrombocytopenia and anisocytosis. Anemia was present in 29 per cent, increased MCHC in 21 per cent, anisocytosis in 60.5 per cent, poikilocytosis in 25 per cent, polychromasia in 23.8 per cent, hypochromasia in 19.7 per cent erythroblastosis in 4.45 per cent, thrombocytopenia in 99.55 per cent, increased MCV in 15.3 per cent, neutropenia in 36.3 per cent, left shift in 21.85 per cent, lymphocytosis in 14.9 per cent and lymphopenia in 7.2 per cent cases.

2.3.1.2 Hepatozoonosis

Gondim *et al.* (1998) studied that the haematological and biochemical findings in canine hepatozoonosisrevealed anemia, leukocytosis with neutrophilia, lymphopenia, monocytosis and elevation in ALP.

Paludo *et al.* (2003) reported normocytic normochromic regenerative anemia and an elevated leucocyte count in canine hepatozoonosis.

Assarasakorn *et al.* (2006) studied clinical haematology and biochemistry of canine hepatozoonosis. The haematological findings demonstrated microcytic hypochromic anemia, variable leucocyte count and normal platelet count. Serum

biochemical values including BUN, creatinine, liver enzymes, total protein, albumin and glucose were within normal ranges. Abnormality in levels of glucose, albumin, globulin and ALP was found in some dogs.

Mundim *et al.* (2008) studied that anemia, neutrophilia, leukocytosis and eosinopenia were also observed in canine hepatozoonosis

2.3.1.3 Ehrlichia

Dagnone *et al.* (2003) determined the prevalence of ehrlichiosis in dogs with anemia, thrombocytopenia or ticks. Out of 129 dogs studied, 68 carried the brown dog tick, 61 had thrombocytopenia and 19 had anemia. 21 per cent dogs with ticks had ehrlichiosis.

Niwetpathomwat *et al.* (2006) studied the clinical haematology and serum biochemistry of dogs infected with ehrlichiosis. The main clinical signs of canine ehrlichiosis are pale mucus membrane, lymphadenopathy, splenomegaly, hepatomegaly, emaciation and hair loss. The clinical haematology showed mild to moderate anemia and severe thrombocytopenia. The red cell count, Hb, Hct, WBC, platelet count and total plasma proteins all were decreased. Twenty percent of cases had an increased BUN level and elevated levels of liver enzymes values (ALP, ALT and AST).

Shipov *et al.* (2008) studied prognostic indicators for monocytic ehrlichiosis. The haematological findings observed were anemia, leucopenia, thrombocytopenia and serum biochemical abnormalities like hypoalbuminemia (90%), increased activities of ALP and lactate dehydrogenase, hyperglobinemia, increased AST, hypokalemia, and increased activity of creatinine kinase.

2.3.2 Immune-mediated haemolytic anemia (IMHA)

Frank *et al.* (1977) reported that in IMHA, red cells are destroyed as a result of anti-erythrocyte antibody production. IgM mediated hemolysis is caused mainly by intravascular complement activation and subsequent intravascular hemolysis. This is in contrast with the IgG mediated hemolysis where hemolysis ismainly caused by macrophages in liver, spleen or both.

Slappendel (1979) stressed the diagnostic significance of the direct antiglobulin test (DAT) in anemic dogs. By using monospecific reagents (coomb's reagent) in 371 dogs, the DAT result was positive in 134 dogs (36.1%) and negative in all 35 control dogs. The following antibodies/complement components were detected in DATs positive dogs; IgG alone in 15(11.2%), IgG and C3b in 41 (30.6%), C3b alone in 74 (55.2%) and IgM and C3b in 2 (1.5%). The study revealed a high incidence of positive DAT in anemic dogs with internal diseases.

Jackson and Kruth (1985) discussed retrospectively studied immune-mediated haemolytic anemia and thrombocytopenia in dogs. Females were slightly over represented and the mean age of dogs was 6.4 years. All the dogs with IMHA presented severe anemia, high reticulocytes count, leukocytosis, neutrophilia, elevated band response and monocytosis.

Klag *et al.* (1993) reported the mean age of dogs that suffered from IMHA as 6.4 years. IgG without complement was reported as the main antibody involved in causing hemolysis. They reported spherocytosis in 67 per cent, hemoglobinuria and hemoglobinemia in 10 per cent, marked bilirubinuria in all the cases, severe anemia in 88 per cent, moderate to severe reticulocytosis in 38 per cent and mild reticulocytosis in 29 per cent cases. A significant seasonal incidence was observed with 40 per cent of all cases diagnosed during the months of May and June. In the study of IMHA in dogs found severe anemia with PCV≤ 20% in 88% cases, moderate to severe reticulocytosis in 38% cases, mild reticulocytosis in 29% cases, mild to severe thrombocytopenia in 67% cases and spherocytosis in 67% of cases, while hemoglobinemia and haemoglobinuria only in 10% cases. Also, they found marked bilirubinuria in all dogs affected with IMHA during the study undertaken at Veterinary Hospital of the University of Pennsylvania (VHUP).

Duval and Giger (1996) opined a temporal association between recent vaccination and IMHA. He observed that vaccination against several diseases can be the cause of IMHA in dogs at the Veterinary Hospital of the University of Pennsylvania (VHUP) from December 1992 to March 1995.

Burgess *et al.* (2000) reported mortality rates up to 70 per cent in dogs suffering from IMHA during the 1st 3 weeks of treatment (The presence of auto-agglutination (Duval and Giger 1996), the degree of reticulocytosis (Klag *et al.* 1993), the severity of anemia (Klag *et al.* 1993), thrombocytopenia (Carr *et al.* 2002), severity of leukocytosis (Burgess *et al.* 2000 and Weinkle *et al.* 2005), increase in bands (Weinkle *et al.* 2005), serum bilirubin concentration (Klag *et al.* 1993, Duval and Giger 1996, Reimer *et al.* 1999 and Carr *et al.* 2002) and increase in prothrombin time (Burgess *et al.* 2000 and Weinkle *et al.* 2005) all are associated with poor prognosis.

Stokol *et al.* (2000) recorded pure red cell aplasia (PRCA) and non-regenerative forms of IMHA in dogs. The chronicity of anemia (based on duration of clinical signs) and response to treatment with immunosuppressivedrugs in these reports favoured an immune-mediated pathogenesis for non- regenerative nature of these disorders**.** PRCA and IMHA were most common in middle aged Labrador and Retriever breeds. All dogs presented severe non-regenerative anemia which was predominantly normocytic and normochromic. The leucocyte counts were within the normal range and band neutrophils were generally found in low numbers. Thrombocytopenia was observed in 22 per cent of cases.

Cotter (2001) stated that immune-mediated hemolysis may also be caused by alloantibodies that are antibodies produced by one individual that react with antigens in another members of same species directed against RBC membrane components e.g. blood group incompatibility, transfusion reactions and neonatal isoerythrolysis.

Scott-Moncrief *et al. (*2001) elaborated that diagnostic imaging in patients with haemolytic anemia may detect underlying diseases that mimic or trigger IMHA. Abdominal radiographs should be obtained to evaluate spleen and liver size as well as to detect metallic (Zinc) foreign bodies and mass lesions. Animals that present with severe tachypnea should be evaluated for pulmonary thromboembolism, a common complication of the hypercoagulability state associated with IHA

Carr *et al.* (2002) proposed approximately one quarter of patients with IMHA in several studies have been found to be vaccinated within 30 days of presentation.

Good (2002) reported 4 to 10 fold increase in liver enzymes indicative of hepatocellular damage and hyperbillirubinemia, reduced blood urea nitrogen (BUN), albumin and cholesterol, indicating reduced hepatic function in a case of IMHA. The dog had significant antibody titre for *Leptospira canicola* which was possibly the cause of hepatic damage.

Miller *et al. (*2004) described that no single finding is pathognomonic for IMHA, but the following have been suggested as adequate criteria for diagnosis. Anemia with Hct < 25 per cent to 30 per cent. Evidence of hemolysis characterised by hemoglobinemia or hemoglobinuria. Evidence of antibodies directed against RBCs, with auto agglutination, spherocytosis or positive result from a direct antiglobulin (Coomb's test). Elimination of other underlying causes of anemia.An appropriate response to immunosuppressive therapy.

Trepanier (2004) studied that several drugs (e.g. sulfa drugs, penicillin, cephalosporins, levamisole, insulin, acetaminophen, tetracycline, phenylbutazone, dipyrone, quinidine, chlorpromazine have been suggested to cause IMHA

Nassiri *et al.* (2005) studied that the hematological and biochemical findings of IMHA included anemia, anisocytosis (61%), spherocytosis (54%), polychromasia (38.5%), thrombocytopenia (54%), hyperbillirubinemia (80%) and increased activity of ALT (46.2%) and ALP (61.5%). The main causes of anemia were found to be infectious diseases (e.g. pyometra) and drug therapy (cotrimoxazole). Study concluded that out of 40 anaemic dogs, 14 cases (35%) were positive for IMHA which were diagnosed with direct Coombs' test. They observed that age of 14 dogs with IMHA ranged from below 2 months to 12 years old, with a mean age of 3.7 years. In that, nine dogs (64%) were female and five (36%) were male. The most frequent breeds were Terriers (four cases) and German shepherds (three cases) and main causes of IMHA were infectious diseases (e.g., pyometra) and drug therapy (e.g., cotrimoxazole) during the investigative study of the prevalence of immune-mediated hemolytic anemia

(IMHA) in anemic dogs referred to the Veterinary Teaching Hospital of the University of Tehran.

Weinkle *et al*. *(*2005) postulated that in young animals with hemolytic anemia, a hereditary disorder, such as pyruvate kinase deficiency (found in Besenjis and other canine breeds), phosphofructokinase deficiency (found in English Springer and American Cocker spaniels), or hereditary RBC osmotic fragility (found in Alaskan malamutes and Miniature Schnauzers), should be considered.

Peik *et al.* (2008) in their study investigated dogs with idiopathic IMHA that had been treated according to a protocol of prednisolone and azathioprine with the objective to determine the treatment outcome and to identify prognostic variables. In their study they concluded that the main predictors of mortality of dogs with idiopathic IMHA are the presence of increased plasma urea concentration, bands, thrombocytopenia and petechiae at the time of diagnosis.

Miller *et al.* (2009) examined all dogs presented at the Animal Medical Center between July 1, 1995, and July 1, 1999, and reported that the Cocker Spaniels specially with blood type DEA 4 had increased risk of IMHA in their case-control study of blood type, breed, sex, and bacteremia in dogs with immune-mediated hemolytic anemia held at New York.

Bovens *et al.* (2014) diagnosed immune-mediated haemolytic anemia (IMHA) in a 10-year-old male neutered small Terrier cross infected with leptospirosis and presumed that IMHA was secondary to the leptospirosis during their study conducted at the Langford Veterinary Services referral hospital, University of Bristol, UK.

Garden *et al.* (2019) described infections, cancer, drugs, vaccines, and inflammatory processes as an underlying cause of immune mediated hemolytic anemia during their study on diagnosis on immune mediated hemolytic anemia in dogs and cats.

Shah *et al.* (2020) confirmed 4 cases of IMHA on the basis of direct Coomb's test, out of those 3 had *E. canis* and one *Leptospira* infection concurrently during the study undertaken at GADVASU, Ludhiana, Punjab.

2.3.3 Immune-mediated thrombocytopenia (IMTP)

Middleton (2005) studied hematological and biochemical abnormalities in a German Shepherd dog presented with immune-mediated thrombocytopenia (IMTP). The CBC revealed a marked thrombocytopenia, moderate anemia and mild leukocytosis. The leukocytosis was characterized by increased segmented and band neutrophils. The biochemical profile showed slightly increased glucose, increased phosphorus, decreased total serum protein and decreased serum globulins. The coagulation profile revealed a normal prothrombin time (PT) and partial thromboplastin time (PTT). This case was unique due to young age of the dog as IMTP is primarily seen in middle aged dogs.

2.3.4 Pancytopenia

Shelly (1988) defined pancytopenia as decreased circulating numbers of all marrow cell lines: myeloid, erythroid and megakaryocytic. Pallor and bleeding tendencies are most common clinical manifestations and are directly related to anemia and thrombocytopenia respectively. Causes of pancytopenia include drug toxicity, neoplasia and infectious diseases.

Weiss *et al. (*1999) reported that in dogs the most common cause of pancytopenia is drug therapy especially chemotherapeutic drugs. Nonetheless, many other causes occur at lower frequency, including parvovirus infection, *E. canis*, neoplasia (malignant histiocytosis, myelodysplastic syndrome) and presumed immune-mediated destruction of hematopoietic precursors

Stern (2005) conducted a study on immune-mediated pancytopenia in dogs presented with moderate non-regenerative normocytic normochromic anemia, and leukopenia consisting of moderate neutropenia, lymphopenia and eosinopenia, and a moderate thrombocytopenia. The most frequent abnormalities in serum chemistry were mild hypokalemia and hypoalbuminemia along mildly elevated alkaline phosphatase and mild hypoferremia.

2.3.5 Aplastic anemia

Steinberg (1970) suggested that it is characterised by leukopenia and thrombocytopenia which usually develops within two weeks of initial marrow

injury. The anemia is usually mild or absent due to long RBC life span. However chronic aplastic anemia is characterised by neutropenia, thrombocytopenia and moderate to severe non-regenerative anemia

Weiss and Armstrong (1984) opined that aplastic anemia is also termed as aplastic pancytopenia in blood and panhypoplasia of bone marrow with marrow space replaced by adipose tissue

2.3.6 Pure red cell aplasia (PRCA)

Glauberg and Beaumont (1978) stated that Pure red cell aplasia (PRCA) is a hematological disorder characterized by severe non-regenerative normocytic normochromic anemia associated with severe depletion of erythroid precursor cells in the bone marrow.

Weiss (2001) studied that granulopoiesis and thrombopoiesis are not affected, so total leucocyte and platelet counts are not decreased in blood, and adequate numbers of precursors are present in bone marrow

2.3.7 Iron deficiency anemia

Weiser and O'Grady (1983) studied hematologic and biochemical features in dogs with iron deficiency anemia attributable to chronic external blood loss. Consistent abnormalities in hemograms included moderate to marked reticulocytosis, decreased MCV, and decreased MCHC. Hypoproteinemia occurred in 33 per cent cases. Consistent blood film findings included hypochromic cells, leptocytes and erythrocyte fragmentation. Biochemical abnormalities indicated decreased serum iron value and percent transferring saturation values. The total iron binding capacity values of these dogs were not significantly different than those of healthy dogs.

Gilson *et al.* (1990) evaluated two commercial kits for detection of occult blood in faeces of dogs and concluded that occult blood testing is useful for detection of blood in faeces at volumes 20 to 50 times less than that required to cause melena.

Smith (1992) elaborated that most of the iron deficiency anemia is regenerative resulting from slow chronic or intermittent blood loss particularly from gastrointestinal tract.

Swenson and Reece (1993) studied that erythrocytes wait for iron needed in synthesis of hemoglobin and after further cell division smaller than normal red blood cells deficient in haemoglobin are produced leading to the development of microcytic hypochromic anemia

Laflamme *et al. (*1994) opined that the common cause of microcytic anemia is generally iron deficiency.

Harrison *et al. (*2002) suggested that iron deficiency anemia is mostly reported in cases of chronic blood loss from blood sucking parasites, gastrointestinal and urinary bladder tumors or hemorrhagic disorders in young animals. Blood feeding hookworms are a leading cause of iron deficiency anemia.

Rockey (2005) stated that occult gastrointestinal bleeding commonly manifests as iron deficiency or faecal occult blood.

Steinberg and Olver (2005) concluded that reticulocyte haemoglobin content (CHr) and decreased reticulocyte MCV are associated with hematologic and serum biochemical abnormalities indicative of iron deficiency in dogs.

Fry and Kirk (2006) compared reticulocyte indices with conventional hematologic and biochemical indices as markers of iron deficiency in dogs.

2.3.8 Anemia of chronic, infectious and inflammatory diseases

The multifactorial aetiology of anemia of chronic disease include malnutrition, chronic blood loss through repeated sampling or gastrointestinal losses, renal failure, coagulopathy, drugs or bone marrow infiltration with malignant cells.

2.3.8.1 Renal failure

King *et al.* (1992) conducted a study to evaluate the incidence, type and aetiology of anemia in chronic renal failure (CRF). A non-regenerative, normochromic, normocytic anemia was seen in 70.6 per cent dogs and they found a direct correlation between the degree of anemia and extent of CRF as assessed by serum creatinine concentrations. Increase in serum parathyroid hormone and phosphorus were not found to correlate significantly with the degree of anemia, although there were significant differences in their concentrations in anemic as compared with non-anemic dogs. There was no change in erythrocytic osmotic fragility with uremia.

King *et al.* (1992) stated that anemia is a common clinical finding in dogs with chronic renal failure (CRF) and a major factor related to anemia appeared to be decreased erythropoietin production by kidneys during their study on anemia of Chronic Renal Failure in Dogs held at Veterinary Hospital of the University of Pennsylvania (VHUP).

Santoro (2002) elaborated that the major cause of anemia in renal failure is an adequate production of a glycoprotein hormone, the erythropoietin which is primary regulator of growth and survival of erythroid progenitor. The introduction of recombinant human erythropoietin has revolutionised the treatment of anemia in chronic renal failure.

Kim *et al.* (2006) reported microcytic hypochromic non regenerative anemia caused due to the membranoproliferative glomerulonephritis in a case of Canine Babesiosis presented to Konkuk University Veterinary Teaching Hospital, Korea.

Buranakarl *et al.* (2008) concluded that in renal azotemic dogs PCV progressively decreased along with degree of renal azotemia and was correlated with degree of renal impairment as measured by plasma creatinine ratio.

Martinelli *et al.* (2016) screened two-thousand two-hundred and seventy-one medical records of dogs presented to the Cardiology Service centre and observed that prevalence of anemia is higher in cases complicated with chronic kidney disease and chronic mitral valve disease during preliminary investigation

of Cardiovascular–Renal Disorders in Dogs at Department of Veterinary Science and Public Health, University of Milan.

Shah *et al.* (2020) studied and found that renal failure as one of the most important causes of anemia during characterization study of hematobiochemical indices in canine anemia at GADVASU, Ludhiana, Punjab.

2.3.8.2 Pyometra

Davis (1944) reported the production of hyperchromic anemia in dogs by administration of choline chloride.

Singh *et al. (2*006) mentioned that pyometra is one of the common causes of death in older female dogs and kidney damage with nephritis is often associated with it. The hematologic abnormalities seen in pyometric bitches include anemia leukocytosis along with increased neutrophil count and decreased lymphocyte count and monocyte count. The biochemical abnormalities include increased values of BUN and hyperproteinemia.

Dorsey *et al.* (2018) stated that in bitches with pyometra, the anaemia may be associated with blood loss or systemic inflammation and discovered in the study that anaemia (PCV ≤ 37%) was documented in 4 of 18 dogs with pyometra; no control dogs were anaemic and this difference in proportion of anaemia was not significant ($p = 0.28$).

Filho *et al.* (2020) stated that (hyperproteinaemia and non-regenerative anaemia) are a consequence of an acute inflammatory response of daily injections of PGF2α and should not be considered a follow-up parameter for pyometra treatment in dogs when prostaglandin protocol is applied.

Anjos *et al.* (2021) established that anaemia was observed in 72% of the animals with pyometra, and 65% of these presented normocytic normochromic anaemia classified. The anaemia may have occurred due to the migration of RBCs into the uterus by diapedesis in a chronic process or due to bone marrow suppression because of endotoxins from the systemic bacterial proliferation.

Paudel *et al.* (2023) reported that low levels of red blood cells, pack cell volume, and haemoglobin indicated that the pyometra-infected dogs were more likely to have normocytic, normochromic, and non-regenerative anaemia.

2.3.8.3 Hepatic diseases

Cotter (2000) stated that the anemia of hepatic diseases occurs as a result of decrease in Adenosine triphosphate (ATP) leading to a shortened red cell life span.

Giger (2000) emphasized that jaundice is a common and easily observed physical examination abnormality. Jaundice is usually first noted on the mucus membranes when the serum bilirubin exceeds 2 to3 mg/dl and effects skin when bilirubin concentrations are higher

Davis (1944) reported the production of hyperchromic anemia in dogs by administration of choline chloride.

Watson and Castle (1945) studied the hematologic effect of choline administration in a human patient with liver cirrhosis and macrocytic anemia which continuously improved during therapy.

Simpson *et al.* (1997) demonstrated microcytosis, hypochromasia, and low mean corpuscular haemoglobin and low serum iron concentrations in dogs with portosystemic vascular anomalies. Serum iron concentration and total iron binding capacity were subnormal in 56% and 20% of dogs with PSVA, respectively. Low serum iron concentration appears to be related to the development of microcytosis.

Casas *et al.* (2009) stated that aplastic anaemia in humans can occur secondary to previous hepatitis, or side effects of treatment of hepatitis with interferon and ribavirin. Significant anaemia (haemoglobin < 10 g/dL) has been observed in 9%-13% of patients receiving interferon and ribavirin; moderate anaemia (haemoglobin < 11 g/dL) occurs in about 30% of patients

Naigamwalla *et al.* (2012) stated that iron deficiency anemia can develop secondary to hepatic diseases.

Elhiblu *et al.* (2015) attributed the anemia seen in liver cirrhosis to the chronic nature of the disease due to increased transient time of erythrocytes through the spleen due to reduced portal blood flow and/or fragility of red blood cells due to high levels of bile acids.

Mazaro *et al.* (2019) described a case of hypochromic macrocytic anemia and hemolytic crisis in a dog with copper associated chronic hepatitis.

Webster *et al.* (2019) stated that individuals with high concentrations of hepatic Cu can rarely undergo an acute necroinflammatory crisis releasing Cu and causing a Coombs negative haemolytic anaemia.

O'Kell *et al.* (2022) documented gastroduodenal ulcer related anaemia in liver disease in dogs. Eight dogs (20%) were anaemic (median Hct, 36.6%; range, 28%-39.6%; reference range, 40%-56%), of which 5 had congenital port vascular anomalies, 1 had cirrhosis, 1 had inflammation without cirrhosis, and 1 had other disease (hepatocellular carcinoma).

2.3.8.4 Infectious diseases

Greene and Appel (1998) documented hematologic abnormalities encountered in canine distemper include lymphopenia, sometimes associated with leukopenia or leukocytosis with left shift, anemia and rarely thrombocytopenia.

Kalin *et al. (*1999) mentioned that other workers reported abnormalities like thrombocytopenia, moderate leukocytosis, marked azotemia and mild elevation in the hepatic enzymes in dogs suffering from leptospirosis. The common sequel of canine leptospirosisis acute liver and renal failure

McDonough (2001) studied that leptospirosis in dogs is characterized by hemolytic anemia, leukocytosis, hemoglobinuria and albuminuria.

Decaro *et al.* (2005) reported leukopenia and lymphopenia in dogs suffering from canine parvovirus infections.

2.3.8.5 Inflammatory, metabolic and other miscellaneous causes of anemia

Jacob *et al.* (1980) reported the abnormalities in dogs suffering from diabetes mellitus as anemia, hypoproteinemia and pancytopenia.

Jurado (1997) opined that anemia of inflammatory disease is a mild to moderate non- regenerative, normocytic normochromic anemia associated with inflammatory processes, chronic infections, traumatic conditions such as tissue injury and fracture and disseminated or necrotising neoplastic disease

Green *et al. (*2001) recorded that in hereditary canine spinal muscular atrophy, regenerative anemia, hypoglobinemia and abnormally high liver alkaline phosphatase activitieswere observed

Ristic and Stidworthy (2002) discussed that inflammatory bowel disease is characterised by severe regenerative microcytic hypochromic anemia

2.4 Clinical assessment of Anemia

Reimer *et al. (*1999) mentioned that physico-chemical examination typically reveals pale mucus membrane, tachypnea, splenomegaly, hepatomegaly, icterus, pigmenturia, fever and lymphadenopathy.

Joshi (2000) reported pallor and dry mucosae, dry muzzle, slight elevated temperature, tachycardia, and hyperpnea, reduced appetite, emaciation, dullness, intolerance to exercise, alopecia, rough hair coat and loss of body weight in anemic

Burgess *et al.* (2000) included 60 cases of immune mediated haemolytic anaemia. The major clinical signs were lethargy (93%), pale mucus membrane (77%), and icterus (45%). Apart from this, 23 dogs (38%) had hepatomegaly and splenomegaly on abdominal palpation, 19 dogs (32%), had haemoglobinuria, 16 dogs (27%) had cardiac murmur, and one dog had petechial haemorrhage on the mucus membranes. Induced canine anemia, with special reference to blood transfusion and its therapy held at MKV, Parbhani.

Furlanello *et al.* (2005) in the study of babesiosis in dogs found clinical signs mainly dehydration, apathy, anorexia and fever in anemic dogs at Italy. The clinical signs in *Babesia* were dehydration (100%), apathy (74%), anorexia or decrease appetite (70%) and fever (68%) as the major clinical signs. The anaemia was present in 74 percent dogs and was classified as mild (35%), moderate (59%)

and severe (6%). In all cases, the anaemia was normocytic and normochromic. Only three dogs were presented with regenerative anaemia.

Helm and Knottenbelt (2010) stated that clinical signs of anaemia included lethargy and exercise intolerance, mucous membrane pallor but normal capillary refill time, tachycardia, low-grade haemic murmur, prominent femoral pulse, tachypnea, episodic collapse and jaundice.

Singh *et al.* (2012) reported the most common clinical signs of anaemia as pale mucous membrane, cyanosis, tachycardia, tachypnea, weakness, lethargy, sometimes jaundice in severe cases. Lethargy, spleen enlargement and heart murmurs were seen in chronic anaemias.

Swann & Skelly (2013) assessed Immune Mediated Haemolytic Anemia (IMHA) based on three criteria viz. **1.** the presence of in-saline agglutination or a significant positive titre in a Coombs' test or the presence of significant spherocytosis, **2.** Analysis of blood samples and imaging not conducted in every case or nature of additional tests performed not reported or unclear was clearly defined but incomplete and **3.** Diagnosis not based on tests suggesting the presence of antibodies specific for endogenous erythrocyte antigens nor spherocytosis or diagnostic criteria unclear at Department of Veterinary Medicine, University of Cambridge, Cambridge, UK.

Elliot (2014) concluded that immune-mediated haemolytic anaemia (IMHA) can occur with haematopoietic and rare solid tumours. Clinical signs included lethargy, weakness, tachycardia, pallor, icterus, hepatosplenomegaly, haemoglobinuria and anorexia.

Kisielewicz *et al.* (2014) designed anemic dog clinical assessment score (ADCAS), evaluating 5 clinical variables. Variables included color of mucus membrane, pulse quality, heart rate, respiratory rate, and mentation and exercise tolerance. Scores were given for each variable based on the severity of the abnormality (brackets indicate score): normal (0), mild (1), moderate (2), and severe (3) during assessment of clinical and laboratory variables as a guide to Packed Red Blood Cell Transfusion of Euvolemic anemic dogs from the Queen

Mother Hospital for Animals, Royal Veterinary College, Hatfield, Hertfordshire, UK.

Mali (2006) observed positive autoagglutination in 8 cases out of 18 cases of anemic group at room temperature and microscopically in eight anemic dogs at 4^0C, room temperature and 37^0C. In 10 cases of anemia agglutination was absent macroscopically at room temperature and microscopically at 4^0C, room temperature and 37^0C.

Mahalingaiah *et al.* (2017) observed fever/pyrexia (77.5%), anorexia (76%), history of ticks presence (73%), lymphadenopathy (68%), depression and lethargy (53%), pale mucous membrane (45.5%), loss of body weight (39.5%), congested mucous membrane (38%), respiratory distress (30%), weakness (18%), ocular discharge (18%), nasal discharge (16.5%), lameness (15%), vomiting (13.5%), diarrhea (7.5%), icteric mucous membrane (7.5%), haematuria (6%) and epistaxis (3%) as characteristic clinical features among 66 positive clinical cases in the prevalence study of canine babesiosis in dog population located in and around Bengaluru city of Karnataka state. In addition to these signs, there was splenomegaly (10.5%), hepatomegaly (9%) based on abdominal ultrasonography and neurological signs such as circling, trembling in 1.5% of cases.

Assenmacher *et al.* (2019) revealed that the most commonly reported clinical signs were lethargy (n = 56 [85%] dogs), anorexia (44 [67%]), pale mucous membranes (20 [30%]), collapse (15 [23%]), and vomiting (14 [21%]) in 66 cases of anemia while studying the clinical features of precursor-targeted immune-mediated anemia in dogs at MSU Veterinary Medical Centre, Michigan.

2.5 Haematological changes in anaemic dogs:

2.5.1 CBC

King *et al.* (1992) screened seventeen dogs with chronic renal failure and reported normocytic normochromic anemia of mild to moderate severity with lowest PCV 22% during their study related to anemia of Chronic Renal Failure in Dogs held at Veterinary Hospital of the University of Pennsylvania (VHUP).

Aird (2000) stated that in a normovolemic animal, anaemia is characterized as having less number of red blood cells than the normal, decreased haemoglobin concentration (Hb), decreased haematocrit (HCT), or decreased packed cell volume (PCV) that causes the oxygen-carrying capacity of the blood, and thus oxygen delivery, to be decreased.

Paltrinieri *et al.* (2000) suggested that metabolic changes like an increasein the activity of pyruvate kinase (PK) and glucose-6-phosphate dehydrogenase (G6PDH), an increase in the concentration of 2, 3-diphosphoglycerate (2,3DPG), and alterations in osmotic fragility were found in dogs with haemolytic anaemia. These changes were mainly caused by the presence of immature red cells found in regenerative anaemias. The author also found abnormalities in osmotic fragility in haemolytic anaemias and in those non-regenerative anaemias in which reticulocyte percentage, but not reticulocyte production index (RPI), increased. The osmotic fragility could be used as an early indicator of erythrocyte regeneration.

Ettinger and Feldman (2005) stated that haemoglobin concentration is the third part of packed cell volume or *vice-versa*. Whereas, total erythrocyte count is the sixth part of packed cell volume or *vice-versa*. Hb concentration, PCV and TEC are dependent on each other. They are closely related to each other. So, when one parameter was affected, it affected other two parameters also. That's why in the anaemic cases, decreased Hb concentrate was associated with decreased TEC and PCV.

Furlanello *et al.* (2005) observed leukopenia in 69% cases, neutropenia in 74% cases and leucocytosis due to mature neutrophilia and lymphocytosis in one dog. Also, study showed activated lymphocytes in 61% of dogs whereas thrombocytopenia and a hyperfibrinogenaemia in all dogs during their clinicopathological study of babesiosis in dogs undertaken in Italy, was recorded.

Nassiri *et al.* (2005) stated that all dogs with IMHA were anemic and hematological findings observed were anisocytosis in eight dogs (61%), polychromasia in five dogs (38.5%), thrombocytopenia in seven dogs s (54%) and spherocytosis in seven dogs (54%). The dogs referred for the study at Veterinary Teaching Hospital of the University of Tehran, were screened.

DeNicola *et al.* (2006) suggested that CBC evaluations in the anaemic dog, changes in erythrocyte indices were unreliable predictors of regeneration. A blood film analysis and reticulocyte count were needed for a more accurate assessment of regeneration. The absolute reticulocyte count was the most objective measure of regeneration in the dog.

Balch and Mackin, (2007) suggested the common haematological changes in anaemic cases as haematocrit less than 25% to 30%, haemolysis characterized by hemoglobinemia or haemoglobinuria, antibodies directed against RBCs, with autoagglutination, spherocytosis, or positive results from a direct antiglobulin (Coombs') test at Las Vegas Veterinary Referral Center, USA.

Niwetpathomwat *et al.* (2007) observed major hematological findings in microfilaremic dogs as mild to moderate anemia, mild to severe thrombocytopenia, marked leukocytosis, moderate to marked neutrophilia, eosinophilia and monocytosis in retrospective study made on canine dirofilariasis cases in an animal hospital in Bangkok, Thailand.

Zygner *et al.* (2007) noticed following hematological changes in babesiosis in dogs. The MCHC was below normal in 9 cases out of 248 dogs, Five out of 248 dogs (2%) had MCV below the normal values. The most common abnormalities of red blood cell morphology were anisocytosis and poikilocytosis. Anisocytosis was present in 150 out of 248 dogs (60.5%), poikilocytosis in 62/248 (25%), polychromasia in 59/248 (23.8%), hypochromia in 49/248 (19.7%) and erythroblastosis in 11/248 (4.4%). Erythroblastosis was noted in dogs within the quantity of 2– 11 erythroblasts for every hundred leucocytes examined. Thrombocytopenia was detected in 247 out of 248 dogs (99.5%). The increase of non-segmented neutrophil counts above the normal values was observed in 54 out of 248 dogs (21.8%). Ninety out of 248 dogs (36.3%) had neutropenia, whereas neutrophilia was noted in two cases (0.4%).

Miller *et al.* (2009) stated that anemia of inflammatory disease in dogs is typically characterized as mild to moderate in degree and normocytic, normochromic, sometimes microcytic can be seen. Also, they recorded the median PCV for the anemic dogs as 35% (25–39%) and the median PCV for the

nonanemic dogs as 46% (40–57%) during study held at the Animal Cancer Centre, Colorado State University.

Shah *et al.* (2009) found that the dogs affected with IMHA were severely anaemic and the common blood smear findings were anisocytosis, hypochromasia, polychromasia, thrombocytopenia and leukopenia, spherocytosis and nucleated RBCs during their study undertaken at GADVASU, Ludhiana, Punjab.

Shah *et al.* (2011) observed non regenerative normocytic normochromic anemian neutrophilic leukocytosis with marked left shift and toxic changes in neutrophils and its precursors during characterization study of hematobiochemical indices in canine anemia due to renal failure during the study undertaken on hematobiochemical changes in natural cases of Canine Babesiosis at GADVASU, Ludhiana, Punjab.

Salem (2014) examined 35 diarrheic dogs and stated that the hematological alterations associated with Canine Parvo Virus and Canine Distemper Virus infections may diverge but anemia, leucopenia, neutropenia and lymphopenia were most commonly seen in dogs referred to Small Animal Medicine Teaching Hospital, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.

Bhadesiya & Raval (2015) evinced lowered platelet counts, Hb levels, TEC, PCV, eosinophils and lymphocytes, as well as increased neutrophil, monocyte counts and MCHC value in 18 dogs positive for naturally occurring ehrlichiosis, based on the Dot-ELISA based rapid diagnostic kit irrespective of their age, breed and sex as compared to healthy dogs during their study on hematobiochemical changes in ehrlichiosis in dogs of Anand region, Gujarat.

Lucidi *et al.* (2017) observed moderate to severe anemia in the majority of dogs, and which was clearly nonregenerative in all dogs at the time of bone marrow evaluation. Most anemias were macrocytic hypochromic (7/25), normocytic normochromic (6/25), or macrocytic normochromic (5/25). Nucleated erythrocytes were reported in 6 dogs. Also, leukocyte findings were variable, i.e., leukocytosis in 40% (10/25) of the dogs, mild leukopenia and neutropenia in 3

dogs, mild neutropenia in 2 others, lymphocytosis in 3, lymphopenia in 4, and monocytosis in 8 dogs. Thrombocytosis was present in 5 dogs. Platelet clumping precluded accurate platelet concentration measurement in 10 dogs, including one dog with thrombocytosis and 3 dogs with platelet concentrations below the lower reference limit.

Melendez-Lazo *et al.* (2017) found mild to moderate non-regenerative anemia (62.7%) and lymphopenia (25.5%) in clinicopathological study of dogs infected with *Leishmania infantum* in Spain.

Radakovich *et al.* (2017) observed that values of hematocrit, MCV, and serum iron decreased with age, indicating possible iron-restricted erythropoiesis (IRE), due to iron deficiency or low-grade chronic inflammation during their study on hematology and biochemistry of ageing, evidence of anemia in older dogs held at Colorado State University's (CSU) Veterinary Teaching Hospital, USA.

Shah *et al.* (2020) reported that the animals affected with babesiosis had normocytic-normochromic anemia and 50% of cases had a PCV less than 20%. The white blood cell counts in Babesia infected dogs ranged from 3.3-11.8xl03 µL-1 and majority (75%) had WBC counts lower than 6.0xl03 µL-1. Leukocyte abnormalities were non-specific as either normal leukocyte count or leukopenia was observed. All the Babesia infected dogs had thrombocytopenia during their study on canine anemia held at GADVASU, Ludhiana, Punjab.

Thongsahuan *et al.* (2020) reported hematological alterations caused by *Ehrlichia* infected anaemic dogs as thrombocytopenia, monocytosis, and eosinophilia. The blood samples of Hepatozoon-infected dogs were characterized by anemia, thrombocytopenia, leukocytosis, neutrophilia, and monocytosis. Further, anemia, thrombocytopenia and eosinopenia were associated with *B. canis* infected dogs during hematological profile of blood parasitic infected dogs in Southern Thailand.

Yadav *et al.* (2021) conducted a study on 30 dogs and revealed significant reduction in mean haemoglobin value along with significant increase in neutrophil count during haematological analysis in investigation of parasitic infestation in

dogs presented to Veterinary Clinical Complex, LUVAS, Hisar, from July, 2021 to August, 2021.

Fonseca *et al.* (2022) concluded that in animals infected with *Babesia* spp., the most observed alterations were anemia (83.87%), normocytic normochromic type (37.9%), leukopenia (45.16%), neutropenia (28.22%), lymphopenia (44.35%), eosinopenia (71.77%), monocytopenia (29.03%), and thrombocytopenia (86.03%), while animals affected with *Hepatozoon* spp., showed anemia (76.52%), normocytic normochromic type (49.69%), leukocytosis (26.22%), neutrophilia (30.49%), lymphopenia (29.57%), eosinopenia (40.55%), monocytopenia (21.04%), and thrombocytopenia (54.27%).

2.5.2 Reticulocytes

Jain (1993) calculated reticulocyte count by counting at least 1000 erythrocytes in smears prepared from a mixture of a few drops of blood and equal to double volume of 0.5% new methylene blue in physiologic saline. The reticulocyte count is essential for assessing the erythropoietic response to anemia. A corrected blood reticulocyte count or reticulocyte index is calculated for a more meaningful assessment of erythropoietic response to anemia in dogs. The corrected reticulocyte count above 1% or an Absolute reticulocyte count greater than 60000/µl of blood in the dog indicates active erythropoiesis i.e. responsive anemia

Paltrinieri *et al* (2000) opined that abnormalities in osmotic fragility were detected in haemolytic anaemias and in those non-regenerative anaemias in which reticulocyte percentage, but not reticulocyte production index (RPI), increased. The osmotic fragility could be used as an early indicator of erythrocyte regeneration.

Weiss and Tvedten (2012) noted that enumerating the absolute number of reticulocytes is the most consistent way to evaluate the strength of erythropoiesis and is recommended as the best single indicator of regeneration in dogs.. He also noted that in anemia the percentage of reticulocytes overestimates the strength of erythropoiesis. The absolute reticulocyte count is a more consistent indicator of

the true reticulocyte response. The absolute reticulocyte count is calculated by multiplying the reticulocyte percentage by the RBC count.

Schaeffer and Stokol (2014) opined that Reticulocyte indices can be altered in dogs with various conditions, and are not specific for iron deficiency. Reference intervals were determined prospectively in 122 healthy dogs, and retrospectively compared between dogs with FeDef (n = 11), AID (n = 12), PSS (n = 12), and BAM (n = 7).

Neiger *et al* (2015) opined that the red blood cell distribution width(RDW),which provides a quantitative measure of the heterogeneity of the red cell population (anisocytosis)in the peripheral blood, mean corpuscular volume (MCV) and a regression model combining both variables were used to assess their predictive accuracy in differentiating 51 dogs with regenerative anaemia from 92 dogs with non-regenerative anaemia, which had been diagnosed on the basis of the corrected reticulocyte count. The RDW and mcv are measured by most automatic haematology analysers and may give the first indication of the bone marrow response of an anaemic dog

Paltrinieri *et al. (*2016) concluded that whereas all reticulocyte parameters identified regeneration in anaemic dogs, the performance of specific parameters was dependent on the method used. Findings suggested that lower cutoffs than published reference limits are preferred for reticulocyte number and production index and higher cutoffs are preferred for reticulocyte percentage. Reticulocyte production index may be useful when the pretest probability of regeneration is moderate.

Lippi *et al* (2021) stated that Anemia is considered a common finding in dogs with chronic kidney disease (CKD), typically as normochromic, normocytic, and non-regenerative. Although anemia can occur at any CKD IRIS (International Renal Interest Society) stage, its severity is related with the loss of kidney function. The aim of the present study was to retrospectively evaluate quantitative and morphological abnormalities of the erythrogram in dogs at different CKD IRIS stages. A total of 482 CBCs from 3643 initially screened were included in the study. Anemia was present in 302/482 (63%) dogs, in the majority of which it

was normochromic, normocytic, and non-regenerative (295/302, 98%). The number of reticulocytes was <60,000/μL in the majority of dogs (248/295; 84%), with a correlation between poor regeneration rate and progression of CKD (p = 0.0001)

Jung *et al.* (2023) suggested that IRF could be a valuable addition to conventional reticulocyte variables for classifying anaemic dogs. The area under the ROC curve of IRF was superior to that of RPI. Data from dogs with regenerative anemia (RA, n = 19), PRA (n = 11), and NRA (n = 15) were retrospectively analyzed. The value of IRF was compared with reticulocyte production index (RPI) using the receiver operating characteristic (ROC) curve.

2.5.3 Free Hb

Bindslev *et al.* (1961) observed that hyperventilation resulting in alkalosis produced a marked increase in the free haemoglobin concentration in plasma in vitro experiments on dogs. This was concluded due to reduction of mechanical resistance of the red blood cell from lowering of the hydrogen concentration in blood.

Rother *et al.* (2005) stated that the clinical consequences of excessive cell-free plasma haemoglobin levels during intravascular haemolysis or the administration of haemoglobin preparations include dystonias involving the gastrointestinal, cardiovascular, pulmonary, and urogenital systems, as well as clotting disorders in humans.

Villagra *et al.* (2007) demonstrated that in vitro exposure of platelets to pathologically relevant concentrations of free haemoglobin blocked inhibitory effects on platelet activation by nitric oxide in human patients with sickle cell disease. The findings suggested pathologic platelet activation that might contribute to thrombosis and pulmonary hypertension in SCD and other disorders of intravascular haemolysis.

Larsen *et al.* (2010) demonstrated that free heme contributed to the pathogenesis of severe sepsis irrespective of pathogen load and compromised host tolerance to infection in reduced serum concentration of HPX protein.

Donadee *et al.* (2011) found that storage of human red blood cells under standard blood banking conditions resulted in accumulation of cell free and microparticle encapsulated hemoglobin due to reduced integrity of erythrocyte membrane and exocytic micro vesicle and microparticle formation and hemolysis. Free hemoglobin reacted with nitric oxide 1000 times faster than with intact erythrocytes.

Patterson *et al.* (2011) demonstrated acute life threatening non immune mediated blood transfusion reactions with hemolysis proved by presence of free hemoglobin, indicating that either lysed RBCs were transfused or intravascular haemolysis occurred after transfusion.

Schaer *et al.* (2013) stated that Multiple hematologic and nonhematologic disease states are associated with RBC lysis, and the adverse effects of free Hb and hemin potentially complicate clinical disease outcome.

Swann and Skelly (2016) concluded that although release of large amounts of free haemoglobin into the circulation could increase the risk of acute kidney injury, this phenomenon has not been reported frequently in the veterinary literature.

Langston *et al.* (2017) concluded that dogs undergoing hemodialysis often require transfusion of blood products like whole blood, packed RBCs, or free hemoglobin.

Gianesini *et al.* (2019) demonstrated the association between canine immune mediated hemolytic anemia and clinically suspected acute pancreatitis and the role of free hemoglobin in development of pancreatitis. In dogs with IMHA, a calculated free hb concentration $\geq$ 0.08 g/dL resulted in an increased relative risk (RR) of having/developing acute pancreatitis (RR = 2.54, 95% CI, 1.51 - 4.29; P = 0.003).

Peng *et al.* (2020) evaluated the effect of hemolysis on complete blood count and declared free hemoglobin value of 1.4 g/L as the value for clinical hemolysis detection

Tothova *et al.* (2020) focused on the evaluation of the differences in serum protein electrophoretic pattern between dogs naturally infected with *B. gibsoni* (17 dogs) and *B. canis* (40 dogs). The mean values of total proteins, β 1 -, β 2 - and γ-globulins were in dogs infected with *B. gibsoni* significantly higher (P < 0.05 and P < 0.001) than in dogs infected with *B. canis.* The relative concentrations of albumin, α 1 -, α 2 -globulins and the A/G ratios were in the *B. gibsoni* infected dogs significantly lower. Lower concentrations of α-globulins in dogs with babesiosis may be caused by free haemoglobin due to intravascular haemolysis caused by the disease.

Idalan *et al.* (2021) compared different in clinic and laboratory methods of direct antiglobulin (Coombs') test (DAT) testing of canine blood samples for immune mediated hemolytic anemia and found consisted free hemoglobin results.

Shin *et al.* (2021) demonstrated development of a simple and rapid point-of-care device is developed for the measurement of plasma free haemoglobin (PFHb) and haematocrit (Hct), based on colorimetry. The device measured free haemoglobin with a detection limit of 0.75 mg/dL in the range of (0–100) mg/dL. The Bland–Altman analysis showed that it was within 95% confidence intervals.

2.6 Biochemical changes in anaemic dogs:

2.6.1 LFT

Cartwright *et al.* (1946) reported that in the anemia of infectious origin there was an increase in erythrocyte protoporphyrin and in serum copper during their study of the anemia of infection, the experimental production of hypoferremia and anemia undertaken at Department of Medicine, University of Utah School of Medicine, Salt Lake City.

Stokol *et al.* (2000) screened 43 cases and found hyperferremia and high percentage saturation of transferrin as biochemical abnormalities in the study of idiopathic pure red cell aplasia and non-regenerative immune-mediated hemolytic anemia held at Department of Biomedical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, New York.

Furlanello *et al.* (2005) studied that in the majority of cases of anemia observed mild elevation of Aspartate Aminotransferase (AST), Alanine Aminotransferase(ALT), Alkaline Phosphatase (ALP), Creatine kinase (CK), total bilirubin andlactic acid and decrease of total iron and total iron binding capacity (TIBC) in the clinicopathological study of babesiosis in dogs held at Italy.

Nassiri *et al.* (2005) reported hyperbilirubinemia in eight dogs (80%) outof the ten evaluated dogs; increased activity of ALT and ALP in six (46.2%) and eight (61.5%) out of thirteen evaluated dogs, respectively during the investigation study of the prevalence of immune-mediated hemolytic anemia (IMHA) in anemic dogs referred to the Veterinary Teaching Hospital of the University of Tehran.

Kim *et al.* (2006) in study of canine babesiosis reported hypoproteinemia and hypoalbuminemia conducted at Konkuk University Veterinary Teaching Hospital, Korea.

Niwetpathomwat *et al.* (2007) observed most common serum biochemical abnormalities in microfilaremic dogs as increased alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase in retrospective study made on canine dirofilariasis cases in an animal hospital population in Bangkok, Thailand.

Naigamwalla *et al.* (2012) studied iron deficiency anemia and observed very low serum iron concentration in anemic animals, mild low to low normal serum iron values in case of anemia of inflammatory disease, elevated serum iron level transiently due to intravascular red blood cell lysis, recent blood transfusion, and iron supplementation. In addition, exogenously administered corticosteroids have also been shown to increase serum iron levels.

Shah *et al.* (2020) reported non-significant serum chemistry which includes BUN, creatinine, total protein, albumin, bilirubin, ALT and AST (remained unaltered) in canine babesiosis, except for an increase in ALP during their study held.

2.6.2 KFT

Reddy *et al.* (2014) studied serum biochemical parameters and observed reduction in total protein, serum albumin, glucose levels along with increased BUN, creatinine, SGPT levels in babesiosis infected dogs during study conducted on clinical and laboratory findings of Babesia infection in dogs at Teaching Veterinary Clinical Complex, College of Veterinary Science, Proddatur.

Radakovich *et al.* (2017) observed increase in total proteins, globulins, cholesterol and platelet counts with increase in age while albumin decreased, suggesting low-grade inflammation. Urea was also increased in older dogs without a concurrent increase in creatinine, which points toward gastrointestinal bleeding or dehydration during their study on hematology and biochemistry of ageing, evidence of anemia in older dogs held at Colorado State University's (CSU) Veterinary Teaching Hospital, USA.

Shah *et al.* (2020) reported non-significant serum chemistry which includes BUN, creatinine, total protein, albumin, bilirubin, ALT and AST (remained unaltered) in canine babesiosis, except for an increase in ALP during their study.

2.6.4 Blood Lactate

Cain (1977**)** elaborated that critically alarming levels of lactate will not be encountered in chronically anaemic subjects, primarily due to increased/greater capacity of the RBCs to carry oxygen as a compensatory mechanism.

Lagutchik *et al.* (1998) emphasized that altered lactate metabolism was associated with derangements in circulation. Lactate concentrations in dogs with major trauma and intoxications, and with cardiopulmonary, gastrointestinal and neurologic problems were significantly higher than in clinically normal dogs and dogs with other problems.

Marino (1998) studied global parameters consistent with tissue hypoxia and included an arterial blood lactate concentration greater than 4 mmol/L.

De Papp *et al.* (1999) analyzed lactate concentration of 102 dogs with GDV. The study successfully predicted the prognosis of survival and non-survival dogs based on serial lactate measurement.

Hughes *et al.* (1999) suggested lactate concentrations do not vary significantly between arterial and venous samples, but have been shown to be lower in jugular compared with cephalic venous samples.

Morisaki and Sibbald (2004) stated that the hyperlactatemia after transfusion was unlikely because of hypoperfusion. It could have resulted from persisting tissue hypoxia caused by anaemia, which might only have resolved hours after transfusion or it was possibly a result of microcirculatory perfusion derangements related to underlying disease.

Nel *et al.* (2004) proposed that mean lactate concentration was higher in non survivors enrolled in their study. Serial lactate concentration provide important insights in guiding therapy and a decrease in lactate concentration within the initial 24 hours of initiating therapy is associated with positive outcome of the patients in a critical care system. A threshold value of > 4.4 mmol/L with serial measurement was suggested to have poor outcome and prognosis by the author.

Savigny (2006) recommended the use of estimating lactate levels in critically ill patients as it proved to be a promising and sensitive indicator for hypoperfusion. Alterations in lactate values were noticed before changes in other parameters of critical patient monitoring thus detecting and addressing perfusion issues sooner, avoiding further complications. Lactate levels can also be of use in guiding therapy in conjunction with predicting prognosis.

Pang and Boysen (2007) reviewed status of lactate levels in samples collected from different sites. In his opinion the significance encountered was small and normal range for plasma lactate values in dogs <2.5 mmol/L regardless of the site of sample collection. The author also suggested a transient increase in lactate concentration, whilst within the normal range, when exposed to stressors. As red blood cells produced lactate even after sampling and increase in lactate concentration may be as high as 20% by each passing hour, it is essential that samples were rapidly (as quickly as 5 mins) to be separated until processed.

Allen and Holm (2008) documented that lactate studies in small animals are mostly limited in dogs although they are widely used. Lactate concentration of venous samples are used with increasing confidence to identify underlying hypoperfusion as well as assessment of response to therapy initiated.

Holahan *et al.* (2010) reported the correlation between blood lactate with age, BUN and ALPwhereas an inverse correlation was obtained with PCV values. The author is suggestive of a plasma lactate concentration <2 mmol/L as a targeted end point resuscitation in critically ill veterinary patients. This retrospective study included 173 subjects, of which 133 (77%) subjects exhibited lactate concentration above the established reference range. The presentation for non survivors was higher in comparison to survivors and an operating curve analysis employed by the author provides an overall accuracy of 73% in predicting outcome He defined 'lactime' asan interval during which blood lactate levels were consistently above normal range. No correlation was established by the author between presenting lactate concentration to number of transfusions undertaken.

Kisielwicz and Self (2014) opined that as a surrogate marker of tissue oxygenation, lactate allows approximation of severity of tissue hypoperfusion and hypoxia, and monitoring serial measurements over time enables assessment of treatment response. Lactate could guide transfusion therapy.

Dabhangi *et al.* (2015) reported a prompt decline in lactate levels and amelioration of lactic acidosis by transfusion of pRBC in absence of other interventions. He also proposed that patient cohorts for validation of same theory in kidney, liver and cardiac dysfunctions is necessary to establish data or reference range.

Yagi and Holowaychuk (2016) described that if blood lactate were elevated prior to the transfusion, post-transfusion decreases in blood lactate can indicate improvement in oxygen-carrying capacity and/or oxygen delivery.

Meléndez-Lazo *et al. (*2017) found hyperproteinemia (52.9%) dysproteinemia (78.4%) and proteinuria (47.8%) in clinicopathological study of dogs infected with Leishmania infantum in Spain.

Zakareviciute *et al.* (2021) suggested that serial blood lactate measurement can improve the prediction of successful blood transfusion and it is useful in monitoring the patient status 24hr post blood transfusion.

2.7 Blood Transfusion in Dogs

Lower (1665) reported of a successful blood transfusion in animals, blood was withdrawn from one dog and replaced with blood from another dog.

Robertson (1941) stated that transfusion of blood stimulates erythropoiesis as transfused erythrocytes reduce the demand on blood-forming organs, thereby permitting a resting period in which the hematopoietic system can recover.

Keskar (1980) studied the usefulness of blood transfusion on microcytic hypochromic anaemia. Hemogram, clinical symptoms and serum bilirubin levels were recorded after transfusion at 24, 48, 72 hours, 7 days and 15 days and they found that all transfusions were well tolerated and transfused dogs did not show any untoward reaction.

Dodds (1991) described potential applications of blood component therapy viz. packed cells can be used for anaemia and trauma, platelet rich plasma for thrombocytopenia, fresh plasma containing clotting factors can be used for cases like von Willebrand's disease or haemophilia A.

Giger *et al.* (1995) stated that blood bags are connected to blood infusion sets that have an in-line microfilter. Microfilter with 170 μm pores can be commonly used to remove clots and larger red cell and platelet aggregates. Finer filters with 40 μm pores will remove most platelets and micro aggregates and clog after 100 ml.

Kristen and Feldman (1995) postulated that mitigation of anaemia, haemostatic dysfunction, hypovolemia, hypoproteinemia, neutropenia, or a combination of these are the main goals of blood component administration.

Bhalerao (1997) stated that although apparent blood transfusion may be needed in IMHA cases it should be avoided because transfused cells are destroyed along with autologous cells and such cases undoubtedly requires proper diagnosis and careful treatment.

Hebert *et al.* (1999) stated that the decision to transfuse and the amount administered should be based on the severity of anaemia, illness severity, and comorbidities rather than a set Hb concentration.

Abram Oggs (2000) discussed blood components and blood derivatives. Blood components are blood products prepared either by centrifugation or, less commonly, by apheresis. The most frequently used components are packed red blood cells (RBCs) and plasma. In many clinical situations a patient in need of a transfusion requires only a specific component of blood. The use of blood components allows several patients to benefit from one blood donation and reduces the risks of transfusion reactions to unnecessary components. As a general rule, whole blood and its components may be transfused at a rate of 5-10 ml/kg/h. The initial rate should be 0.25 ml/kg/h for the first 15-30 minutes to allow for early detection of potentially severe transfusion reactions. The maximum rate of transfusion is 22 ml/kg/h, which is usually only used in an emergency situation.

Hohenhaus (2000) suggested an initial dose of whole blood as 13-22 ml/kg. The initial dose is 6 to 10 mL/kg for pRBC transfusion.

Prittie (2003) opined that when the haemoglobin concentration drops below 3 g/dL or the HCT falls below 12%, human patients are at risk for multiple organ failure, and blood transfusion should not be delayed. In the author's opinion this should apply to small animals as well. In most other situations, the decision to transfuse should be based in part on the patient's haemoglobin concentration but more importantly on the rapidity of the decline in RBCs. The author suggested that blood typing or crossmatching a dog before the first transfusion is not necessary because dogs lack naturally occurring alloantibodies. As sensitization occurs within 3 to 5 days of initial transfusion, subsequent transfusions in both dogs and cats should be preceded by a crossmatch to determine RBC compatibility.

Rutan (2007) opined that transfusions do not cure disease; they stabilize the patient to buy the clinician time to diagnose and treat the underlying cause of the anaemia. The author suggested the simplest rule of thumb was to only replace what was lost.

Tocci and Ewing (2009) recommended that pre-transfusion testing must be standard of care before every RBC transfusion. Optimising patient safety in transfusion medicine is multifaceted. It involves not only using high-quality RBC components but also assuring the integrity of the transfusion process from donor collection through post-transfusion evaluation.

Carless *et al.* (2010) recommended modification of the traditional transfusion triggers of 30/10 (packed cell volume 30% haemoglobin 10g/dL [100g/L]) as in human transfusion medicine in light of a multitude of studies demonstrating that a more conservative transfusion strategy (i.e., transfusing at a lower haemoglobin) is equal, if not superior, to the traditional and more liberal transfusion strategies.

Holahan *et al.* (2010) opined a "transfusion-need scale" was previously designed to guide transfusion decisions using the clinical signs of anaemia; however, it relies heavily on the PCV of the dog, which is an unreliable indicator of the need for transfusion.

Davidow (2013) stated, despite early experiments, small animal blood transfusions have only become common over the last 30– 60 years.

Kisielwicz and Self (2014) stated that blood is best administered intravenously although intraosseous routes could be considered if venous access is not achieved. Intraperitoneal administration results in slow absorption and therefore has delayed effect. The authors also suggested that newer transfusion techniques are being explored such as cell salvage in surgical patients and subsequent autologous transfusion. Xenotransfusions, using blood and blood products between different species, provide an alternative to conventional blood products. There is increasing interest in less conventional blood transfusion techniques which circumvent blood storage considerations, such as cell salvage with autologous transfusion and xenotransfusion, as alternatives to more traditional blood transfusion methods. The blood volume to be transfused (VT) ideally should be guided by goal-directed therapy; historically, it has depended on the severity of anaemia, availability of blood products, body mass and donor PCV. Administering

type-compatible blood to dogs is not necessary for the first transfusion due to the lack of DEA 1.1 alloantibodies.

Mackin (2014) stated that patients exhibiting slide autoagglutination and hemolysis result in a shorter life span of RBCs in the recipient thus making transfusion sub-optimally beneficial, perhaps hazardous.

Bruce *et al.* (2015) observed that administration of antihistamines prior to transfusion decreased the likelihood of allergic transfusion reactions. While premedication prior to blood transfusion is not routinely recommended for veterinary patients, the potential benefits of this practice might warrant future investigation.

Walker (2016) reported that in order to produce blood components on site, a practice must have access to specialized equipment. Most importantly, the preparation of blood products requires access to a large-capacity temperature-controlled centrifuge.

2.7.1 Donor selection and screening

Wardrop (1997) stated that donors should be typed and screened for general health and for endemic infectious diseases. Donor selected for blood collection should be adult (2-8 years), healthy, weigh more than 30 kg, with PCV of 40% or more, fully vaccinated and free of various diseases such as heartworm infection, tick-borne diseases (*Ehrlichia canis*, *Babesia*, *Borrelia burgdorferi, Rickettsia rickettsii*), brucellosis.

Lanevschi & Wardop (2001) concluded that the ideal canine donor should have the characteristics should weigh more than 30 kg, have taunt neck skin that permits easy access to the jugular vein, have a packed cell volume at least 0.40 L/L, good temperament, fit in condition, no previous history of transfusion or pregnancy.

Wardrop *et al.* (2005) concluded that donors should receive a well balanced, high-performance diet that may be supplemented twice weekly with oral ferrous sulfate (Feosol, 10 mg/kg q24h) if the donor is bled every 4 to 6

weeks. PCV or haemoglobin concentration should be more than (40%) and more than 13 g/dL,respectively, in canine donors.

Rutan (2007) stated that donor dogs selected for transfusion should be properly vaccinated, should be docile, have at least (40%) PCV, should not have previous transfusions or had a litter in case of bitches, moreover dogs that have thick skin (Rottweilers) or skin fold on the neck (Bassett hound, Mastiffs) are less preferredthan long-necked dogs (Greyhounds).

Vascellari *et al.* (2016) conducted a blood borne pathogen screening on 150 client-owned blood donor dogs and 338 strays in north east Italy no clinical signs were present at the time of blood collection. Screening was done by serological assays (IFA) for *Leishmania infantum, Ehrlichia canis, Anaplasma phagocythophilum, Babesia canis, Rickettsia conorii, and R. rickettsii*), microscopic blood smear examination, and blood filtration for *Dirofilaria* spp. All blood samples were tested by PCR for *L. infantum, E. canis, A. phagocythophilum*, and *Babesia/ Theileria* and *Rickettsia* spp. All PCR and blood smear examination results were negative. A total of 40 donor candidates (26.7%) and 108 stray dogs (32%) were seropositive to at leastone vector-borne pathogen.

Yagi & Bean (2016) stated that donors should have sufficient blood volume todonate the desired amount of blood without any detrimental effects. The physical characteristics of dogs should also be considered to determine their suitability as a blood donor.

Kumar (2017) stated that all donors should be healthy young adults and never transfused and donors must undergo routine physical, haematological and clinical chemistry evaluations examinations. Proper clinical history of the expected donor should be collected by carefully interviewing the owner to minimize the risk of disease transmission through blood, properly vaccinated and should be tested free of blood parasites and other infectious diseases

2.7.2 Crossmatching

Feldman & Kristensen (1995) reported the two main reasons for the cross match were to decrease the risk of transfusion reactions in previously sensitized patients;in patients with natural iso antibody; and neonatal iso erythrolysis; and to

decrease therisk of sensitizing a patient if more than one transfusion is anticipated or in intact breeding bitches or queens.

Harrell *et al.* (1997) stated that cross matching doesn't provide a guarantee of leukocyte, protein, or platelet compatibility and in some it might be too sensitive to detect anti-erythrocyte antibodies.

Tocci & Ewing (2009) suggested that the major crossmatch is the serologic method designed to determine the compatibility between the donor RBC and the recipient (patient). The key aim of the test is to determine the incompatible transfusions that can result in haemolytic transfusion reactions that are immune- mediated.

Guzman *et al.* (2016) compared the standard blood transfusion in forty-five dogs cross-match technique to a commercial blood transfusion cross-matching kit and concluded that the standard manual cross-match technique remains the gold standard test to determine blood transfusion compatibility.

Odunayo *et al.* (2017) conducted a retrospective study on 169 client-owned dogs on the incidence of incompatible cross match results in dogs with no history of prior blood transfusion and to observed changes in PCV following first blood transfusion in dogs that did and did not have cross-matching performed. Twenty-five of 149 (17%) dogs evaluated by cross-matching were incompatible with 1 or 2 of the3 potential donors.

2.7.3 Blood collection and storage

Nilsson *et al.* (1983) concluded that packed red cell transfusion is preferable over whole blood transfusion as whole blood transfusion can lead to hypervolemia in these patients. The transfusion of packed RBCs is indicated for tissue reoxygenation and is ideal for a normovolemic, anaemic patient.

Kerl & Hohenhaus (1993) stated that collected blood dog erythrocyte antigen (DEA) 1.1 and 1.2 negative grey hound donors by jugular venipuncture (450± 10%) of whole blood in a triple collection unit with 63 mL of citrate phosphate dextrose adenine anticoagulant 1. Plasma was expressed into 1 satellite bag and packed RBC were divided between 2 remaining bags in a closed system

Wardrop *et al. (*1994) mentioned that blood components could be separated by centrifugation immediately after collection, removing supernatant plasma to produce pRBCs containing RBC, leukocytes, platelets, remnant plasma and anticoagulant; this is predominantly used for managing haemolysis and non-regenerative anaemia. Nutrient solutions, sodium chloride-adenine-glucose-mannitol (SAGM), extend storage times to 35–42 days whilst preserving RBC.

Feldman & Kristensen (1995) stated that packed RBC units are made by centrifugation or gravity sedimentation of whole blood and they have less electrolytes, less anticoagulants and less volume than an equivalent amount of whole blood.

Wardrop (1995) elaborated that Acid citrate dextrose (ACD), citrate phosphate dextrose (CPD and CP2D), and citrate-phosphatedextrose-adenine (CPDA-1), in which the added dextrose, phosphate, and adenine favour the viability of RBCs, permitting their storage for up to3 to 5 week, depending on the preservative

Lanevschi & Wardrop (2001) concluded that heparin and citrate are anticoagulants that will not contribute to cell preservation during long- term storage and whole blood collected in these anticoagulants should be used with additive solutions. These solutions contain factors, such as dextrose, adenine, mannitol, and the sodium chloride, needed by RBCs to maintain their energy metabolism and viability during storage. Whole blood may be collected using ACD, CPD or CPDA-1. For dogs, a standard 450 mL CPDA-1 bag enables the collection of one unit of blood. Packed RBC's are prepared by removing 200 to 250 mL of plasma from 450 mL (1 unit) of whole blood after centrifugation. The packed cell volume of the RBC preparation is approximately 0.70 to 0.80 L/L, and the RBCs could be re-suspended in a protein-poor additive solution, such as Adsol, to a packed cell volume of 0.55 to 0.65 LL.

Hogman *et al. (*2002) opined that anticoagulants used for blood products are acid-citrate-dextrose (ACD) and citrate-phosphate-dextrose-adenine (CPDA-1) that are commercially available for collection of blood and have a life span of

21 and 35 days respectively. Additionally, RBC function can be preserved by adding an additive solution that can extend the shelf life up to 42 days

2.7.4 Dose and Administration of Blood

Rutan (2007) reported that RBC products should be transfused with an aim to achieve PCV up to 25 – 30 percent in recipient dogs and to avoid allergic reactions administer diphenhydramine 2 to 4 mg/kg Intra muscular 15 to 20 minutes before the beginning of transfusion (Harrell and Kristensen 1995).

Short *et al.* (2012) studied 37 transfusions the mean pretransfusion PCV (pre- PCV) for all dogs was 16.5% ± 0.76. The median volume of pRBCs transfused was 350 mL (range, 60–450 mL). Nineteen dogs were transfused 350 mL unit of pRBCs, while 10 dogs received a 175 mL unit of pRBCs, and 8 dogs received a fraction of or more than one unit. Eighteen transfusions (49%) were administered due to RBC destruction, nineteen transfusions (51%) were administered due to internal or external blood loss.

Kisielewicz *et al.* (2014) stated that fresh whole blood should be transfused within 4- 6 hours following collection: it contains red blood cells (RBC), platelets, leukocytes and plasma proteins including clotting factors can primarily be used for managing acute, severe haemorrhage from trauma, surgery or coagulopathies. They concluded haemoglobin concentration, haematocrit, increased significantly with transfusion ($P<0.001$) while lactate concentration decreased significantly. Haematocrit was (<17%) in (83 %) of cases and haemoglobin concentration was <5.8g/dL in (80%).

Ognean *et al.* (2015) performed whole blood transfusion in 27 dogs. The dose of blood was calculated according to bodyweight, haematological parameters and severity of anaemia, dehydration status and etiology of anaemia. The mean volume of blood transfused was 7.5mL/kg Bwt. with the maximum volume given was 23 mL/kg Bwt.

Langston *et al.* (2017) administered blood in 47 of the 147 dogs (32%) dogs during the course of renal dialysis. Thirty-one (78%) of the transfused dogs with acutekidney injury (AKI) (28 dogs) or chronic kidney disease (3 dogs) were classified as non-survivors. Of the 82 dogs with AKI (72 dogs) or chronic kidney

disease (10 dogs)that were not administered a transfusion, 39 (48%) dogs survived (38 dogs with AKI and 1 dog with acute on chronic kidney disease). The median PCV before the first dialysis treatment was 30 percent in survivors and non-survivors. Administration of a blood product was associated with a higher likelihood of mortality.

Odunayo *et al.* (2017) compared the mean change in PCV after transfusion was compared between crossmatch-tested dogs (57) and non-crossmatched dogs (20) Mean ± SD change in HCT after transfusion was significantly higher in dogs that had crossmatching performed (12.5 ± 8.6%) than in dogs that did not undergo crossmatching (9.0 ± 4.3%).

Pallavi (2019) transfused blood in 38 dogs volume of blood transfused ranged from 6.98 mL/kg Bwt. to 23.62±2.41 mL/kg Bwt. in different body weight groups. Mean PCV rise from initial after transfusion was (11.61%) for 1-5 kg group, 11.75% for 5-10 kg group, (9.62%) for 10-20 kg group, 2.41% for 20-30 kg group, (8.88%) for 30-40 kg group and (7.85%) for ≥ 40 kg group. Significant improvement in the clinical parameters and haemato-biochemical parameters post-transfusion was recorded. Transfusion was well tolerated except in three cases in which delayed reactions were observed. The survival percentage (≥ 7 days) in the transfusion group was 84.21% and (56.25%) in non-transfusion cases.

2.7.5 Adverse reactions of blood transfusion

Turnwald and Pichler (1985) conducted a study where transfusion related pyrexia was the most common transfusion reaction and is characterized by an increase in body temperature of 1°C or within four hours of the transfusion. Circulatory overload is a common transfusion complication in small animals and is most often associated with the rapid administration of whole blood to patients with cardiac disease and renal disease.

Callan *et al.* (1996) documented hyperthermia and emesis as the main transfusion reactions On the other hand Kerl and Hohenhaus 1993 documented that patients that received erythrocyte concentrate showed acute reactions characterized byemesis, haemolysis, haematuria and jaundice.

Hohenhaus (2000) described the main acute immune transfusion reactions are: red blood cell incompatibility (haemolysis), acute hypersensitivity and the non- haemolytic febrile transfusion reaction.

Lanevschi & Wardrop (2001) concluded that the most serious transfusion reaction is an acute haemolytic reaction immunological response that takes place when the patient has circulating natural or acquired antibodies towards donor erythrocytic antigens. Clinical signs are due to the intravascular haemolysis that results in haemoglobinuria, vasoconstriction, renal ischemia, and DIC. Clinical signs in dogs include fever, tachycardia or bradycardia, hypotension, dyspnea, cyanosis, excessive salivation, tearing, urination, defecation, vomiting, collapse, opisthotonos, cardiac arrest, haemoglobinemia, and haemoglobinuria. The severity of the signs in dogs depends on the volume administered.

Bognato *et al.* (2009) conducted a study from 2006 to 2008 on different type blood products out of which 129 patients received erythrocyte concentrate, 37 received platelet concentrate and 20 received whole blood. The acute adverse reactions were recorded in 28.49 percent (53/186). The adverse effects reported during the transfusion of erythrocyte concentrate were: emesis 59.2 percent, angioedema 18.5 percent, hyperthermia 11.1 percent, dyspnea 11.1 percent, erythema 3.7 percent and tremors 3.7 percent. The main reactions observed with the administration of the platelet concentrates were: angioedema 76.4 percent, emesis 23.5 percent, urticaria 17.6 percent and erythema 5.8 percent. The whole blood transfusion was characterized by: emesis 66.6 percent, angioedema 11.1 percent,hyperthermia 11.1 percent and urticaria 11.1 percent.

Thomovsky & Bach (2014) conducted a study to document the existence and incidence of acute lung injury (i.e., veterinary acute lung injury [VetALI] per the 2007consensus definition) in a population of client-owned 54 dogs receiving transfusions for various clinical reasons. The author concluded that the incidence of VetTRALI (2/54 [3.7%]; 95% confidence interval, (0% to 8.73%) in study dogs was significantlyless than the reported incidence of TRALI in humans (25%) and not significantly different from the reported incidence of ARDS in ill dogs (10%).

Bruce *et al.* (2015) conducted a retrospective study to evaluate the effect of premedication on transfusion reactions (TRs) within 24 hours after blood product. A total of 144 (15%) acute TRs were documented in 136 dogs. The most common TRs were fever alone (53%) and vomiting alone (18%). Six dogs died due to the TR (4%). TR was not associated with age, sex, weight, or premedication. The pRBCs were most likely associated with a TR and plasma least likely. The immune disease was significantly associated with the occurrence of a TR. Transfusions given in the perioperative period had significantly fewer reactions. Most TRs were mild, however, some severe reactions documented were haemolysis, dyspnea, and death in 6 dogs. Packed RBC transfusions were associated with the development of acute TRs. They concluded that the overall occurrence of TR was not significantly altered with premedication; however, when evaluated alone, antihistamines decreased the incidence of acute allergic reactions.

Kumar (2017) stated that probable transfusion reactions might be acute or delayed. Incompatible transfusions may cause acute intravascular haemolysis leading to haemoglobinemia and haemoglobinuria and release of thromboplastic substances that may lead to disseminated intravascular coagulopathy. Delayed haemolysis as evidenced by a decrease in PCV between 2 to 14 days after transfusion.

Maglaras *et al.* (2017) conducted a retrospective study on 210 anaemic dogs to observe any relation between RBC product age and acute transfusion reactions and mortality in dogs. Out of 333 transfusions in 210 patients, 84 transfusion-related complications occurred. Fever was most common (41/333), followed by haemolysis (21/333). Transfusion-related complications were associated with higher product dose, longer duration of administration and immune-mediated disease, but not with the source of product or general category of anaemia. Product age was not associated with increased mortality or fever but associated with an increased risk of transfusion-related haemolysis.

Albernaz *et al.* (2018) recorded TRALI (Transfusion-associated acute lung injury) in a case study of limb amputation and post-operative whole blood

transfusion in a dog observed that approximately eight hours after the transfusion which was manifested by the deterioration of all vital signs, including hypotension and severe hypoxemia, with (PaO2 /FiO2 < 90%) on room air and mixed pattern of bilateral pulmonary infiltration on the thoracic radiograph. TRALI should be differentially diagnosed with circulatory overload, anaphylaxis, bacterial contamination and acute haemolytic transfusion reaction.

2.8 *In-vivo* and *In-vitro* survival of erythrocyte in Dogs

Kirk (1965) stated that stored blood ages, RBCs disintegrate and increasing potassium is released in plasma which may be detrimental to animals with renal disease.

Kerl & Hohenhaus (1993) reported that the normal life span of transfused RBC is 21-48 days. A delayed transfusion incompatibility reaction may shorten the life span to 2-5 days.

Kristen and Feldman (1995) suggested that RBCs require 2, 3-diphosphoglycerate to offload oxygen to tissues, and 2, 3-diphospho-glycerate levels decrease with time in stored blood. Because of this, the longer blood is stored, the less effective it was in oxygen delivery.

Wardrop *et al.* (1997) suggested that the shelf-life of refrigerator stored pRBCs varies depending on the anticoagulant and preservative solution used, but typically is 35 and 42 days for canine and human PRBCs, respectively.

Giger (2000) concluded that in the absence of bleeding and haemolysis, at least (70%) of transfused erythrocytes survive 24 hours and transfused erythrocytes may be thereafter expected to have a normal life-span (approximately 70 days in cats, 110 days in dogs). Monitor response to transfusion by obtaining PCV/TP readings prior to, immediately, and 6 and 24 hours post-transfusion, and consider continued blood loss and/or haemolysis.

Pavenski *et al.* (2012) defined storage lesions as the morphologic changes, metabolic derangements and oxidative injuries that occur with storage, which detrimentally affect RBC viability and function, and ultimately contribute to their decreased survival following transfusion.

Solomon *et al.* (2013) suggested that RBCs stored for 42 days had 24-hour RBC recovery post-transfusion range from 60 to 63%.

Hann *et al.* (2014) referred to the "RBC storage lesion" as the cumulative biochemical and biomechanical changes that occur in RBCs during storage in vitro that decrease their function and survival in vivo. Based on the authors finding in his study involving 3095 dogs receiving pRBC's there was no association between the duration of pRBC storage and overall mortality, suggesting that thus, there seems to be insufficient data at this point to recommend shortening the refrigerator shelf-life of canine PRBCs.

Kisielwicz *et al.* (2014) stated that storage of blood products has improved considerably over time but whilst extended storage times may improve their availability, a phenomenon known as the storage lesion has been identified which affects erythrocyte viability and survival.

Cotter (2019) suggested the anticoagulant of choice is citrate phosphate dextrose adenine (CPDA-1). Commercial blood bags contain the appropriate amount of anticoagulants for a unit. Heparin should not be used as an anticoagulant, because it had a longer half-life in the recipient and causes platelet activation. Blood collected in CPDA-1 with added RBC preservation or nutrient solutions may be safely stored at 4°C for 4 weeks.

Nnmadi *et al.* (2019) suggested that CPDA-1 provided a substrate from which RBCs can synthesize ATP during storage and also improved viability. The dextrose supports continuing ATP generation by glycolytic pathway, while the citrate prevents coagulation by binding ionized calcium which is required for coagulation cascade.

2.9 Plasma proteins in anaemias

2.9.1 Albumin

Harrus *et al.* (1996) suggested that dogs infected with *E. canis* were found to have hypoalbuminemia. Serum protein electrophoresis was performed in 42 dogs with naturally occurring *E. canis* and 15 clinically healthy dogs.

Hoskins (2004) reported that in glomerular disease where hypoalbuminemia is prominent due to smaller size of albumin as compared to globulin and readily leak more due to damaged glomerular membrane.

Camacho *et al*. (2005) studied 58 samples of canine where 19 were of azotemic dog, among the infected dogs it was found significantly elevated concentration of total protein and different globulins and significantly lower level of albumin.

Tothova *et al*. (2020) reported that canine babesiosis may cause several hematological and biochemical changes. The relative concentrations of albumin and A/G ratio were significantly lower.

Asawakarn *et al*. (2021) observed that a single infection of *B. canis*, *E. canis* and *H. canis* revealed significant decrease in albumin levels and A/G ratios.

Yoon and Yu (2021) studied the levels of serum protein by serum electrophoresis method and stated that serum proteins are involved in the inflammatory regulation hereby shows the decreased level of albumin and elevated level of α-globulin and β-globulin in dogs with pyometra.

2.9.2 Globulin

Breitschwerdt *et al.* (1987) stated that *E. canis* antibody titre should be determined in all dogs in which a diagnosis of benign monoclonal gammopathy is contemplated. Clinical signs, hematology and immunological findings of 14 dogs with *E. canis* monoclonal gammopathy were studies retrospectively.

Broek (1987) studied the serum concentration of dogs with confirmed haemorrhagic enteritis were determined by agarose gel electrophoresis before

treatment. The mean concentration and percentage of alpha-2-globulin were significantly increased.

Harrus *et al.* (1996) suggested that dogs infected with *E. canis* were found to have hyperglobenemia. Serum protein electrophoresis was performed in 42 dogs with naturally occurring *E. canis* and 15 clinically healthy dogs.

Hoskins (2004) reported that in glomerular disease where hypoalbuminemia is prominent due to smaller size of albumin as compared to globulin and readily leak more due to damaged glomerular membrane.

Camacho *et al* (2005) they examined 2979 canine blood samples. For all dog samples, serum protein response to albumin, alpha-1-globulin, alph-2-globulin, beta and gamma globulin was measured by capillary electrophoresis. Where beta and gamma globulins in infected animals were decreased and showed significantly higher value of alpha-2-globulin.

Tizard (2020) suggested that polyclonal gammopathies result from chronic infections such as bacterial, viral fungal infections and chronic parasitic disease.

Tothova *et al*. (2020) reported that canine babesiosis may cause several hematological and biochemical changes. The relative concentrations of albumin and A/G ratio were significantly lower. While no any significant difference between different globulin proteins.

Asawakarn *et al*. (2021) observed that a single infection of *B. canis*, *E. canis* and *H. canis* revealed significant decrease in albumin levels and A/G ratios. A monoclonal gammopathy pattern was observed in *E. canis* and *B. canis* single infections, whereas β-γ bridging pattern and increased β and γ globulin fraction were found in *H. canis* single infection.

Yoon and Yu (2021) studied the levels of serum protein by serum electrophoresis method and stated that as serum proteins are involved in the inflammatory regulation hereby shows the decreased level of albumin and elevated level of α-globulin and β-globulin in dogs with pyometra.

2.10 Treatment of anemia

Burgess *et al*. (2000) conducted a review of 60 cases of immune-mediated hemolytic anemia (IMHA) in the dog was performed in order to characterize the disease and to identify potential prognostic indicators. Dogs ranged in age from 1 to 13 years, with a mean age of 6.5 years. The 2 most commonly affected breeds were Cocker Spaniels and Labrador Retrievers. Fifty-two of the 60 dogs tested (87%) were autoagglutination positive and spherocytes were present in 45 (75%). Forty-one (89%) of 46 patients tested positive for the presence of immunoglobulin on the red blood cell surface (Coombs assay). The most common clinical signs at presentation were lethargy, weakness, pale mucous membranes, icterus, hemoglobinuria, and anorexia. PCV less than 25% was present in 59 (98%) dogs. At the time of presentation, 35 dogs (58%) had a nonregenerative anemia, whereas 25 patients (42%) had a regenerative response. Thrombocytopenia was seen in 41 (68%) dogs. Nine of 34 dogs (26%) had a prolonged prothrombin time, 19 of 34 (56%) had a prolonged activated partial thromboplastin clotting time, and 12 of 34 (35%) had abnormal fibrinogen concentrations. All dogs received prednisone at immunosuppressive doses (2.2–4.4 mg/kg PO as a single or divided dose every 24 hours) and cyclophosphamide as primary therapy. Forty-one dogs (63%) received cyclophosphamide at 50 mg/m2 q24h for 4 days, whereas 9 dogs (15%) received an initial high dose (200 mg/m2) followed by 3 days of a lower dose (50 mg/m2 q24h).

Ingole K. H. (2003) studied that treatment with whole blood transfusion was found useful as it supplies all the coagulation factors and improved Hb, PCV, TEC and platelet count.

Weinkle *et al.* (2005) studied that treatment with a combination of glucocorticoids, azathioprine, and ultralow-dose aspirin significantly improved short- and long-term survival in dogs with IMHA. 151 dogs with IMHA not associated with underlying infectious or neoplastic disease were included in the study.

Ettinger *et al.* (2005) opined that recombinant human erythropoietin can be administered @ 100U/kg three times a week subcutaneously until RBC level increase; followed by one time per week thereafter based on response

Assarasakorn *et al*. (2006) conducted a study on clinical use of recombinant human erythropoietin (r-HuEPO) for treatment of anemia in dogs with renal failure and assessed the hematologic and serum biochemical data pre and post-treatment with r-HUEPO. The results were found that means of packed cell volume (PCV), red blood cell count, and hemoglobin concentration were significantly different from the values at week 0 of r-HuEPO treatment until week 8 of treatment. Erythrocyte indices indicated normocytic and normochromic. Other parameters were insignificant during treatment. They concluded that treatment with r-HuEPO stimulated erythrocyte production in dogs with naturally developing anemia of chronic renal failure during 8-week treatment period. PCV reached the target range within 6 weeks of treatment. They also found that exogenous r-HUEPO had no effect on leukocyte, platelet counts, and serum biochemistry in these uremic dogs.

Kaushansky and Kipps (2011) stated that patients with anemia due to chronic kidney disease are ideal candidate to epoeitin alfa therapy. Darbepoietin alfa also approved for use in patient who are anemic secondary to chronic kidney disease. The recommended dose is 0.45 µg/ kg administered intravenously or subcutaneously once a weekly, with dose adjustment depending on response. The most common side effect of therapy is aggravation of hypertension, which occurs in 20 to 30% of patients and most often associated with a rapid rise in hematocrit. Blood pressure usually can be controlled by increasing antihypertensive therapy. He also stated that during erythropoietin therapy, absolute or functional iron deficiency may develop. Functional iron deficiency (i.e. normal feritin levels but low transferrin saturation) presumably results from the inability to mobilize iron stores rapidly enough to support the increased erythropoiesis.

Naigamwalla *et al*. (2012) concluded that blood transfusion, iron dextran, ferrous sulphate is recommended for the treatment of iron deficiency anemia.

Fiocchi *et al.* (2017) opined that Darbepoetin, when combined with treatment of comorbidities, is an effective treatment for anemia secondary to CKD in dogs. Thirty-three client-owned dogs with naturally occurring CKD, including 26 with comorbidities were included in study.

Olsen, G. H. (2000) suggested blood transfusion, erythropoetin stimulating agents, iron supplementation, vitamin supplementation, and immunosuppressives in the therapeutic management of anemia due to viral diseases, parasitic diseases as well as non infectious causes, depending on the hematological parameters and the regeneration status of the anemia.

Swann *et al.* (2019) discussed that anticoagulants, immunosuppressive drugs, pRBC were recommended for the treatment of IMHA in dogs.

Materials and Methods

CHAPTER – III

MATERIALS AND METHODS

The present study was carried out on dogs referred to the Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, Mumbai Veterinary College, Parel and the dogs presented at the Medical ward of Bai Sakarabai Dinshaw Petit Hospital for Animals (BSDPHA) affiliated to Mumbai Veterinary College, Parel, Mumbai- 400012 from March 2021 to September 2023.

3.1 Statutory permission to undertake the research work

The present study was undertaken after the approval from the Board of Studies in Veterinary Clinical Medicine, Ethics and Jurisprudence, Resolution no: VCM/01/2021 and Subject no: VCM/02/2021. Dated: 16/04/2021.

3.2 Design of the Study

The research was planned in three different parts

Part I	Clinico-hematological, Biochemical, and Urine analysis of Hyperglobunamic anaemic dog patients at different time intervals
Part II	Identification of plasma/ serum proteins in hyperglobulinemic anaemic dog patients
Part III	Assessment of RBC survival

3.3 Inclusion Criteria

Anaemic (haemoglobin below 5 gm/dl) and hyperglobulinemic (Serum Globulin more than 4 gm/dl) dogs admitted to BSPDHA of all ages, sexes, weights, and breeds were included.

3.4 Part – I Clinico-hematological, Biochemical, and Urine analysis of hyperglobunamic anaemic dog patients

3.4.1 Clinical Examination

Standard clinical examination and recording of case was done as per the case record sheet (Annexure – I). A detailed information of the enrolled dogs was recorded on before treatment and on Day 0, 3 ,7,15,28 and 35. Clinical data obtained was analyzed for clinical assessment of anemia, (Joshi, 2000).

Table No. 3.1 : Gradation of Clinical Parameters of Anaemic dogs (n=24).

Parameters	Appetence	Body Condition	* Mucous membrane	Skin and Hair coat	Posture and Gait	Behaviour	Lethargy	* Mentation
Grade 1	Normal Appetite	Emaciated	Salmon Pink	Excellent (Shiny coat free from dandruff or greasiness)	Excellent (Normal Posture and gait)	Excellent (Normal Aggregation)	Normal (Active)	Normal (Normal Walking)
Grade 2	Mild Anorexia	Thin	Slightly Pale	Very Good (Greasiness, Dull coat)	Very Good (Mild In-coordination, Limping gait)	Very Good (Quite Alert & Responsive)	Mild (Mild Dullness)	Mild (Quite, Able to walk)
Grade 3	Moderate Anorexia	Ideal (Normal)	Moderately Pale	Good (Greasiness, Dull coat, mild pruritus, Dehydrated)	Good (Moderate In-coordination)	Good (Mild attacking)	Moderate (Moderate Dullness)	Moderate (Lethargic, Able to stand)
Grade 4	Anorexic	Overweight	Severely Pale and / or Icteric	Fair (Loss of Luster, Alopecia/ Dandruff/ mild pruritus)	Fair (Ataxic gait)	Fair (Fear/Phobia, nail-biting etc)	Severe (Dull and Depressed)	Severe (Lethargic, Unable to stand)
Grade 5		Obese	-	Poor (Dull/ Ectoparasites/ Dandruff/ Redness/ Pruritus, smell)	Poor (Sleeping posture, No gait)	Poor (Aggressive/ Very dull)		

Kiselewicz *et al,* (2014) *

Blood Auto- agglutination-

Grade 1- Negative
Grade 2- Positive

Urine Examination

Parameters	Urine Colour	Urine Appearance	Urine pH
Grade 1	Clear (colorless)	Clear	Acidic
Grade 2	Light yellow	Hazy	Neutral
Grade 3	Yellow	Turbid	Alkaline
Grade 4	Deep yellow	Cloudy	-
Grade 5	Reddish brown	-	-
Grade 6	Red	-	-

Grading of anaemic dogs on the basis of Proteinuria and Hematuria

Parameters	Protein In Urine	Quantitative mg ref for Protein in Urine	Occult blood in Urine	Quantitative erythrocytes for Occult blood in Urine
Grade 0	Absent	Less than 30mg/dl	Absent	0
Grade 1	+	30mg/dl	+	10 erythrocytes / µl
Grade 2	+ +	31 to 70 mg/dl	+ +	100 erythrocytes / µl
Grade 3	+ + +	71 to 100mg/dl	+ + +	500 erythrocytes / µl
Grade 4	+ + + +	100 to 500mg/dl	+ + + +	> 500 erythrocytes / µl

The PHAN® diagnostic urine analysis strips by Erba Mannheim were used for the urine analysis.

3.4.2 In-saline agglutination and Haemolysis Test:

The in-saline auto-agglutination test was performed by mixing one drop of blood with 1-3 drops of normal saline (0.9%) with an applicator stick at room temperature on a glass slide (Day, 2008). RBC agglutination was immediately evaluated macroscopically and microscopically (with a 40x objective or 100x oil immersion lens). Differentiation of agglutination and rouleaux (normal, physiological) was carried out by adding one more drop of saline, in which rouleaux dispersed however, agglutination does not.

To confirm the haemolysis following steps were followed (Day and Kohn., 2012)

1. Collect 1-2 ml of blood of the anaemic dog in a serum tube and in an EDTA/heparin tube.
2. Separate the supernatant serum or plasma from red cells by centrifugation at 3000rpm for 10 minutes, or by allowing samples to stand for at least 30 minutes.
3. Transfer the supernatant from the serum tube to a clean, labelled glass tube. Also transfer the plasma to another labelled glass tube.
4. Wash the RBCs three times with normal saline solution, discarding the supernatant after each wash. To wash RBCs: add approximately 4ml saline, mix well and centrifuge for 1-2 minutes. The saline is removed from the supernatant, leaving a packed RBC pellet at the bottom.
5. Make 4 % red cell suspension was made by adding 0.2ml packed red blood cells in 4.8ml, 0.9% saline.
6. Place 0.1 ml aliquot of red blood suspension in three tubes.
7. Add 0.1 ml aliquot of plasma.
8. Incubate the three tubes for 30 minutes:
    a) One tube at 4^0C.
    b) One tube at room temperature
    c) One tube at 37^0C.

9. Centrifuge the tubes for approximately 15 seconds to allow the cells to settle.

10. Check for hemolysis (reddening of the solution) and /or red blood cell agglutination in each tube.

11. Presence of hemolysis or agglutination in any incubated tube indicates an incompatibility.

3.4.3 Haematological estimations

For routine hematologcial studies 2 ml blood aseptically collected from the recipient was collected in Ethylene diamine tetra acetic acid (EDTA) vial and clot activator for the estimation of the parameters mentioned below. The blood was analyzed for complete blood count by a fully automated Mindray BC-3000 haemoanalyzer. Parameters studied were:

1 Haemoglobbin (Hb, g/dl)

2 Packed Cell Volume (PCV, %)

3 Total Erythrocytic count (TEC, ($x10^6/\mu l$)

4 Mean Corpuscular Volume (MCV, fl)

5 Mean Corpuscular Hemoglobin (MCH, pg)

6 Mean Corpuscular Hemoglobin Concentration (MCHC, g/dl)

7 Total Leucocyte Count (TLC, $\times 10^3$/cmm)

8 Differential Leucocytic Count (DLC):

a) Neutrophils (N %),

b) Basophils (B, %),

c) Eosinophils (E, %),

d) Monocyte (M, %),

e) Lymphocyte (L, %)

9. Platelets (PLT, x $10^3/\mu l$)

3.4.4 Biochemical examination

For biochemical studies, 2 ml of serum was harvested was immediately kept inthe deep freeze at -20°C till analysis. Samples were collected from anaemic and apparently healthy control animals. The samples were analyzed for biochemical parameters on semi-automated and fully automated analyzer ARX-100 and FA-200using Erba Manheim kits.

I. Liver function tests

i. Total Bilirubin (TB, mg/dl) (Diazo method, end point)

ii. Direct bilirubin (IB, mg/dl), (Diazo method, end point)

iii. Indirect bilirubin (DB, mg/dl), (Diazo method, end point)

iv. SGOT (IU/L), (IFCC recommended methodology)

v. SGPT (IU/L), (IFCC recommended methodology)

vi. ALP (IU/L), (DGKC-SCE recommended procedure)

vii. Total protein (TP, g/dl), (Direct Biuret method)

viii. Albumin (g/dl), Globulin (g/dl), (Bromocresol green methodology)

ix. Albumin: Globulin (A/G)

II. Kidney Function Tests

i. Serum Blood Urea Nitrogen (BUN, mg/dl), (Urease/GLDHmethodology)

ii. Serum Creatinine (mg/dl). (Modified Jaffe's method)

3.4.5 Blood smear examination

A thorough examination of the stained blood smear was done to determine morphological and staining characteristics of erythrocytes and abnormal inclusions, leucocyte distribution, differentiation (mature and immature neutrophils) and other abnormalities. The blood smears were also examined for the hemoprotozoa (*Babesia canis* and *Hepatozoan canis*) and Rickettsia (*Ehrlichia canis*). The diagnosis of babesiosis was confirmed by the demonstration of the parasite within the infective erythrocyte in Wright-Giemsa-stained thin blood smears (Matijatko *et al.,* 2007).

3.4.6 Reticulocyte count (%):

Reticulocyte count was done by counting reticulocytes in blood smears. A smear was made after incubating 0.5 ml of whole blood with an equal volume of brilliant cresol blue. The reticulocyte number was counted per 1000 RBC under oil immersion and their percentage was calculated.

A corrected reticulocyte count can be determined by using the following equation.

Corrected reticulocyte count = % Reticulocyte count × TEC (Cowgill *et al.*,2003).

A reticulocyte count of more than 1 % indicates regenerative anemia/ active erythropoiesis and a reticulocyte count below 1 % indicates non-regenerative anemia (Jain, 1993).

3.4.7 Free Hb (g/dl)

2 ml blood was collected in an EDTA tube and plasma was harvested by centrifugation at 3000rpm for 10 minutes, or by allowing samples to stand for at least 30 minutes. The plasma was further subjected to estimation of Free Hb on a fully automated Mindray BC-3000 haemoanalyzer. Free Hb was done for all *In-vivo* and *In-vitro* samples). Free Hb of more than 0.1 gm % represents the hemolysis.

3.4.8 Blood lactate levels (mmol/L)

2 ml of blood was withdrawn from the cephalic vein and was collected in a fluoride vial (Grey-capped vial). The method employed in the estimation of blood lactate levels was an enzymatic colorimetric assay.

This assay employs a coupled enzymatic reaction system that combines multiple steps. Initially, lactate oxidase catalyzes the oxidation of L-lactate to pyruvate and hydrogen peroxide (H_2O_2). Peroxidase then catalyzes the reaction of hydrogen peroxide with the Colorimetric Probe to form a pink colored product. The optical density measured at 510nm is directly proportional to the concentration of L-lactate present in the sample.

3.4.9 Iron profiling

(Photometric tests using chromogen ferrozine on EM- 200)

Iron profile was studied on Before Treatment and Day 35 (after treatment).

1. Serum Iron (μg/dl) (Photometric tests using chromogen ferrozine on EM-200)
2. Total Iron binding capacity (TIBC, μg/dl) (Photometric tests using chromogenferrozine on EM-200)
3. Percent Transferrin saturation (%):

(calculated) % TSAT= (Serum iron/ TIBC) x 100

3.4.10 Serum Electrolyte Estimations

Serum electrolytes were done by using a fully automated blood gas analyser (ABL80 FLEX). Electrolyte parameters estimated were-

I. Sodium (Na^+, mEq/L)

II. Potassium (K^+, mEq/L)

III. Chloride (Cl^-, mEq/L)

3.4.11 Urine Analysis

Urine samples were collected by free catch method during urination, in sterile plastic containers. Parameters analyzed were Colour, Appearance, pH, Urinary protein, Urinary creatinine and UPC ratio and occult blood in urine.

All the hematological, biochemical, electrolyte estimations, Urine analysis, Reticulocyte count, Free Hb and Blood lactate were estimated on before treatment, and on Day 0, Day 3,Day 7,Day15, Day 28 and Day 35 while Serum Iron was estimated Before and after treatment of the anaemic dogs. Day 0 was the first treatment day of the anaemic dog in the present study.

3.5 Enrollment and Group formation of anaemic dogs:

The study included 24 anaemic dogs (Appendix V) undergoing transfusion and routine standard treatment as per protocol and 6 healthy control dogs. The anaemic dog patients were randomly selected based on inclusion criteria.

Based on clinical, haemtological, biochemical , urine examination and etiological factors for anaemia, the enrolled dogs were grouped as mentioned in the table. Cases with *Ehrlichia canis* infection were confirmed by using SNAP 4DX® kit (M/s IDEXX Laboratories, New Delhi) that detects *Ehrlichia canis* antibodies. The *Babesia canis* infection was made by demonstrating intraerythrocytic piriform (i.e., pear-shaped or teardrop-shaped) organisms on blood smears. The cases of pyometra were diagnosed based on physical exam findings such as vaginal discharge. The CKD was diagnosed on the basis of clinical signs symptoms, Kidney function tests, urine test, electrolyte study etc.

Anaemic cases re-grouped depending upon their element / etiological factor.

Sr. No.	Group	Particulars	No of Dogs (n=30)
1	Group A	Anaemia dogs due to chronic kidney disease	06
2	Group B	Anaemia dogs due to *Erhilichia canis*	06
3	Group C	Anaemia dogs due to *Babesia canis*	06
4	Group D	Anaemia dogs due to Pyometra	06
5	Group E	Apparently Healthy Control	06

3.6 PART – II Identification and Quantification of Serum proteins in hyperglobulinemic anaemic dog patients

The blood serum samples were subjected to quantification of the specific globulin proteins by capillary/paper zone electrophoresis methods on fully automated Interlab Genios Electrophoresis machine (Plate – 3.9). This machine is used for quantifying different protein peaks/ bands. It is widely used in human protein quantification for detection of different protein bands and multiple myloma detection.

Two ml blood was collected in clot activator and serum was harvested which was further subjected to electrophoresis. The standard procedure for the electrophoresis was followed. The film received in electrophoresis was quantified by reading the bands or graph with special automated algorithm on Interlab

Genios Electrophoresis machine (Plate 3.9).

Protein Electrophoresis study was done Before treatment and on Day 20^{th} of the treatment. With the technique of protein Electrophoresis α1, α2, β1, β2 and γ globulins were quantified. Estimation of IgG and IgM immunoglobulins was also done by Immunoturbidimetry method.

3.7 PART – III: Assessment of erythrocyte survival:

In order to undertake erythrocyte survival study six blood aliquots in each group were collected in three different groups as mentioned below

a. *In-vitro* Sampling of Donor Dog (Before Blood Transfusion)

b. *In-vitro* Sampling of Recipient Dog (Before Blood Transfusion)

c. *In-vitro* Sampling of mixed blood of Donor and Recipient (After Blood Transfusion)

A. ***In-vitro* Sampling of Donor Dog (Before Blood Transfusion)**

1. After the collection of blood from Donor dog in CPD bag, the blood collecting tube was refilled after proper mixing of blood.

2. Six such aliquots (2ml each) were collected by sealing with tube sealer (Plate 3.1). These aliquots were stored at 4^0C in refrigerator till Day 35 of the study.

3. Every single aliquot was subjected to estimation of Hb, TEC, PCV and free Hb on Day 0,3,7,15,28 and 35 of study and observations were recorded

B. ***In-vitro* Sampling of Recipient Dog (Before Blood Transfusion)**

1. Six blood aliquots of 2ml each, were collected from the recipient dog half an hour before blood transfusion. These aliquots contained CPDA-1 as an anticoagulant. (For 300ml blood 49 ml CPDA-1 is required. For 2ml of blood 0.3ml of CPDA-1 was added). (Plate 3.2)

2. These aliquots were stored at 4^0C in refrigerator till Day 35 of the study.

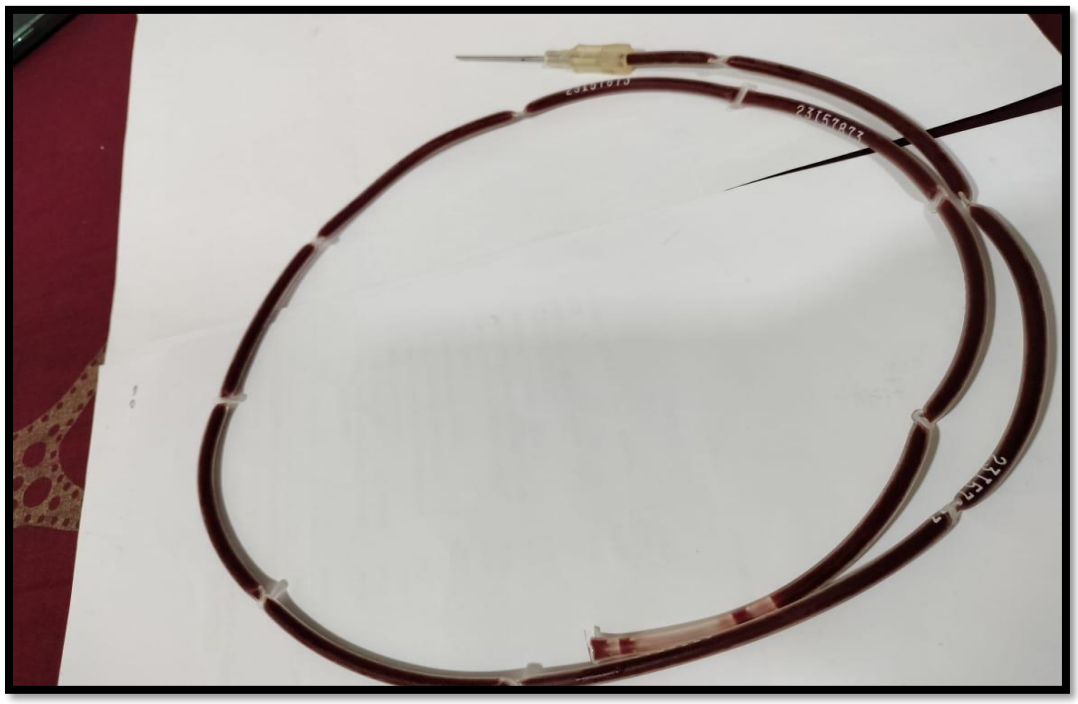


Plate 3.1 Donor Blood aliquots in CPDA for Erythrocytes Survival Study

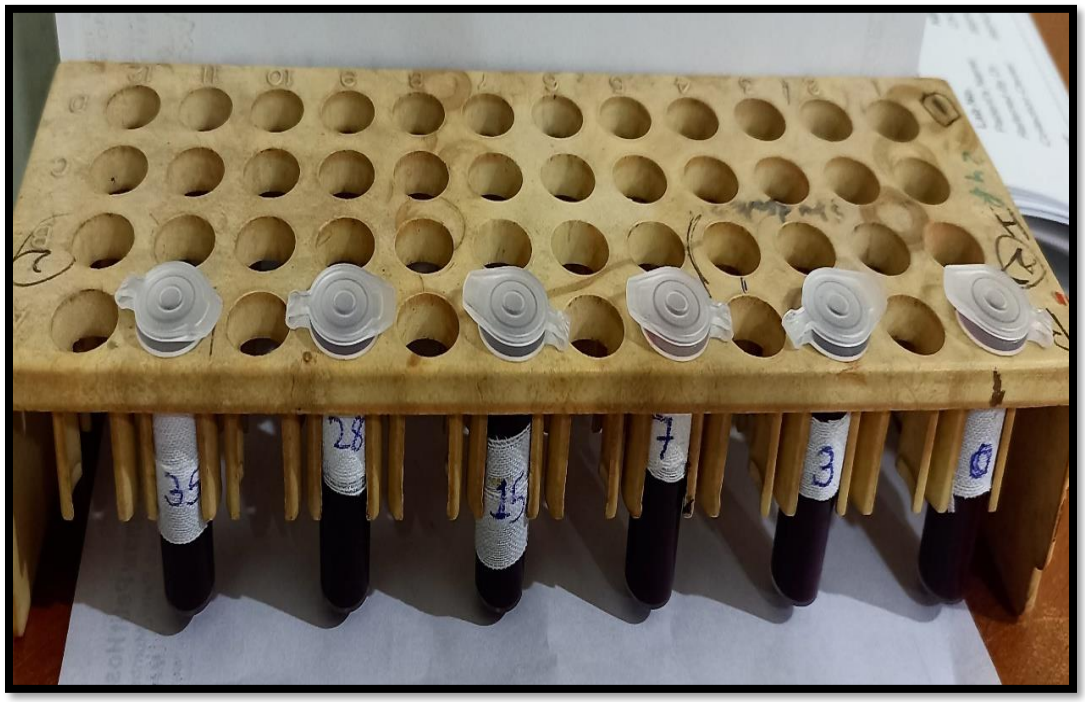


Plate 3.2 Recipient Blood aliquots in CPDA for Erythrocytes Survival Study

4. Every single aliquot was subjected to estimation of Hb, TEC, PCV and free Hb on Day 0,3,7,15,28 and 35 of study and observations were recorded

C. ***In-vitro*** **Sampling of mixed blood of Donor and Recipient (After Blood Transfusion):**

1. Blood collected from the donor dog was transfused to the recipient dog as per the standard procedure (Day and Kohn, 2012)

2. There is an equilibrium phase of half an hour after blood transfusion. Hence half an hour after completion of the blood transfusion, 12ml of blood from the recipient dog was collected in a syringe containing 1.96 ml of the CPDA-1 anticoagulant.

3. Six aliquots of 2.3ml each were prepared in six different sterile containers and were stored at 4^0C in refrigerator. (Plate 3.2)

4. Every single aliquot was subjected to estimation of Hb, TEC, PCV and free Hb on Day 0,3,7,15,28 and 35 of study and observations were recorded

D. **Comparison, Analysis and Finding of Erythrocyte Survival Study**

1. The observations of Hb, PCV, erythrocytes and free Hb both *In-vivo* and *In-vitro* on days 0,3,7,15,28 and 35 were analyzed.

2. The observations of Hb, PCV, erythrocytes and free Hb in *In-vitro* Donor, *In-vitro* Recipient and, *In-vitro* Donor + Recipient were compared and analyzed.

4.8 Calculation of Percent Haemolysis, Ratio and Proportion

The percentage haemolysis *In-vivo* (Donor + Recipient) and *In-vitro* (Donor, Recipient and Donor + Recipient) was calculated by following calculations

=[100-hematocrit (unit) X fHgb (g/L)/ total Hgb (g/L)]

Values of Hb (g/L), RBC (x 10^{12}/L), PCV (L/L) and Free Hb (g/L) are in SI units.

The formulae to calculate the Ratio and proportion

$$\text{Ratio} = \frac{\textit{In-vivo}}{\textit{In-vitro}} \qquad \text{Proportion} = \frac{\textit{In-vivo}}{\textit{In-vivo} + \textit{In-vitro}} \times 100$$

4.9 Transfusion Therapy and Treatment:

4.9.1 Selection of blood donors

The selection and screening criteria for donor :

1. Age bracket: 1-8 years of age
2. Weight: 20 kgs and above
3. Females who are not actively bleeding during the estrus phase.
4. Deworming and vaccination status up to date.
5. Ideally, without a history of tick fever or undertaking serological testing for the same whenever possible. (Ehrlichia *spp.*, Babesia *spp.*)
6. Healthy with no comorbidities.
7. PCV > 45%
8. Not currently on any medication.
9. Calm temperament and docile
10. Should not have had previous history of transfusion in last three months.
11. No history of having a litter in females (Rutan, 2007)

4.9.2 Blood collection

After the initial basic screening of the donor, consent (Appendix II) was obtained from the owner for the collection of blood from the donor dog (15-20 ml/kg BW). The blood was collected (Plate 3.3) in commercially available blood bags (Plate 3.4) in citrate phosphate dextrose adenine (CPDA-1) (Wardrop *et al.*, 1997). Terumo Penpol bags were used with 49 ml CPDA-1 meant for human use. The donors were prepared (Plate 3.5 A) for blood collection in a calm and well-ventilated room. The phlebotomy site was clipped, shaved and prepared

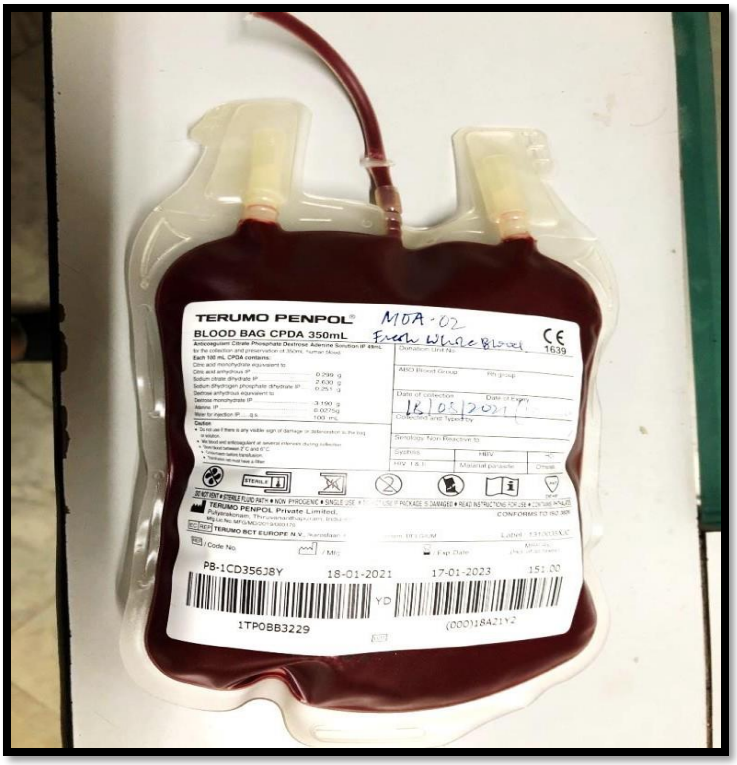
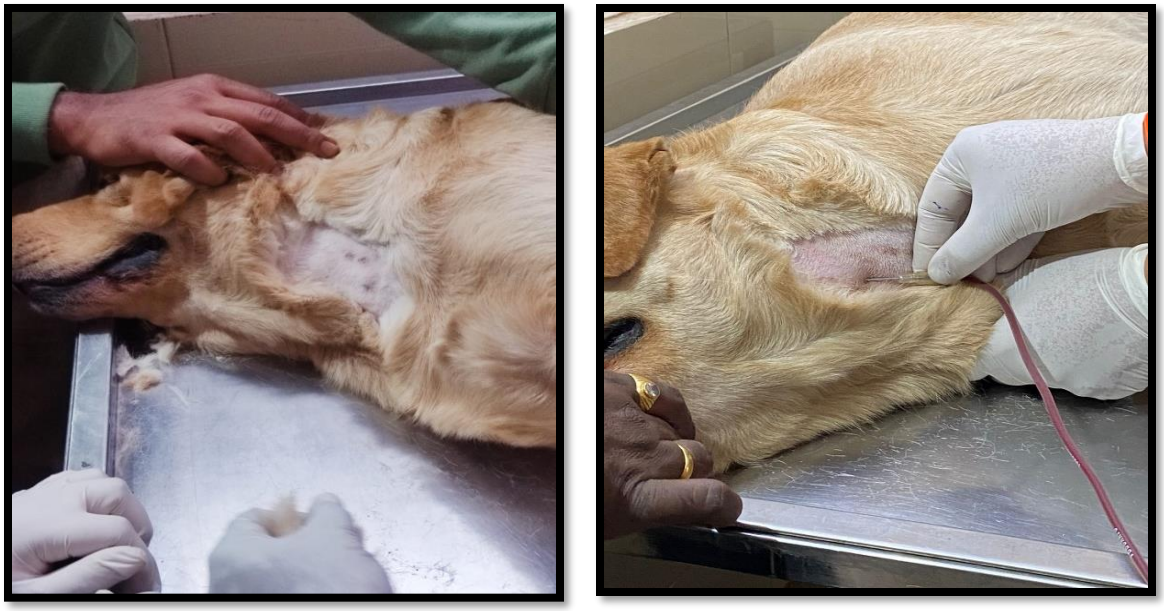


Plate 3.3 One Unit of Whole Blood Collected in CPDA

Plate 3.4 Depicting Empty 350 ml Blood Bag with 49 ml CPDA



(A) **(B)**

Plate 3.5 Depicting (A) preparation of collection site and (B) Phlebotomy Procedure during Blood Transfusion Process

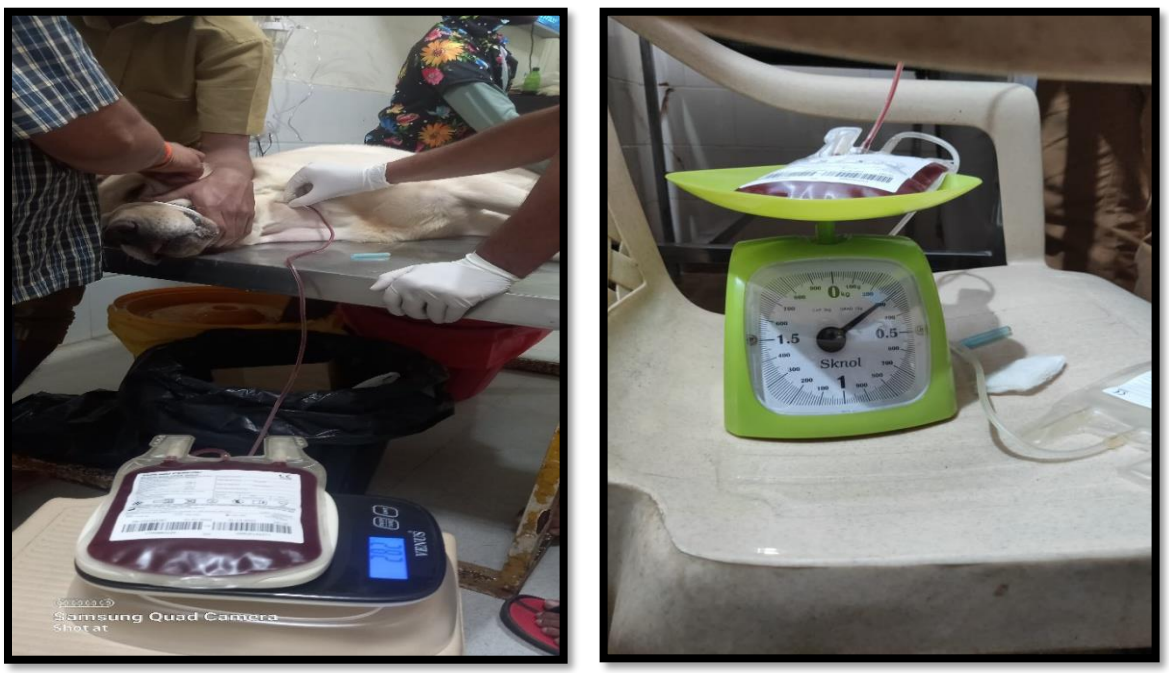


Plate 3.6 Depicting weighing of the amount of blood collected on weighing scale.

Plate 3.7 Blood Bank Refrigerator for storage of Whole blood at 4^0C

Plate 3.8 Biomedical Refrigerator for storage of Plasma at -20^0C

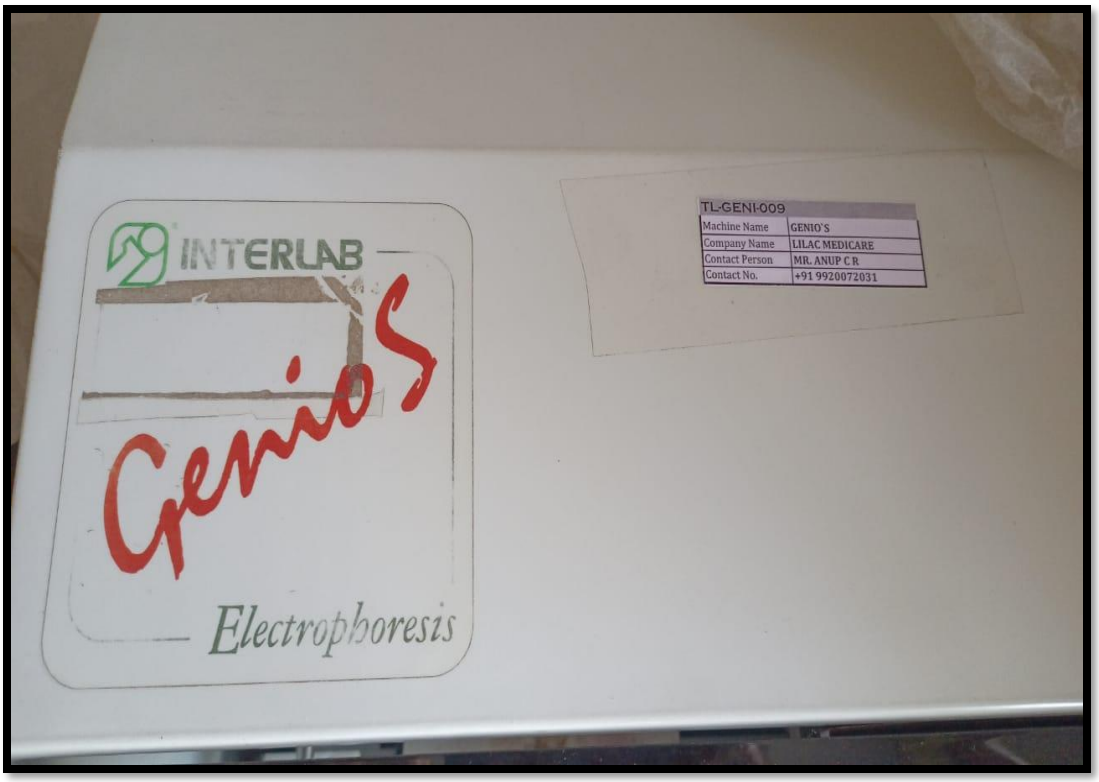


Plate 3.9 Interlab Genios Electrophoresis machine used for Protein Electrophoresis

aseptically. After establishing gravity enabled the free flow of blood via jugular access (Plate 3.5 B), the blood bag was swirled frequently on the palm throughout the collection to ensure optimum mixing of blood and anticoagulant. To ensure the collection of the calculated amount of blood from the donor based on body weight, a weighing scale was used. (Plate 3.6)

4.9.3 Cross-matching

Cross-matching was performed manually at the Animal Blood and Component Bank, Coagulopathy Laboratory established at the Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, Mumbai Veterinary College, Parel, Mumbai according to the guidelines recommended by Bonagura J. D. (2000).

1. Centrifuge EDTA or citrated donor blood at the lowest centrifugal rate for about 10 minutes.
2. Remove 0.2ml of packed erythrocytes and place in 4.8ml of normal (0.9 %) saline. Mix (There are now 5.0 ml in the tube: this procedure essentially replaces the washing step).
3. Place 0.1ml of this mixture into three small test tubes.
4. Place 0.1 ml of patient serum or plasma into each of the three tubes described previously. Each tube now has 0.1ml of the donor red blood cell -saline mixture and 0.1 ml of patient serum or plasma, a total of 0.2ml each.
5. Incubate (for 15 minutes) one tube at 37^0C, one at room temperature (25^0C), and one at refrigerator temperature (4^0C).
6. Centrifuge briskly (fastest centrifugal rate on clinical centrifuge) for 1 minute.
7. Examine the supernatant for any hemolysis. Any hemolysis indicates crossmatch incompatibility.
8. Examine the cell button. Flick or swish the test tube. The fluid in the tube should redden as red blood cells disperse. If the button is agglutinated or microagglutinated (examine a drop under low microscope power),

crossmatch incompatibility exists.

9. To complete the minor crossmatch, use patient red blood cells and donor serum or plasma. To complete the autocontrol, use patient red blood cells and patient plasma. In dogs, the minor crossmatch is useful for a patient receiving multiple plasma product transfusions.

The samples were also analyzed microscopically by examining a drop of sample from each test tube by placing a coverslip under 40x. The test results indicated major and minor compatibility or incompatibility, based on which decision to transfuse blood from that particular donor was made. The clumping of 3 to 5 or more red cells considered incompatible blood.

3.9.4 Administration of blood

Informed consent from the owner explaining the risks associated with the procedure was obtained. (Appendix III). The recipient was premedicated with antihistamines. (Bruce *et al.,* 2015).

IV cannula was secured in the cephalic vein of the recipient and blood was administered through a blood transfusion set which avoids passage of blood clots, if any. The bag was also checked thoroughly for the presence of any visible damage, discoloration or clots as perceived by the naked eye. The transfusion was carried out @ 10ml ml/kg/hour. Transfusion of the entire product was concluded in a maximum of 2 hours to reduce the chances of contamination or introducing infections.

3.9.5 Monitoring of transfusion

The patients were monitored throughout the transfusion process and the details were recorded in a systematic way (Appendix IV) as per guidelines of Pet Blood Bank, United Kingdom. Acute or delayed reactions (upto 10 days) were monitored. Any adverse reaction was recorded and managed with appropriate medical intervention.

3.9.6 Conventional Medical Therapy

All 24 anaemic dogs were evaluated for the type of anaemia based on regeneration status. Transfusion or empirical conventional therapy with fluids and

hematinics was initiated to stabilize the patients to further ascertain the cause of anaemia.

Patients with a spectrum of clinical signs were contemporarily managed by conventional medical therapy. The patients awaiting blood transfusion due to the unavailability of blood/blood products or donors were maintained on the following regimen. Fluid resuscitation was initiated @ 20 ml/kg BW (Inj. D25 and Inj. NS). In the case of iron deficiency anaemia, Inj. Iron dextran @ 10- 20 mg/kg IM every alternate day.

In patients with suboptimal bone marrow response, Inj. Recombinant Human Erythropoietin @ 50-100 IU/kg SC once weekly while repleting iron stores if the deficit was observed. Symptomatic management for gastrointestinal signs, if any, was employed along with supportive therapy catering the individual needs of the patient. The haematological status of the patient was monitored for response to therapy with respect to improvements observed in PCV, Hb and blood lactate levels.

Table No. 3.2: Treatment Protocol of Anaemic dogs (n=24)under study.

Sr. No	Group	Particulars	Treatment
1	Group A	Anaemic dogs having Chronic Kidney Disease (n=6)	• Inj Cresp (erythropoiesis-stimulating protein) @ 0.5 to 1 mcg/kg SC once weekly • Inj. Iron Dextran @ 10 mg/kg, IM, once weekly • Inj B complex containing folic acid and cyanocobalamin (Inj. Vitcofol) – 1ml IM daily • Fluid therapy as per requirement. • Antiemetics Inj Ondansetron at 0.5 mg/kg, IV, b.i.d • Enalapril (ACE inhibitor) @ 0.25mg/kg bid PO. • Phosphorous binders containing Aluminium Hydroxide, Lanthanum Carbonate or Calcium carbonate for hyperphosphatemia

			• In case of leucocytosis or secondary infection, antibiotics such as Enrofloxacin @ 5 mg/kg IM OD. • Oral hematinics containing folic acid, pyridoxine, cyanocobalamin • Fresh Whole Blood
2	Group B	Anaemia dogs having *Erhilichia canis* (n=6)	• Doxycycline @ 5mg/kg PO BID for 28 days • Prednisolone @0.5mg/kg IM OD for 5 days (tapered dose). • Inj. Cresp (erythropoiesis-stimulating protein) @ 0.5 to 1 mcg/kg SC once weekly • Fluid therapy as per requirement. • Inj. B complex containing folic acid and cyanocobalamin (Inj. Vitcofol) – 1ml - IM-od • Inj. Iron Dextran @ 10 mg/kg, IM, once weekly • Oral hematinics containing folic acid, pyridoxine, cyanocobalamin. • Silymarin, S-adenosylmethionine, silybin, milk thistle (hepato-protectants) – PO – bid for 28 days • Carica papaya leaf extract (platelet stimulant) PO – bid for 28 days • Fresh Whole Blood
3	Group C	Anaemia dogs having *Babesia canis* (n=6)	• Doxycycline @ 5 mg/kg PO BID for 28 days • Inj. Imidocarb Dipropionate @ 6 mg/kg SC biweekly 2 doses. (Precede Inj. Imidocarb with Inj. Chlorpheniramine maleate @ 0.2 mg/kg – IM). • Prednisolone @0.5mg/kg BID IM for 5 days (tapered dose) • Inj Cresp (erythropoiesis-stimulating protein) @ 0.5 to 1 mcg/kg SC once weekly • Inj B complex containing folic acid and cyanocobalamin (Inj Vitcofol) – 1ml IM daily.

			• Inj. Iron Dextran @ 10 mg/kg, IM, once weekly • Oral hematinics containing folic acid, pyridoxine, cyanocobalamin • Silymarin, S-adenosylmethionine, silybin, milk thistle (hepato-protectants) – PO – bid for 28 days • Carica papaya leaf extract (platelet stimulant) PO – bid for 28 days • Fresh Whole Blood
4	Group D	Anaemia dogs having Pyometra (n=6)	• Inj Cresp (erythropoiesis-stimulating protein) @ 0.5 to 1 mcg/kg SC once weekly • Inj B complex containing folic acid and cyanocobalamin (Inj Vitcofol) – 1ml IM daily • Inj. Iron Dextran @ 10 mg/kg, IM, once weekly • Oral hematinics containing folic acid, pyridoxine, cyanocobalamin • Inj Enrofloxacin @ 5 mg/kg IM BID • Inj. Metronidazole @ 10 mg/kg – IV – bid • Fluid therapy as per dehydration status and fluid loss • Prednisolone @0.5mg/kg BID IM for 5 days (tapered dose) • Inj. Ondansetron (anti-emetic) @ 0.5 mg/kg – IV – bid • Fresh Whole Blood • Surgical intervention once the patient is stabilized
5	Group E	Apparently Healthy Dogs (Control Group) (n=6)	• No Treatment

3.10. Analysis of Data

The data gathered in the research plan was subjected to statistical analysis to determine whether the results were of significance or not, by methods illustrated

by Snedecor and Cochran (2009). Microsoft Excel was used to determine significances in recorded data via paired t-test.

Results and Discussion

CHAPTER – IV

RESULTS AND DISCUSSION

The present study entitled "Therapeutic Management of Anaemia associated Hyperglobulinemia with special reference to Erythrocyte Survival in Dogs" encompassed twenty-four anaemic dogs (n=24) having hyperglobulinemia and six dogs (n=6) as the healthy control group. The study included 15 (62.50%) males while 9 (37.50%) females with a variety of breeds consisting of Non-descript 4 (16.67 %), Golden Retriever 3(12.50%) then Boxer 2(8.33 %) and German shepherd 2(8.33 %). The single case of Doberman, Crocker spaniel, Siberian husky, Shih Tzu and Rottweiler dog breeds contributed 20.85% as mix breeds. In total 24 dogs fulfilled the inclusion criteria of Haemoglobin less than 5gm/dl and globulin more than 4gm/dl. The overall mean Hb and mean globulin in all the anaemic dogs under study were 3.96$\pm$0.16 and 4.86$\pm$0.25 whereas healthy had mean values of 11.80 ± 0.34 and 3.21 ± 0.04 respectively.

The distribution of anaemic dogs based on haemoglobin values revealed a maximum no of cases i.e. 10 (41.7 %) each in 3.5 to 4.2 g/dl and more than 4.2 g/dl. Two cases (8.34 %) were between 2.7 to 3.4 g/dl and one each in 2 to 2.6g/dl and 1.9 g/dl.

The distribution of the anaemic dogs based on Globumin (g/dl) concentration is illustrated in Table 4.13. The 17 out of 24 (70.89%) anaemic cases contributed a maximum number of anaemic dogs having globulin concentration between 3.4 to 5.0 (g/dl). The histogram showed 3 (12.51%) anaemic dogs each in globulin concentration of 5.1 to 6 g/dl and more than 6 g/dl. Only a single case (4.17 %) of globulin 3.99 (g/dl) was noted.

The dogs that were presented for inspection underwent a comprehensive set of initial tests including clinical, haematological, biochemical, and urine analysis. The serum samples were subjected to protein electrophoresis (SPE) before treatment and on the 20th day of treatment to quantify globulin fractions. The underlying causes responsible for the hyperglobulinemia and anaemia in dogs were CKD, *E. canis, B. canis* and pyometra containing Six (6) cases each. All the dogs were supported with blood transfusion and disease-specific

treatment till recovery or discharge. *In-vitro* studies of the Donor, Recipient and Donor + Recipient blood included estimation of Hb, RBC, PCV and Free Hb before treatment and on Day 0, 3, 7, 15, 28 and 35th day of blood storage.

Part – I: Clinico-hematological, Biochemical and Urine analysis of Hyperglobunamic anaemic dogs at different time intervals.

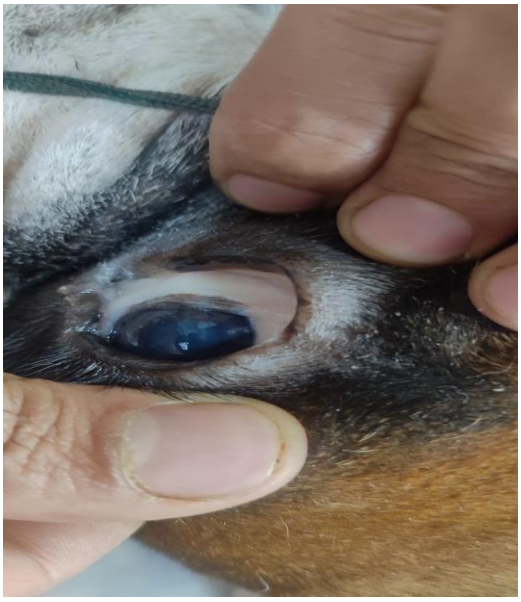
4.1 Clinical and Laboratory Assessment of anaemic dogs:

4.1.1 Clinical Assessment of Anaemic Dogs:

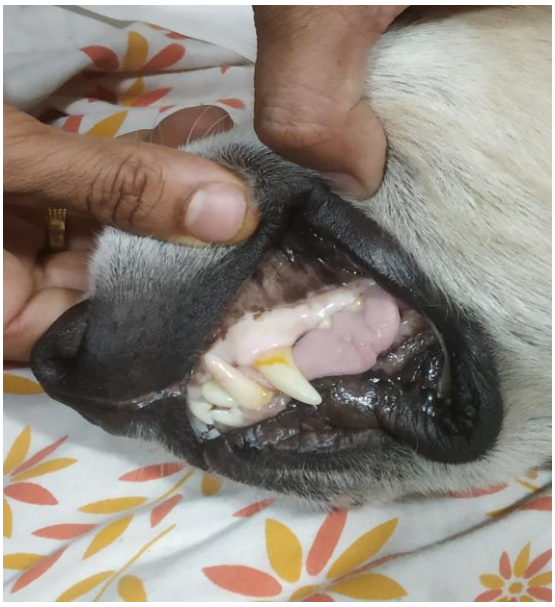
Anaemia was identified by a comprehensive assessment of the patients, which included medical history, physical examination, clinical symptoms and laboratory tests. During the study period, anaemia in dogs was assessed using various parameters including the colour of the visible mucus membrane (Plate 4.1), appetite, body condition, behaviour, skin and hair coat, posture and gait, lethargy, mentation/exercise tolerance, rectal temperature, heart rate and respiration rate. Blood clotting time and autoagglutination (Plate 4.2) were performed to know the coagulopathies. Urine examination contained the colour, appearance, pH, presence of proteins and occult blood. A notable clinical manifestation in anaemic dogs is the observation of pale mucus membranes (MM), particularly the conjunctival MM and penile MM (Plate 4.1), Extensive Epistaxis in *E. canis* dogs (Plate 4.3), Petechial haemorrhages at oral mucosa, penial region (Plate 4.7) and anal region (Plate 4.5).

A detailed clinical assessment of anaemic dogs was performed. The gradation of these parameters and values based on the histogram are mentioned in Table 4.1 (Gradation, frequency and percentage of clinical parameters), 4.2 (Gradation, frequency and percentage of Urine Analysis), 4.4 (Gradation, frequency and percentage of Blood coagulation evaluation of anaemic dogs) and 4.6 (Distribution of Anaemic Dogs based on Severity of Temp (^{0}F), Heart Rate (Per min) and Respiration Rate (Per min).

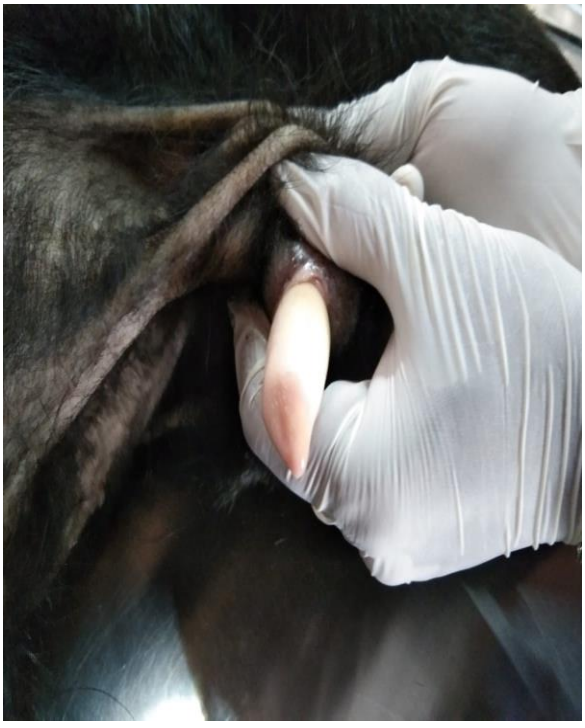
Out of 24 anaemic dogs,17 (7.87 %) dogs were found with severely pale and icteric MM (Plate 4.4) (Grade 4), whereas 7 (3.24 %) dogs had moderately pale MM (Plate 4.1) (Grade 3). None of the dogs had shown either slightly pale mucus membrane (Grade 2) or normal mucus membrane (Grade 1). The inclusion



a. Conjunctival Mucus Membrane



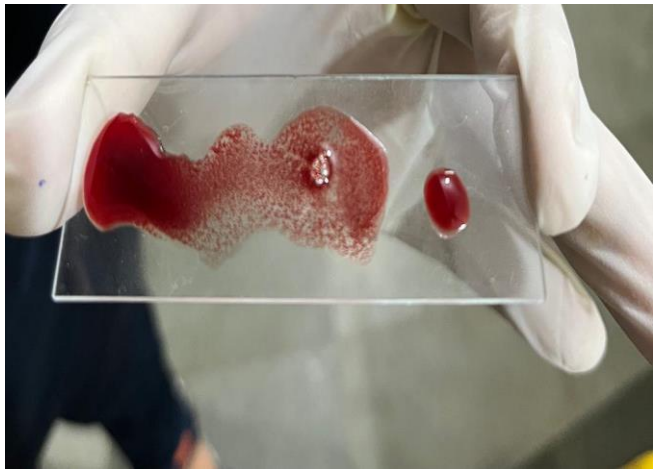
b. Oral Mucosa Membrane



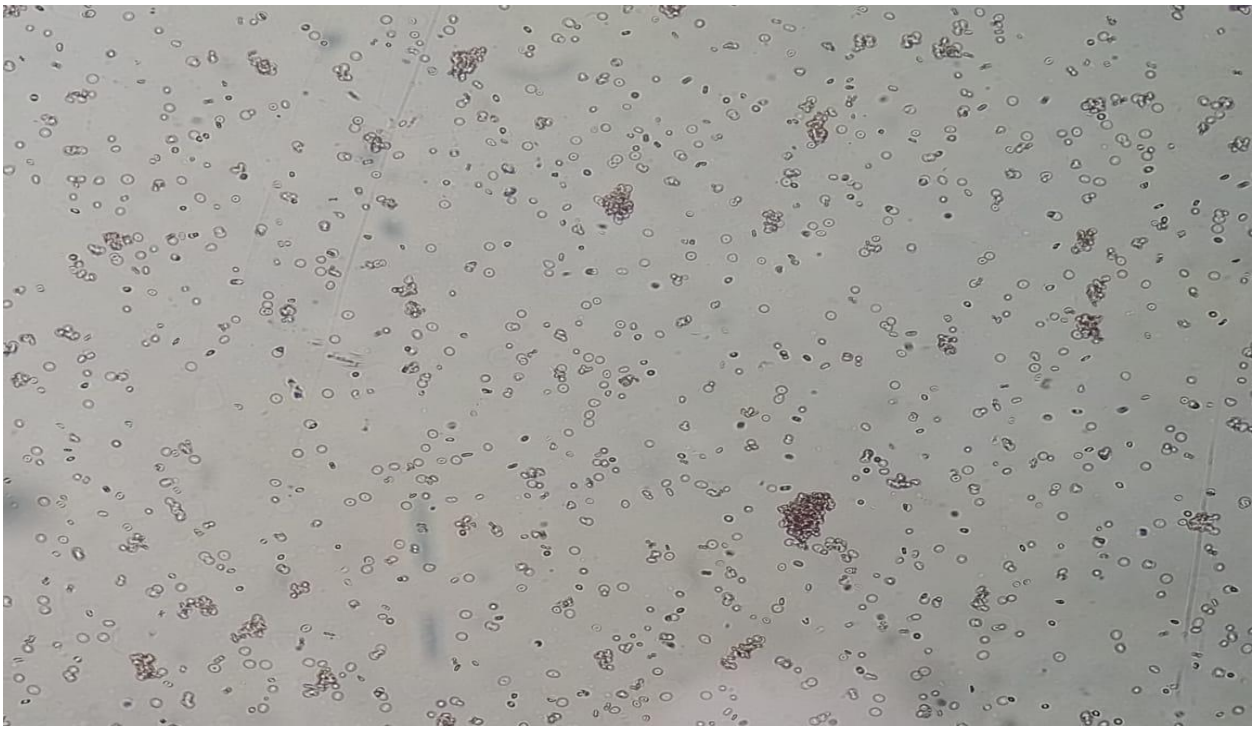
c . Penile mucous membrane

d. Ear Pinna

Plate 4.1 Severely pale mucus membranes of anaemic dogs on presentation (a,b,c,d)



(A)



(B)

Plate 4.2 Saline Agglutination Test (A) Blood Sample on Gross Exam

(B) Under the Microscope (40X)

Plate 4.3 Extensive Epistaxis in *E Canis* Dog on Presentation

Plate 4.4 Generalized Icteric skin Condition of Dog on Presentation

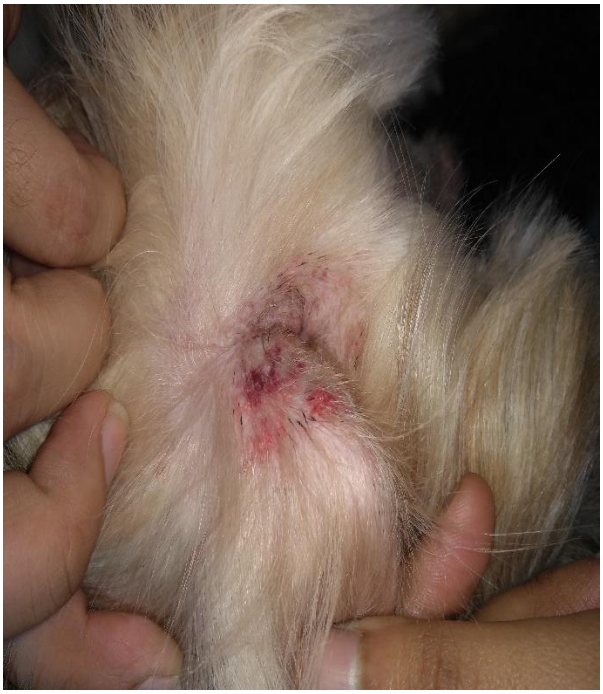
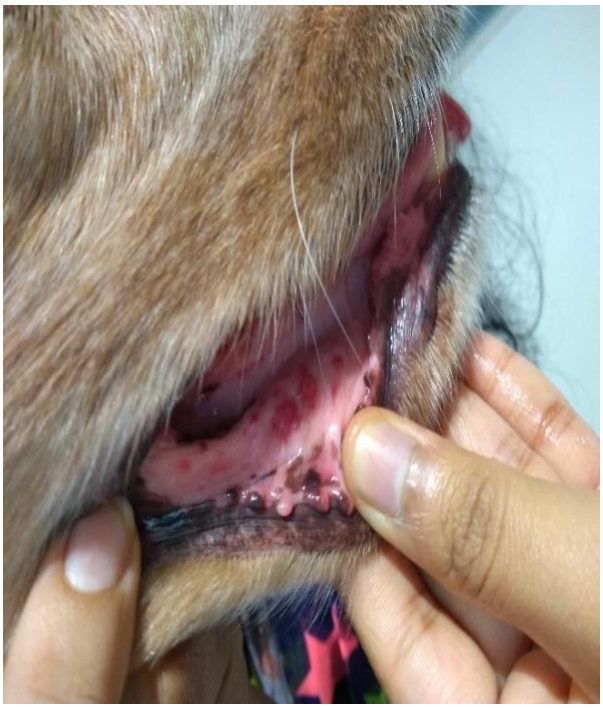


Plate 4.5 Petechiae at anal region of anaemic dog on presentation

Plate 4.6 Haematuria in anaemic dog on presentation



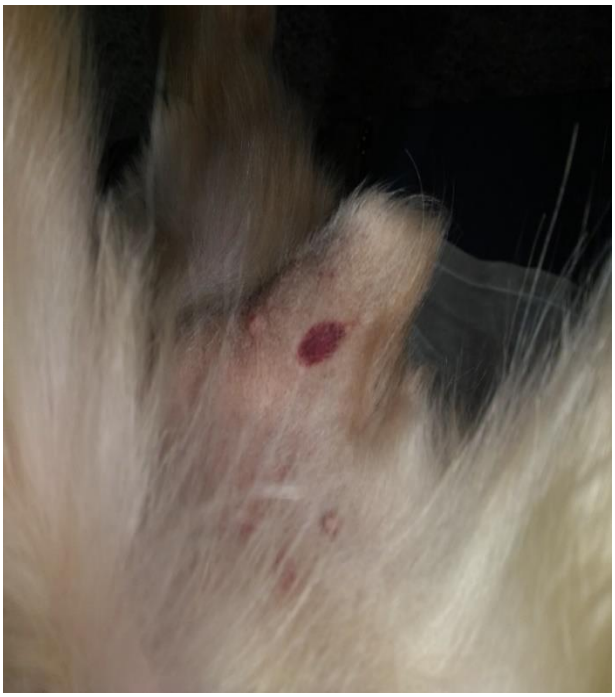


Plate 4.7 Petechial Haemorrhages in Oral mucosa and Penile region in anaemic Dog

criteria of haemoglobin less than 5 g/dl might have exhibited the maximum number of cases in severely pale or icteric grade. Regarding appetite, it was observed that anaemic dogs had a range of symptoms from reduced appetite to complete loss of appetite. Out of 24 anaemic dogs, 18 (8.33%) were anorectic (Grade 4), 4 (1.85%) were mildly anorectic (Grade 2) and 2 (0.93%) were moderately anorectic (Grade 3). Moderate fever in anaemic dogs might have caused the decreased appetite in the anaemic dogs. The cumulative means of Haemoglobin and Globulin in different grades of mucus membranes were (4.07 and 4.40 in Grade 3) and (3.91 and 5.04 in Grade 4) respectively.

Table 4.1: Gradation, Frequency and Percentage of Clinical Parameters of Anaemic Dogs (n=24). (Based on Histogram Distribution)

Sr. No	Parameter	Gradation	Gradation Short form	Before Treatment (n=24)		Day 35 (n=24)	
			Parameter	Freq^n	%	Freq^n	%
1	Appetite	Normal Appetite	AN Gr. 1	0	0.00	17	7.87
		Mild Anorexia	AN Gr. 2	4	1.85	5	2.31
		Moderate Anorexia	AN Gr. 3	2	0.93	2	0.93
		Anorexic/ Inappetence	AN Gr. 4	18	8.33	0	0.00
2	Body Condition	Emaciated	BC Gr. 1	1	0.46	0	0.00
		Thin	BC Gr. 2	10	4.63	11	5.09
		Ideal (Normal)	BC Gr. 3	11	5.09	12	5.56
		Overweight	BC Gr. 4	2	0.93	1	0.46
		Obese	BC Gr. 5	0	0.00	0	0.00
3	* Mucus Membrane	Salmon Pink (Normal)	MM Gr. 1	0	0.00	1	0.46
		Slightly Pale (Mild)	MM Gr. 2	0	0.00	9	4.17
		Moderately Pale (Moderate)	MM Gr. 3	7	3.24	14	6.48
		Severely Pale and/or Icteric (Severe)	MM Gr. 4	17	7.87	0	0.00
4	Skin and Hair Coat	Excellent (Shiny coat, free from dandruff or greasiness)	SH Gr. 1	0	0.00	0	0.00
		Very Good (Greasiness, Dull coat)	SH Gr. 2	0	0.00	5	2.31
		Good	SH Gr. 3	18	8.33	17	7.87

		(Greasiness, Dull coat/ Dehydration, Mild Pruritus)					
		Fair (Loss of Lustre, Alopecia/ Dandruff/ Mild Pruritus)	SH Gr. 4	6	2.78	2	0.93
		Poor (Dull/ Ectoparasites/ Dandruff/ Redness/ Pruritus, Odour)	SH Gr. 5	0	0.00	0	0.00
5	Posture And Gait	Excellent (Normal Posture and gait)	PG Gr. 1	0	0.00	0	0.00
		Very Good (Mild lameness, limping gait)	PG Gr. 2	5	2.31	10	4.63
		Good (Moderate lameness)	PG Gr. 3	16	7.41	13	6.02
		Fair (Ataxic gait)	PG Gr. 4	3	1.39	1	0.46
		Poor (Sleeping posture, No gait)	PG Gr. 5	0	0.00	0	0.00
6	Behaviour/ Mentation	Excellent (Normal Aggregation)	Bh Gr. 1	0	0.00	1	0.46
		Very Good (Quite Alert & Responsive)	Bh Gr. 2	7	3.24	10	4.63
		Good (Mild attacking)	Bh Gr. 3	13	6.02	13	6.02
		Fair (Fear/Phobia, nail-biting etc)	Bh Gr. 4	4	1.85	0	0.00
		Poor (Aggressive / Very dull)	Bh Gr. 5	0	0.00	0	0.00
7	Lethargy	Normal (Active)	Lt Gr. 1	1	0.46	14	6.48
		Mild (Mild Dullness)	Lt Gr. 2	3	1.39	9	4.17
		Moderate (Moderate Dullness)	Lt Gr. 3	7	3.24	1	0.46
		Severe (Dull and Depressed)	Lt Gr. 4	13	6.02	0	0.00
8	* Exercise Tolerance	Normal (BAR Walking)	Ex. Int. 1	1	0.46	17	7.87

		Mild (Quiet, Able to walk)	Ex. Int. 2	7	3.24	3	1.39
		Moderate (Reluctant to walk)	Ex. Int. 3	11	5.09	4	1.85
		Severe (Unable to stand)	Ex. Int. 4	5	2.31	0	0.00
9	Melena	Negative	Mel Gr. 1	11	5.09	16	7.41
		Positive	Mel Gr. 2	13	6.02	8	3.70
			Total	**216**	**100**	**216**	**100**

(*Kisielewicz *et al*., 2014)

Lethargy was one of the most significant clinical manifestations observed in anaemic dogs after pale mucus membrane as out of 24 anaemic dogs, 13 (6.02%) were lethargic (Grade 4), followed by 7 (3.24%) moderately dull (Grade 3) and 3(1.39%) had mild dullness (Grade 2). Only a single dog (0.46%) had shown none of the signs of lethargy (Grade 1) despite anaemia (# Case No 9, *E canis*) might be due to robust breed (Boxer) characteristics. The cumulative means of Haemoglobin and Globulin in different grades of lethargy were (3.6 and 5.3 in Grade 1), (3.53 and 4.56 in Grade 2), (3.93 and 4.35 in Grade 3) and (4.06 and 5.19 in Grade 4) respectively.

Regarding body condition score 11 (5.09 %) dogs exhibited ideal body condition score (Grade 3), while 10 (4.63%) dogs were thin (Grade 2) and 1 (0.46%) was emaciated (Grade 1). Two dogs (0.93%) were reported overweight despite the anaemic condition (Grade 4) might be due to hereditary. The cumulative means of Haemoglobin and Globulin in different grades of body condition scores were (3.88 and 5.34 in Grade 2), (3.90 and 4.23 in Grade 3) and (4.33 and 4.58 in Grade 4) respectively. Thin body condition could be due to protein losing uropathy arising from CKD (4 out of 10) and *E. canis* (3 out of 10), *B. canis* (2 out of 10) infection. There are chances of intravascular haemolysis and further hemolytic crisis.

In the case of posture and gait of anaemic dogs, 16 (7.41%) had moderate In-coordination (Grade 3), 5 (2.31%) had limping gait (Grade 2) and 3 (1.39%) dogs were with an ataxic gait (Grade 4). The serum chemistry panel revealed elevated alkaline phosphatase (ALP) (182.74 ± 37.05) which has a direct relation with postural reactions and reflexes (Andrade, 2022)

Out of 24 anaemic dogs, 18 (8.33%) dogs were dehydrated and had rough skin and hair coat with mild pruritus (Grade 3) while 6 (2.78%) dogs were observed with lusterless skin coat with alopecia dandruff and mild pruritus (Grade 4). The hypoalbuminemia (1.97 ± 0.10) in the present study might have affected the skin coat of the anaemic dogs.

The behaviour pattern of the anaemic dogs showed 13 (6.02%) dogs with good behaviour with a mild attacking nature (Grade 3). 7 (3.24%) dogs showed quiet, alert and responsive behaviour (Grade 2) while 4 (1.85%) dogs showed the behaviour of fear, phobia and nail-biting (Grade 4).

The exercise tolerance examination in anaemic dogs showed 11 (5.09%) dogs that were reluctant to walk (Grade 3)and 7 (3.24%) dogs that showed mild exercise intolerance (Grade 2) that could stand and walk. 5 (2.31%) dogs exhibited severe exercise intolerance with inability to stand (Grade 4). Only a single dog (0.46 %) had normal exercise tolerance (Grade 1). The cumulative means of Haemoglobin and Globulin in different grades of exercise tolerance were (4.1 and 4.8 in Grade 1), (4.21 and 4.91 in Grade 2), (3.84 and 4.69 in Grade 3) and (3.84 and 5.13 in Grade 4) respectively. Low haemoglobin is one of the major causes of increased tolerance to exercise.

The melena, one of the major clinical findings was reported in 13 (6.02 %) dogs (Grade 2) while the 11 dogs (5.09 %) didn't exhibit the signs of melena (Grade 1) in the present study. Melena (dark, tarry faeces) is caused by blood in the lumen of the stomach or proximal intestinal tract, resulting in black (digested) blood appearing in the faeces. Gastrointestinal bleeding due to varied reasons in the present study might have recorded the melena in 13 (6.02%) dogs. The cumulative means of Haemoglobin and Globulin in different grades of melena were (4.21 and 4.80 in Grade 1) and (3.74 and 4.9 in Grade 2) respectively. All six cases of *B. canis* anaemic dogs (Cases no 5,6,13,14,21 and 22), Four cases of *E. canis* anaemic dogs (Cases no 3,4,9 and 12) and two cases of CKD (Cases no 11 and 16) were found to be positive for melena where chances of autoimmune haemolytic crisis were more. None of the cases of pyometra was found to be positive melena.

The major predominant clinical signs in anaemic dogs noted were

Inappetance, Severely pale and/or Icteric mucus membrane, Lethagy, Exercise Tolerance and Melena.

The detailed urine analysis is presented in Table 4.2 (Gradation, frequency and percentage of Urine Analysis) which comprises urine colour, appearance, pH, presence of protein and presence of occult blood. Out of 24 urine samples, the urine colour of 7 (5.83%), 4(3.33 %), 4 (3.33 %), 1(0.83 %) and 5(4.17 %) anaemic dogs had yellow (Grade 3), light yellow (Grade 2), deep yellow (Grade 4), reddish brown (Grade 5) and red coloured urine (Plate 4.6) (Grade 6) respectively. The decreased GFR, and increased creatinine and BUN levels might have changed the urine colour to yellow. The reddish brown colour of the urine in the present study might be due to CKD and *E. canis* infections. The rest of the 3(2.50 %) urine samples were clear (Grade 1). The urine appearance in anaemic dogs was hazy (Grade 2) in 2 (1.67%), turbid in 5 (4.17%) (Grade 3) and cloudy in 7 (5.83%) (Grade 4) while the rest of the 10 urine samples showed a clear appearance (Grade 1). An equal distribution of 12 (10.00 %) as acidic (Grade 1) and 12 (10.00%) as alkaline (Grade 3) urine pH was noted in 24 anaemic dogs. Table 4.3 describes the correlation of Urine appearance with Urine pH in Anaemic Dogs. It revealed that out of 10 cases of clear appearance of urine 9 had acidic pH and and 1 had alkaline pH. There were a total of 14 cases of urine having Hazy (02), Turbid (05) and cloudy (07) appearance of which 11 had alkaline pH. Urinary tract infections caused by bacteria that produce urease can lead to an elevation in urine pH, resulting in alkaline urine. These bacteria have the ability to convert urea into ammonia. If a dog has alkaline pee, it is important to evaluate whether there are white cells and/or bacteria present, which may frequently be seen in the urine sediment. The urine pH in healthy dogs is mostly influenced by their food and if they have undergone fasting. Diets rich in animal protein, which are often taken by dogs and cats, result in a decreased urine pH, making the urine more acidic. Animals consuming milk diets tend to have acidic urine.

Protein-losing nephropathy was documented in 6 anaemic dogs (5.00%) (Grade 4) which showed the highest + + + + protein followed by 7 (2.08%) + + + protein (Grade 3), 1 (0.30%) + + protein and 3(2.50 %) showed + of protein in the

Table 4.2: Gradation, Frequency and Percentage of Urine Analysis Parameters of Anaemic Dogs (n=24) (Based on Histogram Distribution)

Sr. No	Parameter	Gradation	Gradation Short form	Before Treatment (n=24)		Day 35 (n=24)	
			Parameter	Freq^n	%	Freq^n	%
1	Urine Color	Clear (Colourless)	U Cl Gr. 1	3	2.50	3	2.50
		Light yellow	U Cl Gr. 2	4	3.33	4	3.33
		Yellow	U Cl Gr. 3	7	5.83	15	12.50
		Deep yellow	U Cl Gr. 4	4	3.33	2	1.67
		Reddish brown	U Cl Gr. 5	1	0.83	0	0.00
		Red	U Cl Gr. 6	5	4.17	0	0.00
2	Urine Appearance	Clear	UA Gr. 1	10	8.33	10	8.33
		Hazy	UA Gr. 2	2	1.67	13	10.83
		Turbid	UA Gr. 3	5	4.17	1	0.83
		Cloudy	UA Gr. 4	7	5.83	0	0.00
3	Urine pH	Acidic	UpH Gr. 1	12	10.00	18	15.00
		Neutral	UpH Gr. 2	0	0.00	0	0.00
		Alkaline	UpH Gr. 3	12	10.00	6	5.00
4	Protein in Urine	Absent	PrU Gr. 0	7	5.83	13	10.83
		+	PrU Gr. 1	3	2.50	0	0.00
		++	PrU Gr. 2	1	0.83	10	8.33
		+++	PrU Gr. 3	7	5.83	1	0.83
		++++	PrU Gr. 4	6	5.00	0	0.00
5	Occult blood in Urine	Absent	OBU Gr. 0	16	13.33	19	15.83
		+	OBU Gr. 1	1	0.83	0	0.00
		++	OBU Gr. 2	1	0.83	4	3.33
		+++	OBU Gr. 3	1	0.83	1	0.83
		++++	OBU Gr. 4	5	4.17	0	0.00
			Total	**120**	**100**	**120**	**100**

Table 4.3: Correlation of Urine appearance with Urine pH in Anaemic Dogs (n=24).

Urine Appearance	Acidic	Alkaline	Total
Clear (Grade 1)	9 (Case No 1,4,5,6,9,14,20,22, and 23)	1 (Case No 21)	10
Hazy (Grade 2)	2 (Case No 7 and 10)	0	02
Turbid (Grade 3)	0	5 (Case No 2,3,11,12 and 13)	05
Cloudy (Grade 4)	1 (Case no 8)	6 (Case No 15,16,17,18,19 and 24)	07
Total	**12**	**12**	**24**

urine (Grade 1) while proteinuria was absent in 7 (5.83%) (Grade 0). Proteinuria was absent (Grade 0) in 4 cases of *E. canis* (# Case No 1,4,9 and 23) and one case each of CKD (# Case No 20) and *B. canis* (# Case No 5). In Grade 3, out of 7 cases, two of each were of CKD (# Cases No 2 and 11), *E. canis* (# Cases No 3 and 12), *B. canis* (# Case No 13 and 22) and one case of pyometra (# Case no 17). In Grade 4 proteinuria, out of 6 anaemic dogs, 5 (# Case no 8,15,18,19 and 24) were of pyometra while one was of CKD.

Severe protein loss from the body, most significantly albumin; glomerular proteinuria is persistent and its magnitude can be quite high (4+ on test strip pad). Typically, urine contains minimal or negligible amounts of protein. The glomerulus generally does not filter larger plasma proteins, such as albumin and globulins. However, it does filter smaller proteins, which are then reabsorbed in the proximal tubules of the kidneys. This process is usually unaffected unless there is a significant increase in the amount of these proteins or there is a problem with the reabsorption in the renal tubules.

The observations of occult blood in 24 anaemic dogs showed 5 (4.17%) dogs with + + + + occult blood (Grade 4), 1 (0.83%) dog with + + + occult blood (Grade 3), 1 (0.83%) dog with + + occult blood (Grade 2) and 1 (0.83%) dog with + occult blood (Grade 1). The remaining 16 (13.33%) anaemic dogs had no evidence of occult blood in the urine (Grade 0). The presence of heme in urine might originate from either haemoglobin or myoglobin. Haemoglobin that is not bound to any other molecules is derived either from ruptured red blood cells or from undamaged red blood cells. Hematuria is the most prevalent cause of positive occult blood findings, rather than hemoglobinuria.

The evaluation of blood coagulation in anaemic dogs is illustrated in Table 4.4 (Gradation, frequency and percentage of Blood coagulation evaluation of anaemic dogs). Out of 24 anaemic dogs, 15 (31.25 %) blood samples showed negative slide agglutination (Grade 1) and microscopic agglutination (Plate 4.8) while the remaining 9 (18.75%) dogs showed positive slide agglutination test (Grade 2) and microscopic agglutination where immune aggregation of clumping of 3 to 5 more red blood cells (Grape like clusters) was observed (Plate 4.9).

Autoagglutination was observed as red speckles in EDTA-anticoagulated blood from a dog with severe IMHA. Since autoagglutination is only seen with high antibody levels, a negative slide agglutination test does not rule out IMHA (Bennett, 1981).

The analysis of the blood clotting time exhibited 16 (33.33 %) dogs which showed the blood clotting time as 6 or less than 6 minutes (Grade 1) while 8 (16.66 %) dogs showed blood clotting time as more than 6 minutes (Grade 2).

The correlation of Slide agglutination and blood clotting time was established in Table 4.5. All 8 cases of dogs positive for autoagglutination found to be have hypercoagulopathy i.e. blood clotting time between 1.6 to 3.5 min. Such hypercoagulability may be a precursor to clinically evident thrombosis as a complication of the disease process. Also, 11 anaemic dogs (45.87 %) were found to have comparatively higher (3.6 to 7.5 min) blood clotting time that has negative auto-agglutination.

In the case of rectal temperature (Table 4.6), the distribution of the anaemic dogs based on the histogram revealed a maximum no of cases i.e 11 (41.7 %) in the range of 102.7^0F to 104.3^0F followed by 6 (25.02 %) in the range of 104.3^0F, 5 (20.85 %) in the range of 101^0F to 102.6^0F and one case (4.17 %) each in the range of 99.3^0F and 99.4 ^{0}F to 100.9^0F.

In the case of heart rate (Table 4.6), the distribution of the anaemic dogs based on histogram revealed maximum no of cases i.e 9 (37.53 %) in more than 129.7/min range followed by 7 (29.19 %) in the range of 114.6/min to 129.7/min, 6 (25.02 %) in the range of 99.3 to 114.5/min and one case (4.17 %) each in the range of 84/min and 84.1/min to 99.2/min.

In the case of respiration rate (Table 4.6), the distribution of the anaemic dogs based on histogram revealed a maximum no of cases i.e 8 (33.36 %) in the range of 37.8/min to 46.5/min followed by 7 (29.19 %) in the range of 29.1/min to 37.7/min, 5 (20.85 %) in the range of 46.6/min to 55.2/min, 3 (12.51 %) in the range of more than 55.2/ min and one (4.17 %) in 29/min. The increased respiratory rate observed in anaemic dogs, as opposed to the normal group, can be attributed to the heightened demand for oxygen due to a decreased number of

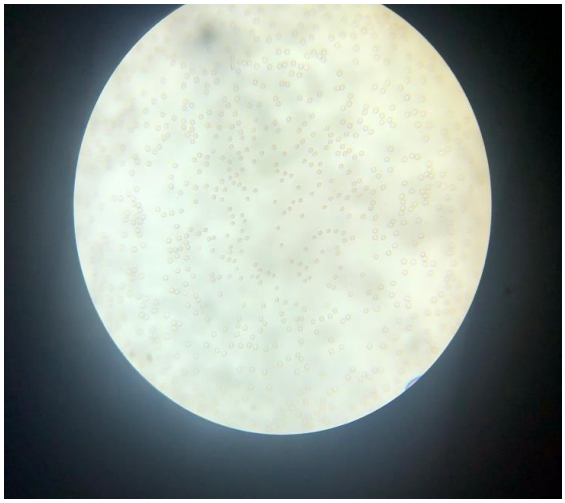


Plate 4.8 Illustrating microscopic compatible crossmatch

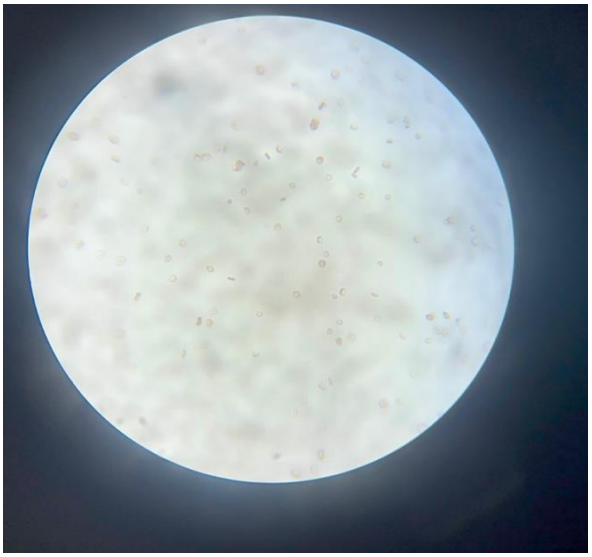


Plate 4.9 Illustrating microscopic agglutination (Clumping of 3-5 or more red cells) and incompatible crossmatch

erythrons. This phenomenon can be elucidated by a compensatory process, wherein the heart rate and respiratory rate are augmented in reaction to anaemia.

Table 4.4 : Gradation, Frequency and Percentage of Blood Coagulation Evaluation of Anaemic Dogs (n=24)

(Based on Histogram Distribution)

Sr. No	Parameter	Gradation	Gradation Short form	Before Treatment (n=24)		Day 35 (n=24)	
			Parameter	Freq^{n}	%	Freq^{n}	%
1	Slide Autoagglutination	Negative	Ag Gr. 1	15	31.25	24	50.00
		Positive	Ag Gr. 2	9	18.75	0	0.00
2	Blood Clotting Time (Min)	6 or less than 6 minutes	BCT Gr. 1	16	33.33	12	25.00
		More than 6 minutes	BCT Gr. 2	8	16.66	12	25.00
			Total		**100**		**100**

Table 4.5 – Correlation of Blood clotting time with Slide Auto-agglutination in anaemic dogs. (n=24).

Sr. No	Blood Clotting Time (Min)	No of dogs	Slide Auto-agglutination	
			Grade 1 (Negative auto agglutination)	Grade 2 (Positive Auto agglutination)
1	1.5	1 (4.17 %)		14
2	1.6 to 3.5	10 (41.7%)	7,16	1,3,4,6,9,12, 21, 23
3	3.6 to 7.5	11 (45.87 %)	2,8,10,11,15, 17,18,19,20, 22,24	
4	More than 7.5	02 (8.34 %)	5,13,	
		24	**15**	**09**

Table 4.6: Distribution of Anaemic Dogs (n=24) on the basis of Severity of Temp (^{0}F), Heart Rate (Per min) and Respiration Rate (Per min)

(Based on Histogram Distribution)

Rectal Temperature (^{0}F)	**99.3^{0}F**	**99.4^{0}F to 100.9^{0}F**	**101^{0}F to 102.6^{0}F**	**102.7 ^{0}F to 104.3^{0}F**	**More than 104.3^{0}F**
Frequency	01 (4.17 %)	01 (8.34 %)	05 (20.85 %)	11 (41.7%)	6 (25.02 %)
Heart Rate (Per min)	**84/min**	**84.1/ min to 99.2/min**	**99.3/min to 114.5/min**	**114.6/min to 129.7/min**	**More than 129.7/min**
Frequency	1 (4.17 %)	1 (4.17 %)	6 (25.02 %)	7 (29.19%)	9 (37.53 %)
Respiration Rate (Per min)	**29/min**	**29.1/min to 37.7 /min**	**37.8 to 46.5/min**	**46.6/min to 55.2/min**	**More than 55.2/min**
Frequency	1 (4.17%)	7 (29.19 %)	8 (33.36 %)	5 (20.85 %)	3 (12.51%)

Several researchers have documented prominent clinical signs and symptoms indicative of anaemia in dogs. According to the literature reviewed, Joshi (2000), Furlanello *et al.*, (2005), Swann and Skelly (2013), Kisielewicz *et al.*, (2014), Mahalingaiah *et al.*, (2017), and Assenmacher *et al.*, (2019), all observed similar clinical symptoms in anaemic dogs. These symptoms include anorexia, lethargy, pale mucus membranes, depression, high rectal temperature, increased heart rate, dyspnea, deep breathing, and dehydration. The clinical observations made in 24 anaemic dogs during the study period can be supported by the data documented by previous researchers. The study indicated that statistically, characteristics such as mucous membrane, rectal temperature, heart rate, respiration rate, anorexia, lethargy, physical condition, behaviour, and skin and hair coat were found to be significant. However, parameters such as posture and gait were found to be non-significant. These findings indicate that anaemic subjects typically exhibit pale mucus membranes, anorexia, and lethargy. Furthermore, anaemic dogs exhibit no clinical signs despondent behaviour, and a

compromised body condition characterized by a poor physique. There is no significant correlation between anaemia and the condition of the skin and hair coat.

On comparison among the four groups, these clinical manifestations showed increased intensity in CKD, *E. canis*, *B. canis* and pyometra groups than the apparently healthy control group.

4.1.2 Age-wise distribution of Anaemia in dogs:

The Age-wise distribution of anaemia is shown in Table 4.7 (Figure 4.1). The maximum no of anaemic cases in the present study were noted in Geriatric dogs i.e. in the age group of 74 to 109 months old i.e. 8 (33.33 %) and age group between 39 to 74 months old 7 (29.16 %) followed by more than 109 months old 4(16.66 %) and 5 to 39 months old 4 (16.66 %). Only a single dog (4.16 %) of less than 5 months old age was noted to be suffering from anaemia.

Table 4.7: Age-wise Distribution of Anaemic dogs (n=24)

Sr. No.	Age in Months	No of animals (n=24)
1	5 Months	1 (4.16 %)
2	5 to 39 Months	4 (16.66 %)
3	39 to 74 Months	7 (29.16 %)
4	74 to 109 Months	8 (33.33 %)
5	> 109 Months	4 (16.66 %)

The elderly canines have a higher likelihood of developing immune-mediated haemolytic anaemia, whereas older dogs are more susceptible to developing anaemia as a result of renal failure (Cotter, 2000), which aligns with the findings of the current study. In their study, Tandel *et al.,* (2019) observed a comparable result indicating a higher occurrence rate, specifically 58.83%, of anaemia in adult dogs.

In the present study, the anaemia due to *E. canis* and *B. canis* was higher in a middle-aged group of animals. According to Comazzi *et al.,* (2006), viral and parasite infections are more common in young and middle-aged dogs due to the ongoing growth of their bone marrow.

The results on the age of dogs with anaemia in this study contradicted the findings of Hinton and Jones (1977), who reported that anaemia was more prevalent in dogs under one year old and over ten years old. However, in the current study 50 % (12/24) dogs belong to the adult age group.

4.1.3 Gender-wise distribution of Anaemia in dogs:

The gender-wise distribution of anaemia is depicted in Table 4.8. (Figure 4.2). A higher percent of cases of anaemia were observed in males 15 (62.50%) than in females 9 (37.50%), out of 24 cases of anaemia.

Table 4.8: Disease-wise and Gender-Disease wise Distribution of Anaemic dogs (n=24)

Sr. No.	Name of Disease	Male	Female	Total
1	CKD	5	1	6
2	*E. canis*	6	0	6
3	*B. canis*	4	2	6
4	Pyometra	0	6	6
	Total	**15 (62.50 %)**	**9 (37.50 %)**	**24 (100 %)**

The current study's findings align with previous research undertaken by Brahmbhatt *et al.,* (2015), Tandel *et al.,* (2019), Meshram *et al.,* (2019), and Shah *et al.,* (2020), who also reported a significant finding of anaemia in males. The pyometra, being a disease of females, contributed cent per cent by female gender. Excluding pyometra cases, the per cent distribution of males was 83.33% (15/18) and females were 16.67% (3/18) in CKD, *E. canis* and *B. canis* anaemic dogs.

In the present study, 5 out of the 6 dogs suffering from chronic renal failure and subsequent anaemia were males. Renal insufficiencies are commonly reported in males, as supported by earlier investigations (Behrend *et al.,*1996). Data further shows that 6 out of 6 dogs in *E. canis* and 4 out of 6 dogs in *B. canis* were males.

The results indicating the anaemia due to *E. canis* and *B. canis* are in consistent with the findings of Vijayalakshmi (2011), who observed that intact males and neutered females were more susceptible to severe tick-borne infections.

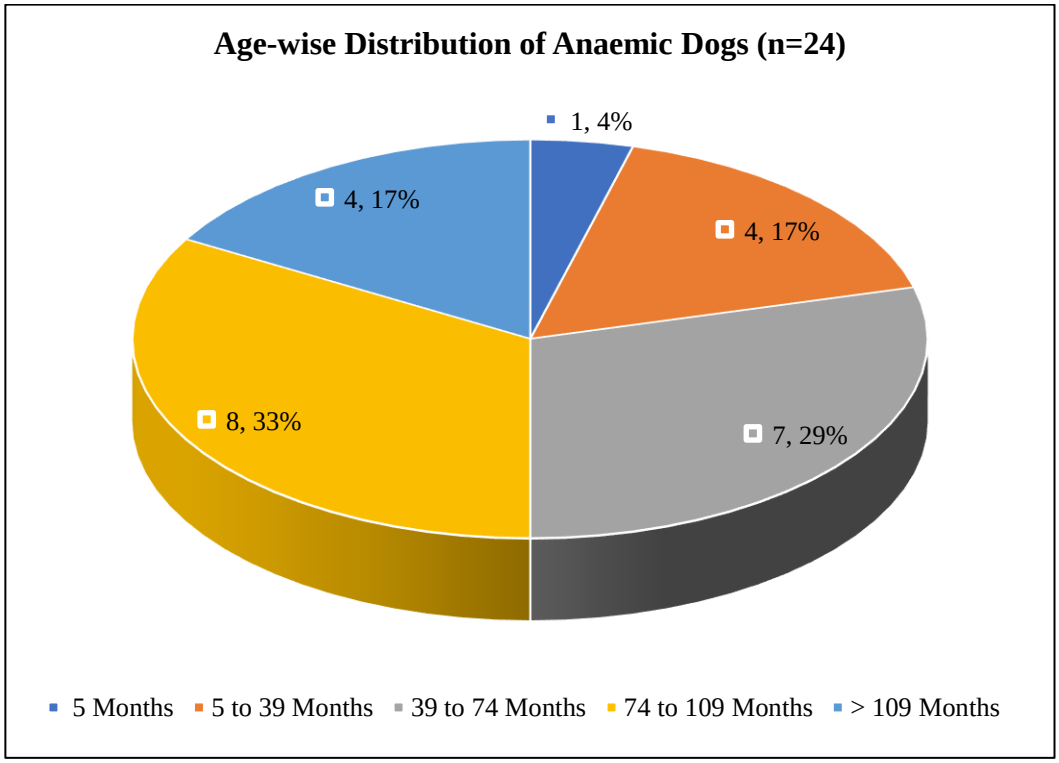


Figure 4.1 Age-wise Distribution of Anaemic Dogs (n=24)

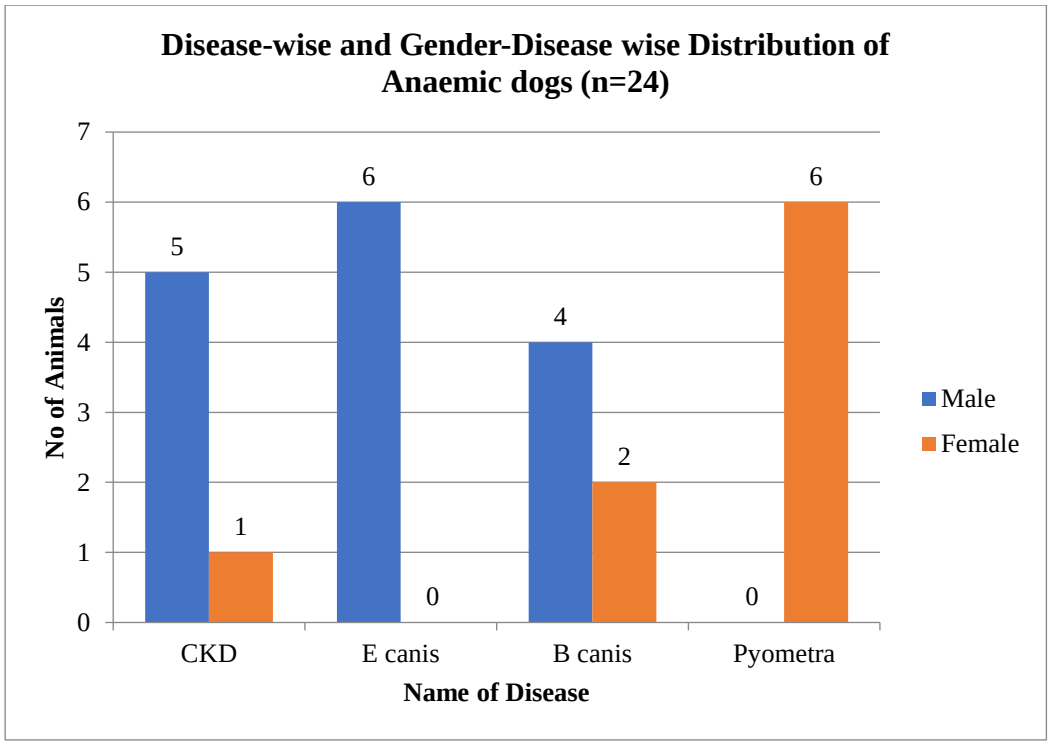


Figure 4.2 Disease-wise and Gender-Disease wise Distribution of Anaemic dogs (n=24)

It is important to examine both the geographical distribution and the preference for male dogs among the Indian people. However, due to the limited sample size in the current investigation, it is not possible to generalise the gender-specific cases from the available data.

4.1.4 Breed-wise distribution of Anaemia in dogs:

The Breed-wise distribution of anaemia is shown in Table 4.9 (Figure 4.3). Percent distribution of anaemia was found highest 8 (33.33%) in the Labrador breed of dog, followed by Non-descript 4(16.67 %), Golden Retriever 3(12.50%) then Boxer 2(8.33 %) and German shepherd 2(8.33 %). The single case of Doberman, Crocker spaniel, Siberian husky, Shih Tzu and Rottweiler breeds of dogs contributed a total of 20.83% as mix breeds.

Table 4.9: Breed-wise Distribution of Anaemic dogs (n=24)

Sr. No.	Name of the Breed	No of Animals
1	Labrador	8 (33.33 %)
2	German Shepherd	2 (8.33 %)
3	Non-Descript	4 (16.67 %)
4	Golden Retriever	3 (12.50 %)
5	Boxer	2 (8.33 %)
6	Doberman	1 (4.17 %)
7	Cocker Spaniel	1 (4.17 %)
8	Siberian Husky	1 (4.17 %)
9	Shih Tzu	1 (4.17 %)
10	Rottweiler	1 (4.17 %)
	Total	**24 (100%)**

The current study's findings on breed-wise distribution of anaemic dogs were in consistent with those reported by Singh *et al.,* (2012), Brahmbhatt *et al.,* (2015), Meshram *et al.,* (2019), Shah *et al.,* (2020), and Swami (2023).

The higher predominance of Labradors in the total canine community can be attributed to their assumed mild and even temperament, easy-going attitude, agility, friendliness, docility, and low maintenance requirements compared to other non-descript canines. These potential factors may have led to the significantly greater occurrence of anaemia in these breeds in the current

investigation.

The variances in breed predilection may be attributed to pet owners' preferences and inclinations while selecting breeds as their pets (Singh *et al.*,2012).

Out of Six (6) *E. canis*-caused anaemia, three (3) cases were recorded in the Labrador dog breed. Labradors were more prone to *E. canis* infection compared to other breeds owing to their genetic causes and strain variation (Buhles, 2016).

4.1.5 Distribution of anaemic dogs based on Erythrocytic indices:

The categorization of anaemic dogs according to erythrocytic indices, specifically MCV (fl), MCH (pg), and MCHC (g/dl) is presented in Table 4.10 (Figure 4.4 and Figure 4.5). The anaemic cases were classified into different categories, including normocytic, macrocytic, microcytic, normochromic, hypochromic and hyperchromic based on morphological changes and erythrocytic calculations using an automated haemoanalyzer. (Allen *et al*., 2008).

Table 4.10: Distribution of Anaemic Dogs based on Erythrocytic Indices and Bone marrow activity (n=24)

Sr. No	Type of Anaemia (Erythrocytic Indices)	No of dogs	On Bone marrow Activity Basis	
			Regenerative	Non-Regenerative
1	Normocytic Normochromic	17 (70.83 %)	17	00
2	Macrocytic Hypochromic	6 (25.00 %)	06	00
3	Microcytic Hypochromic	1 (4.17%)	00	01
	Total	24 (100 %)	23	01

In the present study, 70.83 % (17/24) of anaemic dogs were found to exhibit normocytic normochromic anaemia whereas 25.00% (6/24) showed macrocytic hypochromic anaemia. A single (4.17%) case of microcytic hypochromic anaemia was noted in the present study. Normocytic normochromic blood picture was evident in the anaemic cases of CKD, *E. canis* and pyometra dogs contributing 66.67% (16/24) except the single case of pyometra as

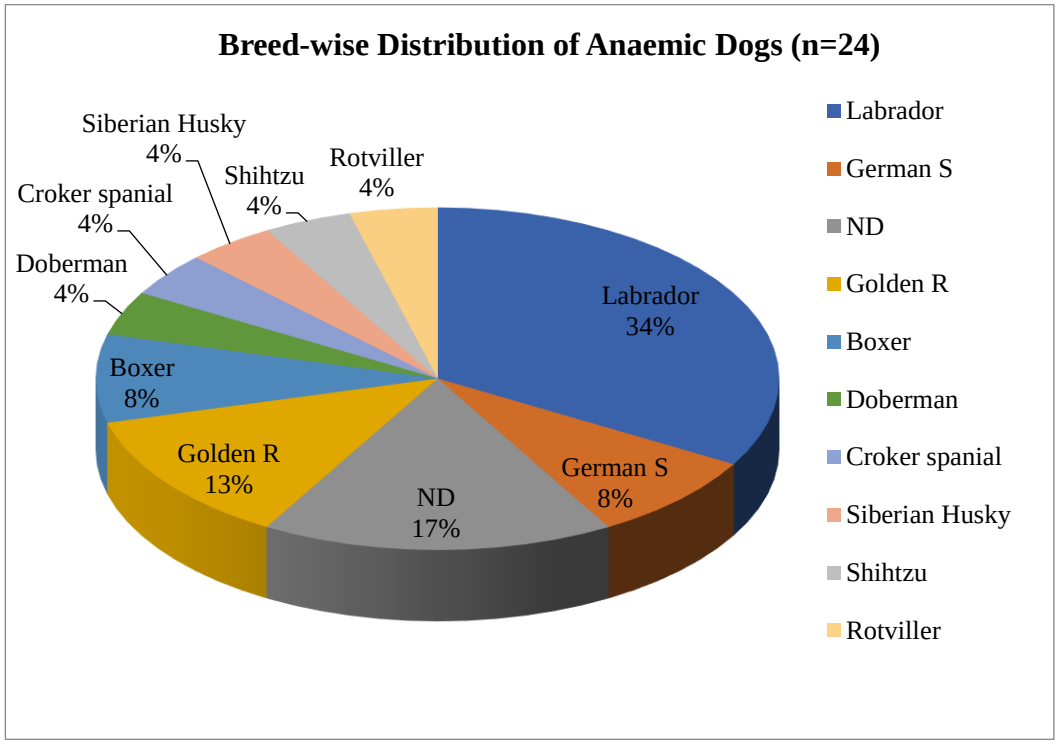


Figure 4.3 Breed-wise Distribution of Anaemic Dogs (n=24)

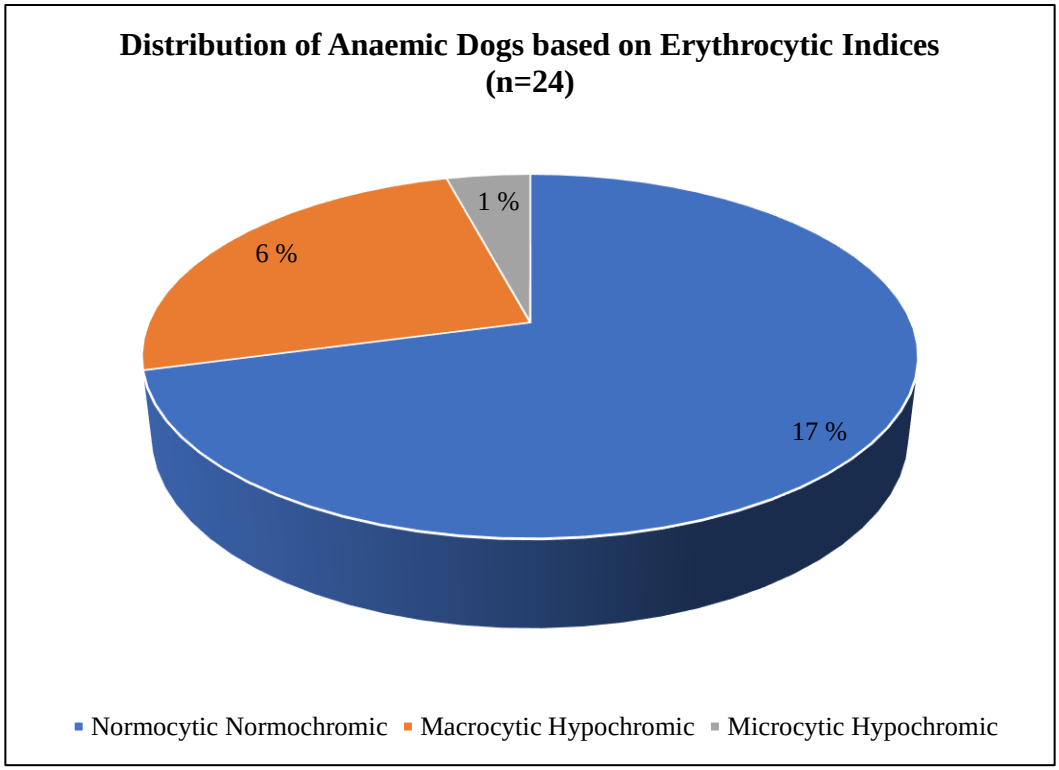


Figure 4.4 Distribution of Anaemic Dogs based on Erythrocytic Indices (n=24)

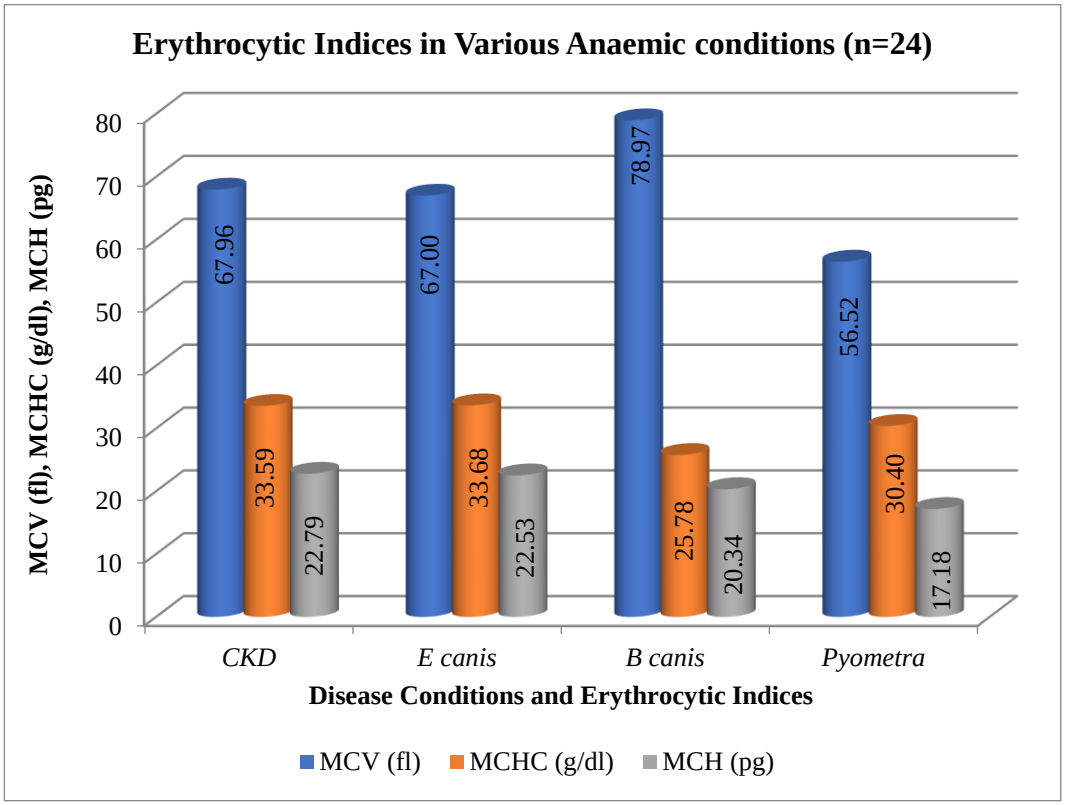


Figure 4.5 Erythrocytic Indices in Various Anaemic conditions (n=24)

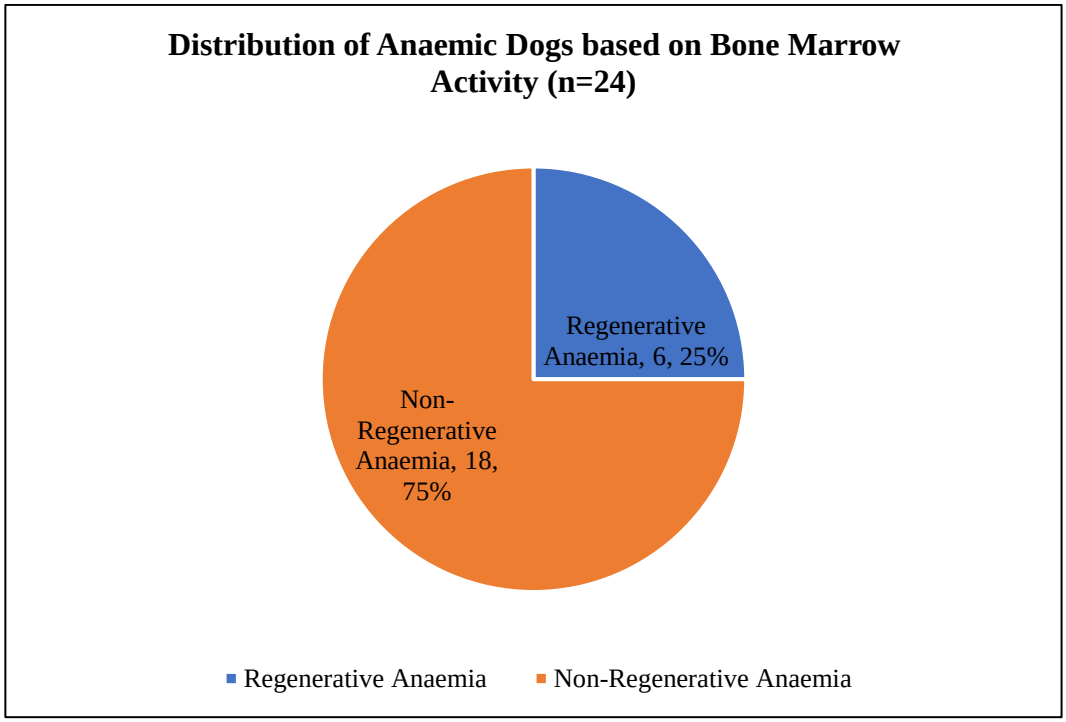


Figure 4.6 Distribution of Anaemic Dogs based on Bone Marrow Activity (n=24)

microcytic hypochromia (# Case no 15). Chronic blood loss due to pyometra was reported in the 10-year-old non-descript female which might have resulted in the exhibition of microcytic hypochomic anaemia. All cases (6/6) of *B. canis*-caused anaemia showed macrocytic hypochromic anaemia type of blood picture.

The presence of microcytic hypochromic anaemia is commonly associated with iron deficiency, requiring further diagnostic testing to confirm a state of sufficient iron levels. The presence of normocytic normochromic anaemia indicated a non-regenerative cause.

Salutgi (2022) suggests that macrocytosis can often be associated with storage artefacts or infectious organisms, similar to how vitamin B12 deficiency may not always be evident in clinical cases of anaemia in dogs. The findings were in line with the results reported by Paltrinieri (2014) and Paltrinieri *et al.,* (2000). Several other researchers, such as King *et al.,* (1992), Shah *et al.,* (2011), and Lucidi *et al.,* (2017), have also shown normocytic normochromic anaemia to be the most common type of anaemia in their studies, which is similar to the current research.

The haematological findings of the present study corroborate the findings of Irizarry-Rovira *et al.,* (2001) who examined the haematological alterations in a dog infected with *B. gibsoni*. The findings revealed a severe regenerative anaemia characterised by normocytic to macrocytic normochromic red blood cells (RBCs) with poikilocytosis, polychromasia, anisocytosis, and a significant increase in nucleated RBCs.

The observations of chronic renal failure in anaemic dogs are akin to the findings of King *et al.,* (1992) who researched to assess the frequency, nature, and causes of anaemia in individuals with chronic renal failure (CRF). 70.6 % of dogs had a non-regenerative, normochromic, normocytic anaemia. They discovered a clear relationship between the severity of anaemia and the amount of chronic renal failure, as determined by blood creatinine levels.

4.1.6 Distribution of the anaemic dogs based on Bone Marrow Activity:

The distribution of the anaemic dogs based on bone marrow activity is presented in Table 4.11 (Figure 4.6) revealing maximum cases i.e. 18 (75%) of

non-regenerative anaemia type containing CKD, *E. canis* and Pyometra group dogs while 6 (25%) cases of regenerative anaemia of *B. canis* group. The RPI was the basis of regenerative and non-regenerative anaemia. The RPI in CKD, *E. canis* and pyometra dogs had lower RPI i.e. 0.13 ± 0.01, 0.32 ± 0.20 and 0.15 ± 0.02 respectively while *B. canis* had 2.01 ± 0.10.

Table 4.11: Distribution of the Anaemic Dogs based on Bone Marrow Activity (n=24)

Sr. No.	Type of Anaemia	No of dogs
1	Regenerative Anaemia	6 (25%)
2	Non-Regenerative Anaemia	18 (75%)
	Total	**24 (100 %)**

The most common aetiologies of non-regenerative anaemia are inflammation, infectious illnesses, and metabolic disorders. Nevertheless, in certain cases of non-regenerative anaemia, the bone marrow's functioning may be adversely affected by a systemic disease (Paltrinieri, 2014). King *et al.,* (1992), Shah *et al.,* (2011), and Lucidi *et al.,* (2017) discovered that normocytic normochromic anaemia is a prevalent form of anaemia based on their research findings. This finding in their study is likewise consistent with the findings in the present investigation.

Absolute reticulocyte count in dogs over 60000 to 80000/µl or larger than 1 to 1.5 per cent often implies regeneration, according to Cowgill *et al.,* (2003). In cases with significant regenerative anaemias, the blood test results may show a kind of anaemia characterised by larger red blood cells and lower levels of haemoglobin (known as macrocytic and hypochromic anaemia). These results suggest that the reticulocytes, which are immature red blood cells, are larger in size and have lower levels of haemoglobin. A more discerning measure is the amalgamation of an increased mean corpuscular volume (MCV) and red cell distribution width (RDW) (Neiger *et al.,* 2002). Hemolysis and loss of red blood cells are factors contributing to regenerative anaemia.

Non-regenerative anaemia is often encountered and results from diminished erythrocyte synthesis. The anaemia in question often manifests as

either normocytic and normochromic or microcytic and hypochromic. Various diseases can cause non-regenerative anaemias, with the most common cause being anaemia of chronic disease (ACD). Inflammatory processes, chronic infections, and disseminated neoplasias can lead to a decrease in iron availability, erythrocyte survival, and response to erythropoietin (Wanner and Harrus 2000). Other factors that can cause non-regenerative anaemia include myelofibrosis, myelopthisis, chronic renal disease, infectious diseases that impact the development of red blood cells (such as leukaemia virus and *E. canis*), toxic effects of certain drugs (such as oestrogen compounds, doxorubicin, and vincristine), and immune-mediated destruction specifically targeting erythroid precursors rather than mature red cells (Barger, 2003).

4.1.7 Distribution of the anaemic dogs based on Albumin (g/dl) concentration:

The distribution of the anaemic dogs based on Albumin (g/dl) concentration is shown in Table 4.12 (Figure 4.7). Hypoproteinemia concurrent with hypoalbuminemia was the major finding in all 24 cases of anaemic dogs. The histogram showed 50% (12/24) cases having albumin concentration between 1.5 to 2 (g/dl) followed by 7 (29.17%) dogs having albumin concentration of 2 to 2.5 (g/dl) and 3 (12.50%) more than 2.5 (g/dl). Each single case having albumin concentration of 1 (g/dl) and 1 to 1.5 (g/dl) was noted in the present study. Glomerular proteinuria typically causes significant loss of albumin from the body (Theresa E. Rizzi, 2014). Microalbuminuria was recorded in CKD anaemic dogs with UPC 1.84 ± 0.55.

Table 4.12: Distribution of the Anaemic Dogs based on Albumin (g/dl) Concentration (n=24)

Sr. No	Albumin (g/dl)	No of Dogs
1	1	1 (4.17 %)
2	1 to 1.5	1 (4.17 %)
3	1.5 to 2.00	12 (50.00 %)
4	2.00 to 2.50	7 (29.17 %)
5	More than 2.50	3 (12.50 %)
	Total	**24 (100 %)**

4.1.8 Distribution of the anaemic dogs based on Globulin (g/dl) concentration:

The distribution of the anaemic dogs based on Globumin (g/dl) concentration is illustrated in Table 4.13 (Figure 4.8). As per the inclusion criteria of the study, all the selected anaemic cases had globulin more than 4g/dl. 17 out of 24 (70.89%) anaemic cases contributed the maximum number having globulin concentration between 3.4 to 5.0 (g/dl). The distribution showed 3 (12.51%) anaemic dogs each in globulin concentration having between 5.1 to 6 g/dl and more than 6 g/dl. Only a single case (4.17 %) of globulin 3.99 (g/dl) was noted.

Table 4.13: Distribution of the Anaemic Dogs based on Globulin (g/dl) Concentration (n=24)

Sr. No	Globulin (g/dl)	No of Dogs
1	3.99	01 (4.17 %)
2	3.4 to 5.0	17 (70.89 %)
3	5.1 to 6.0	03 (12.51 %)
4	More than 6.0	03 (12.51 %)
	Total	**24 (100 %)**

Table 4.14: Distribution of the Anaemic Dogs based on Haemoglobin (g/dl) Concentration (n=24).

Sr. No.	Haemoglobin (g/dl)	No of Dogs.
1	1.9	01 (4.17 %)
2	2.0 – 2.6	01 (4.17 %)
3	2.7 – 3.4	02 (8.34 %)
4	3.5 – 4.2	10 (41.7 %)
5	More than 4.2	10 (41.7 %)
	Total	**24 (100 %)**

In the present study, significant hypoalbuminemia was noted in all the anaemic dogs, hence may act as a compensatory mechanism for the hyprgloblulinemic state to maintain the oncotic pressure and prevent an increase in the blood viscosity. Hyperglobulinemia arises when antigenic stimulation prompts the synthesis of a diverse range of immunoglobulin types that specifically target different epitopes. Each of these distinct immunoglobulins has unique migration patterns and disperses widely during electrophoresis.

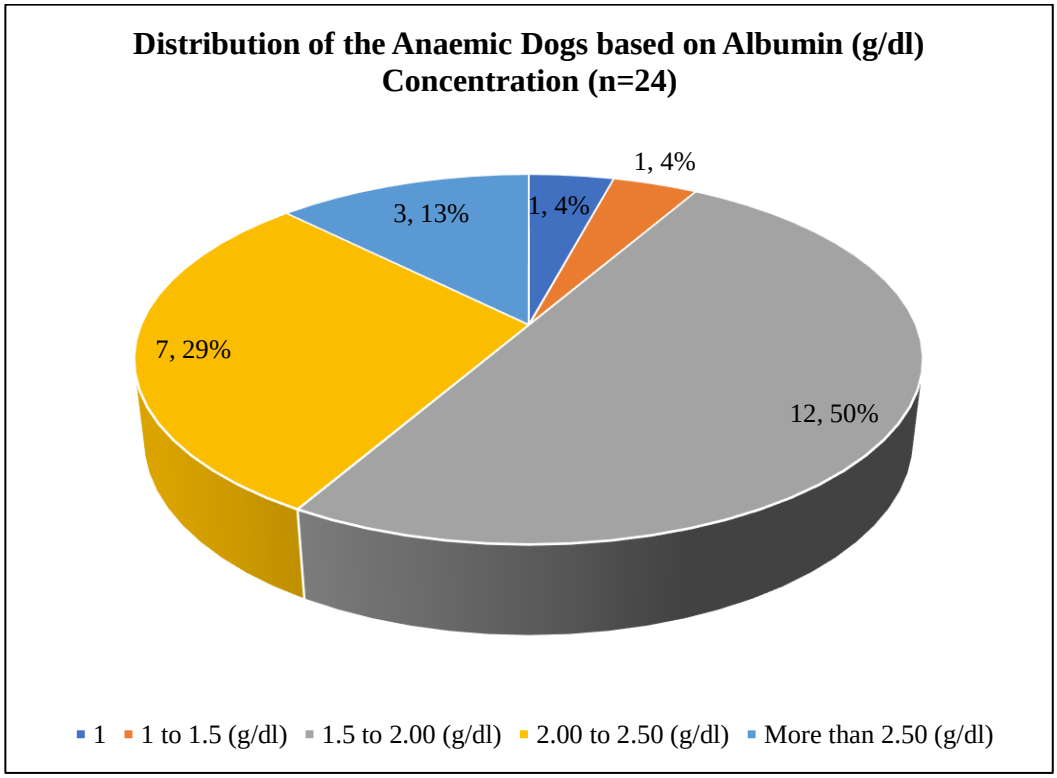


Figure 4.7 Distribution of the Anaemic Dogs based on Albumin (g/dl) Concentration (n=24)

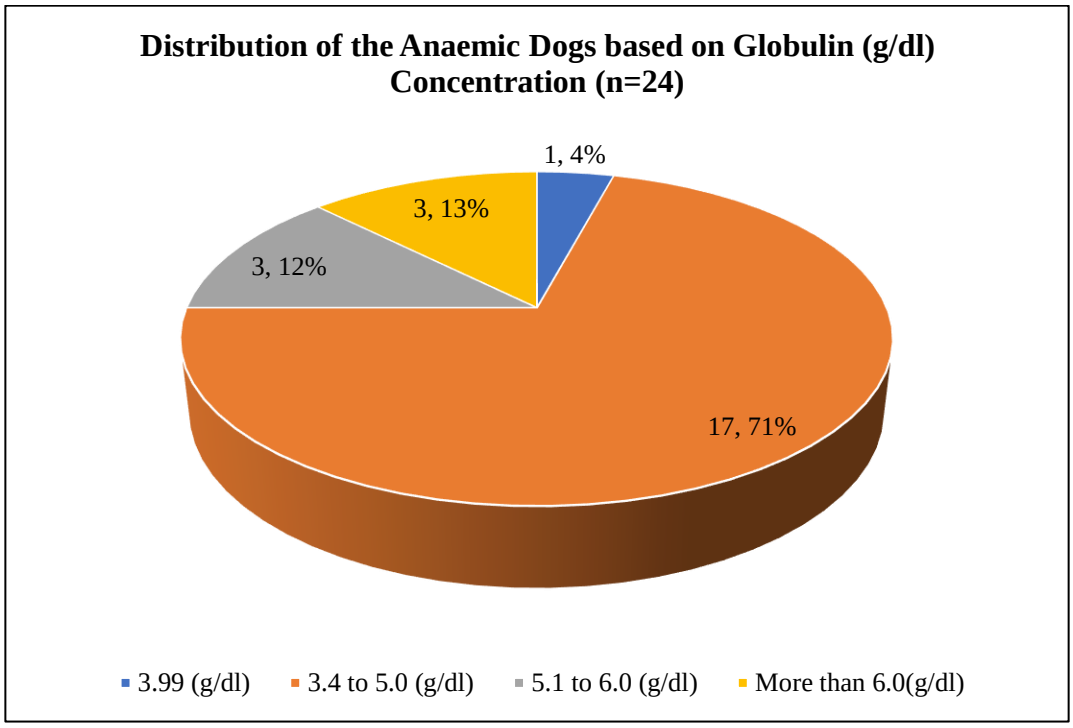


Figure 4.8 Distribution of the Anaemic Dogs based on Globulin (g/dl) Concentration (n=24)

Table 4.15 Haematologial and Biochemical alterations in Healthy control (n=6) and Anaemic dogs (n=24)

Sr. No.	Parameter	Healthy (n=6)	Anaemic (n=24)	t-Test
1.	Hb (g/dl)	11.80±0.34	3.96±0.16	20.92**
2.	Erythrocyte (x 10^6/µl)	5.75±0.36	1.83±0.09	10.69**
3.	PCV (%)	36.83±1.61	12.98±0.67	13.67**
4.	RDW (%)	16.26 ± 0.85	19.62 ± 1.38	2.07 ^{NS}
5.	MCV (fl)	64.36±1.36	71.00±1.33	3.48**
6.	MCH (pg)	20.80±1.09	21.90±0.45	0.92^{NS}
7.	MCHC (g/dl)	32.23±1.19	31.02±0.73	0.87^{NS}
8.	Total WBC (x 10^3/µl)	11.65±1.59	19.45±3.86	1.86^{NS}
9.	Neutrophil (%)	74.66±2.02	73.75±2.47	0.28^{NS}
10.	Eosinophil (%)	2.00±0.00	3.63±1.02	1.58^{NS}
11.	Lymphocyte (%)	20.16±1.64	17.38±2.21	1.02^{NS}
12.	Monocyte (%)	3.16±0.60	5.25±1.69	1.16^{NS}
13.	Platelet (x 10^3/µl)	238.83±20.05	125.42±20.68	3.93**
14.	Free Hb (g/dl)	0.00±0.0	0.20±0.03	5.87**
15.	ESR (mm/hr)	4.50±0.22	41.96±2.87	12.99**
16.	Serum BUN (mg/dl)	21.50±1.62	67.45±9.17	4.93**
17.	Serum Creatinine (mg/dl)	1.05±0.15	3.51±0.60	3.98**
18.	Bilirubin total (mg/dl)	0.53±0.07	2.46±0.70	2.74*
19.	Bilirubin Direct (mg/dl)	0.31±0.05	1.31±0.38	2.57*
20.	Bilirubin Indirect (mg/dl)	0.20±0.04	1.16±0.33	2.79*
21.	SGOT (IU/L)	57.66±7.65	138.74±40.60	1.96^{NS}
22.	SGPT (IU/L)	55.33±4.67	139.67±42.78	1.95^{NS}
23.	Alkaline Phosphatase (IU/L)	111.66±18.74	182.74±37.05	1.71^{NS}
24.	Total Proteins (g/dl)	6.41±0.04	6.85±0.28	1.55^{NS}
25.	Albumin (g/dl)	3.19±0.02	1.97±0.10	12.25**
26.	Globulin (g/dl)	3.21±0.04	4.86±0.25	6.43**
27.	A: G Ratio	0.99±0.02	0.42±0.02	18.20**
28.	Lactate (mmol/L)	0.86±0.14	4.80±0.31	11.50**
29.	Reticulocyte (%)	0.75±0.10	4.66±1.33	2.92**
30.	Absolute Reticulocyte (thousand cells/µl)	44.86±9.35	74.58±19.10	1.39^{NS}
31.	RPI	0.34±0.06	0.66±0.17	1.70^{NS}
32.	Sodium (mEq/L)	147.66±2.15	139.91±5.05	1.41^{NS}
33.	Potassium (mEq/L)	4.23±0.09	5.03±0.23	3.26**
34.	Chloride (mEq/L)	106.50±0.56	107.54±0.95	0.94^{NS}
35.	Urinary Protein (mg/dl)	15.08±1.00	41.23±11.76	2.21*
36.	Urinary Creatinine (mg/dl)	65.50±7.10	76.43±9.61	0.91^{NS}
37.	UPC Ratio	0.24±0.02	0.64±0.20	1.98^{NS}
38.	Serum Iron (µg/dl)	104.12±3.56	98.52±3.15	0.93 ^{NS}
39.	TIBC (µg/dl)	210.58±15.34	233.03±7.95	1.35 ^{NS}
40	Iron Saturation %	50.54±3.30	42.80±1.57	2.31*

* Significant at 0.05% level ** Significant at 0.01% level NS - Non-significant
Table t Value, 0.05%=2.06, 0.01%=2.79

4.1.9 Haematological Changes in Healthy Control and Anaemic Dogs in Different Disease Conditions:

Hb, RBC, PCV, MCV, MCH, MCHC, Platelets:

Table 4.15 shows a detailed analysis of anaemic dogs (n=24) and their comparison with healthy control dogs (n=6). The haemoglobin (g/dl), erythrocyte (X 10^6/µl), PCV (%), MCV(fl), MCH (pg), MCHC (g/dl) and platelet count (X 10^3/µl) in healthy and anaemic dogs were (11.80 ± 0.34, 3.96 ± 0.16), (5.75 ± 0.36, 1.83 ± 0.09), (36.83 ± 1.61, 12.98 ± 0.67), (64.36 ± 1.36, 71.00 ± 1.33), (20.80 ± 1.09, 21.90 ± 0.45), (32.23 ± 1.19, 31.02 ± 0.73) and (238.83 ± 20.05, 125.42 ± 20.68), respectively. These values are in agreement with the findings of Tandel *et al.,* (2019) who reported at par values in 78 anaemic dogs.

Overall data of the anaemic dogs (n=24) revealed a statistically significant ($p \leq 0.01$) decrease in Hb (g/dl), TEC (X 10^6/µl) and PCV (%) and platelet (x10^3/µl) count and a statistically significant ($p \leq 0.01$) increase in MCV (fl) when compared with healthy dogs (n=6). Non-significant difference was noted in MCH (pg) and MCHC (g/dl) values between anaemic and healthy dogs.

Table 4.16 shows a detailed analysis of haematological and biochemical alterations in anaemic dogs (n=24) in different disease conditions and their comparison with healthy control dogs (n=6). The Mean ± SE for haematological parameters viz. Hb (g/dl), TEC (x10^6/µl), PCV (%), MCV (fl), MCH (pg), MCHC (g/dl), and platelets (X 10^3/µl) for anaemic dogs in **CKD** (4.10 ± 0.31), (1.90 ± 0.18), (13.40 ± 1.60), (70.10± 2.19), (21.88 ± 0.98), (31.37 ± 1.68) and (148.18 ± 36.30), in ***E. canis*** group (3.90 ± 0.16),(1.67 ± 0.08), (11.83 ± 0.36), (71.40 ± 2.66), (23.44 ± 0.51), (32.95 ± 0.81) and (112.58 ± 29.86) in ***B. canis*** group (3.47 ± 0.46), (1.70 ± 0.21), (12.95 ± 1.95),(74.82 ± 3.00), (20.40 ± 0.40), (27.47 ± 1.17) and (39.17 ± 6.79) in **pyometra** (4.38 ± 0.25),(2.05 ± 0.19), (13.73 ± 1.12), (67.70 ± 2.49), (21.89 ± 1.21), (32.28 ± 1.09) and (201.75 ± 53.11) were noted. Individual disease-wise data showed a significant ($p \leq 0.01$) decrease in Hb (g/dl), TEC (X 10^6/µl), PCV (%) and platelets (x 10^3/µl) in CKD, *E. canis*, *B. canis* and pyometra anaemic groups over healthy control dogs.

Table 4.16 Haematological and biochemical values (Mean ± SE) in anaemic dogs (n=24) in different disease conditions and their comparison with healthy control dogs (n=6)

Sr. No.	Parameter	Healthy (n=6)	CKD (n=6)	*E. canis* (n=6)	*B. canis* (n=6)	Pyometra (n=6)
1.	Hb (g/dl)	11.80±0.34	4.10±0.31	3.90±0.16	3.47±0.46	4.38±0.25
	t-Test		14.01**	22.86**	13.70**	12.95**
2.	Erythrocyte (x 10^6/µl)	5.75±0.36	1.90±0.18	1.67±0.08	1.70±0.21	2.05±0.19
	t-Test		9.88**	10.34**	10.23**	12.85**
3.	PCV (%)	36.83±1.61	13.40±1.60	11.83±0.36	12.95±1.95	13.73±1.12
	t-Test		10.86**	14.91**	10.45**	12.89**
4	MCV (fl)	64.36±1.36	70.10±2.19	71.40±2.66	74.82±3.00	67.70±2.49
	t-Test		1.43^{NS}	1.08 ^{NS}	3.54*	1.58
5.	MCH (pg)	20.80±1.09	21.88±0.98	23.44±0.51	20.40±0.40	21.89±1.21
	t-Test		0.64^{NS}	1.88 ^{NS}	0.28	2.83*
6.	MCHC (g/dl)	32.23±1.19	31.37±1.68	32.95±0.81	27.47±1.17	32.28±1.09
	t-Test		0.43 ^{NS}	0.95 ^{NS}	2.43*	0.05
7.	Total WBC (x10^3/µl)	11.65±1.59	13.94±4.60	30.59±11.75	5.78±1.79	27.48±5.52
	t-Test		0.51 ^{NS}	1.71^{NS}	1.83 ^{NS}	3.59*
8.	Neutrophil (%)	74.66±2.02	80.00±2.23	81.33±4.42	58.00±3.43	75.67±2.52
	t-Test		1.88 ^{NS}	1.84 ^{NS}	4.27**	0.36**
9.	Eosinophil (%)	2.00±0.00	3.83±2.45	3.67±2.49	2.33±0.76	4.67±2.41
	t-Test		0.74 ^{NS}	0.66 ^{NS}	0.43 ^{NS}	1.10 ^{NS}
10.	Lymphocyte (%)	20.16±1.64	14.67±3.75	11.17±3.99	25.83±3.97	17.83±4.49
	t-Test		1.49 ^{NS}	2.31 ^{NS}	1.35 ^{NS}	0.51 ^{NS}
11.	Monocyte (%)	3.16±0.60	1.50±0.34	3.83±2.84	13.83±4.86	1.83±0.40
	t-Test		2.19 ^{NS}	0.24 ^{NS}	2.29 ^{NS}	1.58 ^{NS}
12.	Platelet (x10^3/µl)	238.83±20.05	148.18±36.30	112.58±29.86	39.17±6.79	201.75±53.11
	t-Test		1.98 ^{NS}	4.94**	10.28**	1.05
13.	Free Hb (g/dl)	0.00±0.00	0.07±0.02	0.38±0.03	0.32±0.03	0.03±0.02
	t-Test		3.16*	12.47**	10.30**	1.58
14.	ESR (mm/hr)	4.50±0.22	43.00±4.69	40.00±5.64	43.00±7.18	41.83±6.72
	t-Test		8.17**	6.08**	5.49**	5.58**
15.	Serum BUN (mg/dl)	21.50±1.62	128.28±13.10	41.40±11.79	56.03±9.38	44.07±11.71
	t-Test		7.55**	1.64 ^{NS}	3.21**	2.03
16.	Serum Creatinine (mg/dl)	1.05±0.15	7.30±0.57	1.59±0.25	1.87±0.40	3.28±1.36
	t-Test		12.92**	1.63 ^{NS}	2.10 ^{NS}	1.52 ^{NS}
17.	Bilirubin total (mg/dl)	0.53±0.07	0.33±0.08	4.90±1.16	3.85±0.58	0.76±0.37
	t-Test		1.52 ^{NS}	2.09 ^{NS}	3.16*	0.45

Sr. No.	Parameter	Healthy (n=6)	CKD (n=6)	*E. canis* (n=6)	*B. canis* (n=6)	Pyometra (n=6)
18.	Bilirubin Direct (mg/dl)	0.31±0.05	0.13±0.02	2.60±1.16	1.98±0.58	0.52±0.37
	t-Test		3.48*	2.01 NS	2.73*	0.52 NS
19.	Bilirubin Indirect (mg/dl)	0.20±0.04	0.21±0.06	2.30±1.02	1.87±0.51	0.24±0.11
	t-Test		0.10 NS	2.05 NS	6.01**	0.50 NS
20.	SGOT (IU/L)	57.66±7.65	50.73±10.47	288.00±145.80	142.29±33.74	73.94±31.17
	t-Test		0.83 NS	1.59 NS	2.72 NS	0.53 NS
21.	SGPT (IU/L)	55.33±4.67	50.11±8.01	209.67±159.90	112.23±30.89	186.68±62.47
	t-Test		0.59 NS	0.97 NS	1.86 NS	2.15 NS
22.	Alkaline Phosphatase (IU/L)	111.66±18.74	65.73±15.77	226.00±82.09	186.55±30.86	252.67±114.96
	t-Test		3.81*	1.60 NS	3.61*	1.11 NS
23.	Total Protein (g/dl)	6.41±0.04	7.57±0.76	7.58±0.51	5.90±0.08	6.37±0.39
	t-Test		1.55 NS	2.19 NS	9.96**	0.11 NS
24.	Albumin (g/dl)	3.19±0.02	1.95±0.14	2.19±0.15	1.74±0.07	2.01±0.30
	t-Test		8.180**	6.60**	20.85**	3.78**
25.	Globulin (g/dl)	3.21±0.04	5.51±0.70	5.39±0.57	4.16±0.04	4.36±0.20
	t-Test		3.32*	3.65*	14.61**	5.84**
26.	A:G Ratio	0.99±0.02	0.36±0.03	0.43±0.05	0.42±0.02	0.46±0.07
	t-Test		14.67**	9.29**	18.01**	8.34**
27.	Lactate (mmol/L)	0.86±0.14	6.12±1.05	4.85±0.20	4.56±0.15	3.67±0.08
	t-Test		5.27**	15.29**	21.21**	22.68**
28.	Reticulocyte (%)	0.75±0.10	0.84±0.06	2.33±1.49	14.49±2.07	0.99±0.13
	t-Test		0.51 NS	1.04 NS	6.54**	1.39 NS
29.	Absolute Reticulocyte (thou/µl)	44.86±9.35	15.77±2.02	38.28±24.57	224.34±7.75	19.92±2.92
	t-Test		2.82*	0.24 NS	11.84**	2.98*
30.	RPI	0.34±0.06	0.13±0.02	0.32±0.20	2.02±0.11	0.16±0.02
	t-Test		2.88*	0.08 NS	11.93**	2.96*
31.	Sodium (mEq/L)	147.66±2.15	142.75±3.01	126.67±11.03	119.88±5.88	170.33±1.43
	t-Test		1.05 NS	1.96 NS	3.65*	11.56**
32.	Potassium (mEq/L)	4.23±0.09	3.57±0.16	5.88±0.34	5.97±0.09	4.70±0.12
	t-Test		4.51**	3.94**	11.24**	6.13**
33.	Chloride (mEq/L)	106.50±0.56	105.15±3.00	108.68±1.52	109.87±1.39	106.47±0.81
	t-Test		0.40 NS	1.31 NS	3.04*	0.02 NS
34.	Urinary Protein (mg/dl)	15.08±1.00	116.93±30.33	11.18±2.69	14.12±4.33	22.67±7.38

Sr. No.	Parameter	Healthy (n=6)	CKD (n=6)	*E. canis* (n=6)	*B. canis* (n=6)	Pyometra (n=6)
	t-Test		3.26*	1.47 ^{NS}	0.25 ^{NS}	1.07 ^{NS}
35.	Urinary Creatinine (mg/dl)	65.50±7.10	77.57±26.98	67.72±10.68	85.70±26.58	74.75±10.77
	t-Test		0.53 ^{NS}	0.13 ^{NS}	0.70 ^{NS}	0.75 ^{NS}
36.	UPC Ratio	0.24±0.02	1.85±0.55	0.17±0.04	0.23±0.06	0.29±0.06
	t-Test		2.93*	1.34 ^{NS}	0.06 ^{NS}	0.61 ^{NS}

* Significant ($p \leq 0.05$) ** Significant ($p \leq 0.01$)

Table t Value 0.05%=2.57 0.01%=4.03

The concentration of haemoglobin is the third constituent of packed cell volume, or packed cell volume is the inverse of haemoglobin concentration. Conversely, the total number of erythrocytes is equal to one-sixth of the packed cell volume. The levels of haemoglobin (Hb), packed cell volume (PCV), and total erythrocyte count (TEC) are interconnected. These entities demonstrate a robust association with each other. Modifying a single parameter resulted in a consequential effect on the other two parameters. Hence, in instances of anaemia, a decrease in the concentration of haemoglobin is linked to a decline in both the total erythrocyte count (TEC) and packed cell volume (PCV) (Ettinger and Feldman, 2005).

The majority of dogs in the study displayed anaemia in conjunction with a persistently unwell condition, likely resulting from ineffective erythropoiesis. This particular attribute is also observable during the per-acute hemorrhagic phase, or in instances when there are decreases in peripheral red blood cells as a result of bone marrow malfunction.

The erythrocytic indices found in the anaemic dogs in this study align with the distinctive and diagnostic markers of anaemia, as shown by Das and Konar (2013), Reddy *et al.,* (2014), and Nalubamba *et al.,* (2015) who also found that there were a notable decrease in the average haemoglobin and total erythrocyte count (TEC) levels seen in dogs with anaemia, as compared to the dogs in the group classified as apparently healthy. Several studies, including those conducted by Bhadesiya and Raval (2015), and Yadav *et al.,* (2021), have reported a noteworthy decrease in the average haemoglobin levels among dogs with

anaemia. The results of the current investigation align with the observations documented by all of these authors.

In the present, the insignificant difference was recorded in MCV (fl), MCH (pg) and MCHC (g/dl) in CKD and *E. canis* groups. In the *B. canis* group highly significant ($p \leq 0.01$) MCV, significant ($p \leq 0.05$) MCHC and non-significant MCH values were recorded. In the pyometra group, only MCH was significant while MCV, and MCHC were non-significant. We found that RBC indices did not alter significantly ($p \leq 0.05$). It is widely recognized that non-regenerative anaemia is linked to normochromic normocytic red cell indices. A normocytic normochromic pattern was anticipated that showed a higher-than-average no of cases of nonregenerative anaemia in the present study population.

In the present study, 66.67 % (16/24) of anaemic dogs from the CKD group, *E. canis* and pyometra group were found to exhibit normocytic normochromic anaemia whereas macrocytic hypochromic anaemia was evident in *B. canis* group indicating regenerative anaemia.

The occurrence of microcytic hypochromic anaemia in the present study was in agreement with Showkat, 2009. The occurrence of microcytic hypochromic anaemia was typically linked to iron deficiency, necessitating additional diagnostic testing to establish an iron-replete state.

Normocytic normochromic anaemia was consistent with a non-regenerative aetiology. According to Salutgi (2022) storage artefacts or infectious agents may frequently be linked to macrocytosis, much as vitamin B_{12} deficiency in humans is not always present in clinical cases of anaemia in dogs. These results were consistent with those of Paltrinieri *et al.,* (2000) and Paltrinieri (2014).

Several researchers, including King *et al.,* (1992), Shah *et al.,* (2011), and Lucidi *et al.,* (2017), identified normocytic normochromic anaemia as the predominant kind of anaemia in their investigations which is akin to the present research work where 17 out of 24 cases containing CKD, E canis and pyometra were of normocytic-normochromic anaemia.

Nambi (1993) found 73.11 ± 6.31 (fl) for mean corpuscular volume, 19.78+0.69 pg for mean haemoglobin, and 28.72+1.93 percent for concentration

in healthy dogs. Tasker (1966), Feldman (1981), and Jain (1986) defined anaemia types using erythrocyte indices. Jain (1986) recommended rapid blood transfusion for microcytic hypochromic anaemia as a supportive treatment. In anaemic dogs, Vetrimalai (1992) found mean corpuscular volume, haemoglobin, and concentration to be 51.02+2.00 fl, 18.22+0.72 pg, and 31.58+0.69 %, respectively. The erythrocyte indices in normal and anaemic dogs matched the findings of the authors (Table 4.15). We selected severely sick anaemic dogs with packed cell volume < 20% for the investigation. Thus, the mean corpuscular volume and haemoglobin concentration were extremely low.

Tasker (1966) stated that the degree of anaemia has to be evaluated by determining the packed cell volume, haemoglobin and total erythrocyte count. In his opinion, the packed cell volume is the test of choice that can be easily performed and is more reliable. Further, it permits the direct inspection of the plasma. Weiser (1981) asserts that the typical MCV values range from 62 to 77 femtoliters, whereas the MCH content falls between 33 and 36 pg. The Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin Concentration (MCHC) are the most valuable indicators for diagnosing anaemia.

The normochromic normocytic image often indicates a decrease in the production of red blood cells which can be caused by several factors such as chronic inflammation, nephritis, hormone deficit, neoplastic conditions, poisoning, and radiation exposure. The presence of macrocytic hypochromic implies significant blood loss, which may be caused by factors such as medication toxicity, haemoprotozoan parasites, or congenital conditions. The microcytic hypochromic form of anaemia is commonly found in cases of iron insufficiency or in diseases where there is impaired utilization of iron, apart from B6 deficiency.

The anaemic alterations observed in the current investigation align with the findings reported by the aforementioned author.

There was statistically significant difference ($p \leq 0.05$) in the platelet count between anaemic and healthy dogs. In their respective studies, Shah *et al.,* (2011), Guadarrama-Olhovich *et al.,* (2013), and Vishnurahav *et al.,* (2014) observed a

statistically significant drop in platelet counts (thrombocytopenia) in anaemic dogs, with a p-value less than 0.05.

Thrombocytopenia may be caused by inflammatory changes in blood vessel endothelium, increased splenic sequestration, and immunologic damage that reduces platelet life (Pierce *et al.*,1977; Kakoma *et al.*,1978) All thrombocytopenic groups had anaemia and low PCV/TEC. Similarly, De Gopegui *et al.*, (2007) found that all babesiosis canines had thrombocytopenia and 20% developed Disseminated Intravascular coagulopathy.

In dogs with babesiosis, Furlanello *et al.*, (2005) reported thrombocytopenia, hyperfibrinogenemia, and variable severity anaemia. According to Birkenheuer *et al.*, (1999), Babesia can cause severe haemolytic anaemia, thrombocytopenia, and subclinical infections. Bulla *et al.*, (2004) found thrombocytopenia in 146 *E. canis*-infected samples (less than 200 000/µl). According to Waner *et al.*, (1995), thrombocytopenia is the most prevalent and consistent haematological aberration in dogs spontaneously or experimentally infected with *E. canis*. Smith *et al.*, (1975) found that platelet survival time dropped from 9 to 4 days 2 to 4 days after *E. canis* infection. Our study found identical findings to Thongsahuan *et al.*, (2020). Ehrlichia infections produce anaemia, thrombocytopenia, monocytosis, and eosinophilia. Canine babesiosis is characterised by haemolytic anaemia and thrombocytopenia, according to Jacobson and Clark (1994). Increased osmotic fragility, decreased lifespan, and erythrophagocytosis destroy RBCs.

The recent investigation found marked thrombocytopenia in chronic renal disease patients, *E. canis*, *B. canis* and pyometra anaemic dogs. Supriya (2019) found decreased platelet counts in 24 of 90 renal failure dogs. Dorgalaleh *et al.*, (2013) found anaemia and thrombocytopenia in renal failure patients. Mann (2013) discovered a mean total platelet count of $145.12\pm20\times10^3$/cu mm in dogs with renal failure, with 10 out of 46 dogs showing thrombocytopenia. Devipriya *et al.*, (2018) reported that 77 of 150 renally insufficiency dogs had reduced PCV, Hb, TEC, TLC, BUN, and creatinine. Mann (2013) reported a mean BUN of 134.65±14.27 (g/dl) in renal patients.

Even though over-bleeding signs were not the presenting complaints in all *E. canis* and *B. canis-affected* dogs, the anaemia in these non-overt DIC could be attributed to increased macrophage, erythrophagocytic activity, oxidative fragility, immune-mediated intravascular hemolysis of erythrocytes, hemodilution and sequestration of erythrocytes as described by Furlanello *et al.*,(2005), Schetters *et al.,* (2009) and Ayoob *et al.,* (2010).

Previous studies have described thrombocytopenia as a major consequence of disseminated intravascular coagulation (DIC) in canine ehrlichiosis (Varela *et al.,* 1997; Harrus *et al.,*1999; Mohammed *et al.,*2006) and babesiosis (Pantoanowitz 2003; Vijayalakshmi 2011). Thrombocytopenia in dogs with DIC owing to ehrlichiosis and babesiosis can be caused by increased platelet sequestration, consumption, reduced production, and sepsis (Silverstein, 2015; Larkin *et al.,*2016).

4.1.10 Leucocytic changes in healthy control and anaemic dogs in different disease conditions:

Table 4.15 presents the Mean ± SE data for the leucocyte count and differential leucogram. The Mean ± SE values for total leukocyte count (TLC) (X 10^3/µl), Neutrophils (%), Eosinophils (%), Lymphocytes (%), Monocytes (%) and Basophils (%), in healthy and anaemic dogs were (11.65 ± 1.59, 19.45 ± 3.86), (74.66 ± 2.02, 73.75 ± 2.47), (2.00 ± 0.00, 3.63 ± 1.02), (20.16 ± 1.64, 17.38 ± 2.21) and (3.16 ± 0.60, 5.25 ± 1.69), respectively.

Comparison of all anaemic dogs (n=24) with healthy dogs (n=6) revealed leucocytosis, at par values of Neutrophils and Eosinophils, lymphopenia and monocytosis, however, the statistical difference between them was non-significant. Poongodi (1997) reported a significant difference in total leucocytic count (16.06 X 10^3 and 9.91 X 10^3) and neutrophil count (12.60 X 10^3 and 6.50 X 10^3) while the non-significant difference in Lymphocyte, Monocyte and Eosinophil when anaemic dogs were compared with normal group dogs, respectively.

Table 4.16 shows a detailed analysis of haematological and biochemical alterations in anaemic dogs (n=24) in different disease conditions and their

comparison with healthy control dogs (n=6). The mean ± SE values for total leukocyte count ($\times 10^3$/cmm), Neutrophils (%), Eosinophils (%), Lymphocytes (%) and Monocytes (%) in **CKD** group were (13.94 ± 4.60), (80.00 ± 2.23), (3.83 ± 2.45), (14.67 ± 3.75), (1.50 ± 0.34), in ***E. canis*** group were (30.59 ± 11.75), (81.33 ± 4.42), (3.67 ± 2.49), (11.17 ± 3.99) and (3.83 ± 2.84) in ***B. canis*** group were (5.78 ± 1.79), (58.00 ± 3.43), (2.33 ± 0.76), (25.83 ± 3.97), (13.83 ± 4.86) and (0.0 ± 0.0), in **pyometra** group were (27.48 ± 5.52), (75.67 ± 2.52), (4.67 ± 2.41), (17.83 ± 4.49) and (1.83 ± 0.40) respectively.

Individual disease-wise data observed that there was marked leucocytosis in CKD and *E. canis* dogs and leucopenia in *B. canis* anaemic dogs when compared with healthy dogs but the difference between them was statistically non-significant. Statistically significant ($p \leq 0.05$) leucocytosis was noted in pyometra anaemic dogs over healthy control dogs. The present findings align with the findings reported by Chhabra *et al.,* (2013) and Das and Konar (2013).

The differential leucocytic count in the anaemic group revealed a non-significant difference in anaemic dogs when compared with healthy control group dogs in respective disease conditions except for statistically significant ($p \leq 0.01$) difference in neutrophil count in *B. canis* and pyometra groups.

Though there was an insignificant difference in Leucocytic count and differential leucocytic count, CKD anaemic dogs revealed leucocytosis, neutrophilia, eosinophilia, lymphopenia and monocytopenia. *E. canis* anaemic dog revealed leucocytosis, neutrophilia, eosinophilia, lymphopenia and mild monocytosis. In *B. canis* anaemic dogs there was leucopenia, neutropenia, mild eosinophilia, lymphocytosis and marked monocytosis. The pyometra anaemic dogs revealed leucocytosis, mild neutrophilia, eosinophilia, lymphopenia and monocytopenia.

The haematology of canine pyometra cases (n=4) revealed massive leukocytosis/ leukemoid reaction, massive neutrophilia with marked left shift, toxic changes in neutrophils and their precursors, besides anaemia (Showkat, 2009).

Salem (2014) and Lucidi *et al.*, (2017) identified neutropenia and

lymphopenia as significant observations in their investigation of anaemia in dogs using DLC examination. Furlanello *et al.,* (2005) and Lucidi *et al.,* (2017) saw an increase in lymphocyte count, while Fonseca *et al.,* (2022) documented a decrease in neutrophil and lymphocyte counts, as well as a decrease in eosinophil and monocyte counts.

Monocytosis was observed among all the dogs infected with *B. canis* in the present study which was also observed in the previous studies (Irwin, 2010 and Hackner, 2010). Monocytosis is often found in such dogs because of the movement of monocytes from the marginating to the circulating pool in chronic infections (Schmidt, 2015).

The study conducted by Ruiz de Gopegui *et al.,* (2007) found that thrombocytopenia, leucopenia, neutropenia, and monocytosis were the main abnormalities observed in the total blood count of dogs with babesiosis, which aligns with the current findings.

The leucocytic findings of the present study are akin to the findings of Singh *et al.*,(2006) who noted that pyometra is a frequent cause of mortality in elderly female dogs, sometimes accompanied by renal impairment and nephritis. The hematologic abnormalities observed in pyometric female dogs consist of anaemia, leukocytosis, elevated neutrophil count, and reduced lymphocyte and monocyte counts.

4.1.11 Reticulocyte count (%) in healthy control and anaemic dogs in different disease conditions:

The overall reticulocyte count (%) of both control and anaemic dogs, which is used to evaluate regeneration, is presented in Table 4.15. This table also includes the associated values of absolute reticulocyte count and Reticulocyte Production Index (RPI), expressed as Mean ± SE.

The values of reticulocyte and reticulocyte production index (RPI) in healthy control and anaemic dogs groups were (0.75 ± 0.10, 4.66 ± 1.33), (0.34 ± 0.06, 0.66 ± 0.17). There was statistically significant increase in reticulocyte while an insignificant increase in reticulocyte production index in anaemic dogs when compared with healthy control dogs.

The mean ± SE values for reticulocyte count (%), absolute reticulocyte count (thousand cells/µl)), and reticulocyte production index (RPI) in different disease conditions causing anaemia were measured and documented in Table 4.16. For healthy dogs, the observed values were 0.75 ± 0.10, 44.86 ± 9.35 and 0.34 ± 0.06 respectively. These values in the CKD group were 0.84 ± 0.06, 15.77±2.02 and 0.13 ± 0.02, in *E. canis* group were 2.33 ± 1.49, 38.28 ± 24.57 and 0.32 ± 0.20, in *B. canis* group, were 14.49 ± 2.07, 224.34 ± 7.75 and 2.02 ± 0.11, in pyometra group 0.99 ± 0.13, 19.92 ± 2.92 and 2.0.16 ± 0.02 respectively.

Reticulocyte count revealed a non-significant difference in CKD, *E. canis* and pyometra groups while a highly significant difference ($p \leq 0.01$) of increment was observed in the *B. canis* anaemic group of dogs.

The reticulocyte production index showed a highly significant ($p \leq 0.01$) increase in the *B. canis* group (2.01±0.10). It might have caused due to intravascular haemolysis. Significant ($p \leq 0.05$) increase was noted in CKD (0.13±0.01) and pyometra (0.15 ± 0.02) groups of the anaemic dogs. The non-significant difference was recorded in the *E. canis* group of dogs when compared with the apparently healthy control groups. Erythrocytic indices revealed a Macrocytic hypochromic picture of the blood in the *B. canis* group of dogs which signifies that there is regenerative anaemia. The values of the RPI (2.01 ± 0.10) in *B. canis* are correlated with the trend of regeneration of RBCs in the present study. According to Cowgill *et al.*, (2003), the recommended reference range for reticulocyte percentage in healthy dogs is 0-1.5% which is akin to the present findings. In the case of the *E. canis* group, the results indicated that the reticulocyte count fell within the reference interval for both groups. Nevertheless, it was noted that the proportion of reticulocytes in dogs with *E. canis* anaemia was slightly higher, but this disparity did not reach statistical significance ($p \leq 0.05$). Dogs typically have a favourable tolerance towards compensated and chronic anaemias due to the potential inadequacy of the hypoxic signal to effectively stimulate erythropoietin synthesis and subsequently cause reticulocytosis. According to Fernandez and Grindem (2000), the non-regenerative form of anaemia can emerge in the later phases of the disease, usually within 10 to 14 days following its onset, even while the bone marrow shows increased production

of mature red blood cells. According to Ewing *et al.*, (1972), normal dogs had reticulocyte levels ranging from 0.0-1.5%. Similar findings were found by Coles (1986) and Sastry (1989). According to Tasker *et al.*, (1969), the presence of immature erythrocytes in the blood was a typical response to anaemia by erythropoiesis. According to Harvey and Smith (1994), polychromasia and reticulocytes are reliable indicators of red blood cell renewal in dogs. Gomathi (1995) found a variance of 10-12% in reticulocytes in anaemic dogs under experimental conditions. The research results align with Tasker *et al.,* (1969) and Harvey *et al.,* (1994) for anaemic dogs, and Ewing *et al.,* (1972), Coles (1986), and Sastry (1989) for normal values.

4.1.12 Biochemical Changes in healthy control and anaemic dogs in different disease conditions:

Table 4.15 presents the Mean ± SE values for liver and renal function tests in healthy control and anaemic dogs. The overall mean ± SE values for in the healthy control group and an anaemic group of dogs were as follows: (Total Bilirubin - 0.53 ± 0.07, 2.46 ± 0.70 mg/dl), (Direct Bilirubin 0.31 ± 0.05, 1.31±0.38 mg/dl), (Indirect Bilirubin - 0.20 ± 0.04, 1.16 ±0.33 mg/dl), (SGOT - 57.66±7.65, 138.74±40.60 IU/L), (SGPT -55.33±4.67, 139.67±42.78 IU/L), (Alkaline Phosphatase - 111.66±18.74, 182.74±37.05 IU/L) (Total Proteins - 6.41 ± 0.04, 6.85 ± 0.28 g/dl), (Albumin 3.19 ± 0.02, 1.97 ± 0.10 g/dl), (Globulin - 3.21 ± 0.04, 4.86 ± 0.25 g/dl), (A: G Ratio- 0.99 ± 0.02, 0.42 ± 0.02),(Serum BUN - 21.50 ± 1.62, 67.45 ± 9.17 mg/dl) and (Serum Cratinine 1.05± 0.15, 3.51 ± 0.60 mg/dl) respectively.

Overall anaemic dogs revealed that there was a statistically significant ($p \leq 0.05$) increase in total bilirubin (mg/dl), direct bilirubin (mg/dl), Indirect bilirubin (mg/dl)), Globulin (g/dl), BUN (mg/dl)), and Creatinine (mg/dl) while statistically significant ($p \leq 0.05$) decrease in albumin (g/dl), A: G ratio in anaemic dogs when compared with healthy control dogs. Also, there was an increase in the SGOT, SGPT, alkaline phosphatase and total protein values in anaemic groups over healthy control groups but the difference between anaemic and healthy control dogs was statistically non-significant.

Table 4.16 shows a detailed analysis of haematological and biochemical alterations in anaemic dogs (n=24) in different disease conditions and their comparison with healthy control dogs (n=6). The Mean ± SE values for Total Bilirubin (mg/dl)), Direct Bilirubin (m(g/dl)), Indirect Bilirubin (m(g/dl), SGOT (IU/L), SGPT (IU/L), ALP (IU/L), Total Protein (g/dl), Albumin (g/dl), Globulin (g/dl), A/G, BUN (mg/dl)), and Creatinine (mg/dl)) in the anaemic group of dogs were as follows: **CKD Group**: 0.33±0.08, 0.13±0.02, 0.21±0.06, 50.73±10.47, 50.11±8.01, 65.73±15.77, 7.57±0.76, 1.95±0.14, 5.51±0.70, 0.36±0.03, 128.28±13.10 and 7.30±0.57 respectively; ***E. canis* Group**: 4.90±1.16, 2.60±1.16, 2.30±1.02, 288.00±145.80, 209.67±159.90, 226.00±82.09, 7.58±0.51, 2.19±0.15, 5.39±0.57, 0.43±0.05, 41.40±11.79 and 1.59±0.25 respectively; ***B. canis* Group**: 3.85±0.58, 1.98±0.58, 1.87±0.51, 142.29±33.74, 112.23±30.89, 186.55±30.86, 5.90±0.08, 1.74±0.07, 4.16±0.04, 0.42±0.02, 56.03±9.38 and 1.87±0.40 respectively; **pyometra Group:** 0.76±0.37, 0.52±0.37, 0.24±0.11, 73.94±31.17, 186.68±62.47, 252.67±114.96, 6.37±0.39, 2.01±0.30, 4.36±0.20, 0.46±0.07, 44.07±11.71 and 3.28±1.36.

A significant ($p \leq 0.05$) increase in the total bilirubin was noted in *B. canis* group over the healthy control group while a non-significant increase was noted in *E. canis* and pyometra anaemic dogs and non-significant decrease in the CKD group.

The values of direct bilirubin revealed that there is significant decrease ($p \leq 0.05$) in the CKD group while significant increase in the *B. canis* group. The non-significant increase in direct bilirubin was reported in pyometra and *E. canis* group.

The non-significant increase in indirect bilirubin was noted in all the anaemic groups except *B. canis* anaemic groups where a statistically significant ($p \leq 0.01$) increase was noted.

The dogs afflicted with *E. canis*, *B. canis* and pyometra anaemia exhibited high to moderate bilirubinemia, as observed in the current investigation (Table 4.16). The elevated levels of total bilirubin observed in canine babesiosis in this study can be attributed to the proliferation of parasites within mononuclear cells,

as well as the infiltration of cytokine-mediated diseases in the spleen and liver's mononuclear phagocytic tissues (Sharma *et al.,* 2016). According to Eichenberger *et al.,* (2016), dogs with CKD, *E. canis*, *B. canis*, and pyometra had a higher death risk due to hyperbilirubinemia. Research has shown a significant correlation between immune-mediated hemolytic anaemia (IMHA), *B. canis*, and hyperbilirubinemia in dogs (Goggs *et al.,* 2012).

The serum enzymatic analysis revealed a nonsignificant increase in SGOT and SGPT concentrations in all diseased anaemic dogs over healthy control dogs except the CKD group non significant decrease has been reported. The alkaline phosphatase was found to be statistically ($p \leq 0.05$) decreased in the CKD group while statistically increased in the *B. canis* anaemic group over healthy control values. In *E. canis* and pyometra groups, the increase in alkaline phosphatase was statistically non-significant. Nassiri *et al.,* (2005), Niwetpathomwat *et al.,* (2007), and Reddy *et al.,* (2014) observed elevated levels of serum ALP in dogs with anaemia throughout their respective studies. The outcomes of the current investigation were similar to the findings of these researchers. The heightened enzymatic activity seen in the dogs may be attributed to modifications in the lipid membrane of the hepatocytes, resulting from liver damage, inflammation, or infection, which might potentially be the underlying cause of the anaemia.

There was a nonsignificant increase in total protein values in CKD and *E. canis* anaemic dogs while non-significant decrease in pyometra anaemic dogs. A statistically significant decrease was noted in the *B. canis* group of dogs.

Contrary to the present findings Singh *et al.,* (2006) recorded hyperproteinemia in pyometra cases stating that it may be due to acute phase reaction in pyometric bitches as also documented by Singh *et al.,* (2006). Low total protein in canine babesiosis could be attributed to the loss of intravascular protein due to increased vascular permeability as a consequence of concomitant inflammation (Dvorak, 2010; Hackner, 2010). The reason behind the comparatively higher values of TP among the dogs with *E. canis* could be monoclonal/polyclonal gammopathy in this group of dogs (Breitschwerdt, 2005) which may lead to hyperviscosity syndrome (Varela *et al.,* 1997). Monoclonal

gammopathy is a condition of very high gamma globulin concentrations associated with canine ehrlichiosis as an atypical response which can interfere sufficiently with platelet function *In-vivo* to produce severe haemorrhage with a subsequent rise in total protein and globulin concentration (Varela *et al.*, 1997; Harrus *et al.*,1998).

The albumin, one of the major protein portions in the serum was found to be decreased in all the anaemic groups viz, CKD, *E. canis, B. canis* and pyometra when compared with the healthy control dogs and the decrease was statistically highly significant ($p \leq 0.01$).

An opposite pattern of highly significant ($p \leq 0.01$) difference of increment was noted in globulin values in all the anaemic groups i.e. in CKD, *E. canis, B. canis* and pyometra diseased dogs over the healthy control group.

The Albumin to Globulin ratio (A: G ratio) was found to be decreased in all the anaemic groups over healthy control values under study and the decrease was statistically ($p \leq 0.01$) significant.

The observed alterations that demonstrated statistical significance ($p \leq 0.05$) were found to be connected with the levels of albumin and the albumin-to-globulin ratio (A/G). The anaemic group exhibits suboptimal levels of albumin, which can be explained by the presence of comorbid conditions such as kidney dysfunction, gastrointestinal issues, and trauma-related blood loss. These factors contribute to hypoalbuminemia in this subset of dogs, leading to a significant reduction in albumin levels ($p \leq 0.05$). The study conducted by Guadarrama-Olhovich *et al.*, (2013) found that the ratio of albumin to globulin (A/G) is influenced by the levels of albumin and globulin. Specifically, a decrease in albumin levels caused by a primary illness results in elevated globulin levels. This relationship is particularly evident in inflammatory conditions. The researchers observed that these changes in albumin and globulin levels led to statistically significant reductions in the A/G ratio ($p \leq 0.05$).

The presence of hyperglobulinemia indicates an excessive immunological response that is not sufficiently efficient (Woody and Hoskins, 1991; Moore and Williams, 2004). Furthermore, a simultaneous decrease in albumin levels may

occur as a consequence of protein deficiency (caused by anorexia in our study), proteinuria, blood loss, peripheral loss of fluid owing to vasculitis (ascites) in all the anaemic group, and as a compensatory response to increased globulin levels (Varela *et al.*,1997). Consequently, there was a substantial decrease in the A: G ratio between the healthy and sick groups. A decreased A:G ratio may indicate excessive synthesis of globulins, nephrotic syndrome, and sepsis. This indicates that dogs with CKD had a high level of pathogenesis, followed by *B. canis, E. canis*, and pyometra in anaemic dogs. Kim *et al.,* (2006), Reddy *et al.,* (2014), and Radakovich *et al.,* (2017) observed hypoalbuminemia, hyperglobulinemia, and hypergammaglobulinemia as the primary biochemical abnormalities seen in dogs that were infected with *E. canis* which aligns with the findings of the current study. Hypoalbuminemia can occur as a result of either an acute phase response or poor hepatic function, leading to reduced albumin production. Albuminuria may occur during haemorrhage or in cases with protein-deficient nephropathy.

Serum BUN values were found to be significantly ($p \leq 0.01$) increased in CKD and *B. canis* anaemic dogs while an insignificant difference of increment was recorded in *E. canis* and pyometra groups. The serum creatinine values were found to be statistically increased in CKD anaemic dogs while an insignificant difference of increment was noted in *E. canis*, *B. canis* and pyometra anaemic dogs.

The values of increased BUN and creatinine are akin to Showkat (2009). A marked increase in the values of BUN and creatinine in pyometra cases indicated serious renal dysfunction/renal failure. Pyometra is one of the common causes of death in older female dogs and kidney damage with nephritis is often associated with it (Renton *et al.,* 1971). Tandel *et al.,* (2019) noted that hematobiochemical changes in a sample of 78 anaemic canines in the Gujarat area and mentioned that elevated levels of creatinine and blood urea nitrogen (BUN) can potentially be attributed to glomerulonephritis, which suggests renal involvement accompanied by anaemia. The presence of chronic emaciation resulted in elevated blood urea nitrogen (BUN) values. Dogs that were suspected and then confirmed to have hemoprotozoan infection exhibited renal pathology, which was indicated by higher levels of blood urea nitrogen (BUN) and

creatinine. Singh (2017) discovered elevated levels of blood urea nitrogen (BUN) of 58.44 (mg/dl) in dogs with renal failure. Similarly, Srinivasan *et al.,* (1993) documented a high average BUN level of 78.18 (mg/dl) in dogs with renal failure. Elevated blood urea nitrogen (BUN) levels are linked to heightened breakdown of proteins, reduced urine output, vomiting, or gastrointestinal bleeding (Bartges and Polzin, 2011). The current study observed a notable elevation in blood urea nitrogen (BUN) and serum creatinine (Cr) levels in all the groups with anaemia. However, the levels of azotemia were more elevated in anaemic dogs with chronic kidney disease compared to the low levels observed in anaemic dogs with *E. canis*, *B. canis*, and pyometra. These findings align with the discoveries provided by Breitschwerdt (2005), Lobetti (2005), Ayoob *et al.,* (2010), and Vijayalakshmi (2011). The azotemia may be caused by renal tubular ischemic injuries resulting from excessive clots in microvessels, the formation of renal methemoglobin due to oxidation of haemoglobin in the bladder, a decrease in glomerular filtration rate (GFR), sepsis, and hypovolemic shock (Lobetti, 2005; Lupu *et al.,* 2014).

The studies conducted by Reddy *et al.,* (2014) and Radakovich *et al.,* (2017) both observed an elevation in the average levels of creatinine, which aligns with the results of our current investigation. Nephropathy leads to insufficient erythropoietin production, resulting in reduced erythrocyte formation and subsequent anaemia. Protein loss nephropathy is a contributing factor to anaemia in dogs.

4.1.13 Blood Lactate levels (mmol/L) in healthy control and anaemic dogs in different disease conditions:

Table 4.15 presents the overall Mean ± SE values of blood lactate levels in healthy control dogs and anaemic dogs and the values were 0.86± 0.14 and 4.80 ± 0.31, respectively where significant ($p \leq 0.01$) increase in the anaemic dogs was recorded. The study presented the Mean ± SE values of blood lactate (mmol/L) for, CKD, *E. canis*, *B. canis* and pyometra anaemic dogs as 6.12±1.05, 4.85$\pm$0.20, 4.56$\pm$0.15, and 3.67$\pm$0.08 respectively (Table 4.16)

A significant ($p \leq 0.01$) increment was noted in blood lactate throughout all the anaemic groups when compared with the healthy control group. Impairment of

oxygenation is a common consequence in individuals with anaemia, and the presence of hyperlactatemia in these patients, as compared to healthy canines, indicates disturbances in the amounts of circulating oxygen. The values align with the findings of Holahan *et al.,* (2010), and Kisielewicz *et al.,* (2014), and have significant implications for clinical interpretation in the context of hypoxia.

4.1.14 Electrolytes in healthy control and anaemic dogs in different disease conditions:

Table 4.15 presents the Mean ± SE values of electrolyte levels in healthy control dogs and anaemic dogs. The overall concentrations of electrolytes viz. Sodium (mEq/L), Potassium (mEq/) and Chloride (mEq/) in anaemic dogs were 139.91 ± 5.05, 5.03 ± 0.23 and 107.54 ± 0.95 respectively. Statistically non-significant decrement in sodium, non-significant increment in chloride and significant ($P< 0.01$) increment in potassium was noted in anaemic dogs over healthy control dogs.

On perusal of Table 4.16, it revealed that the values of Sodium (mEq/L), Potassium (mEq/) and Chloride (mEq/) in **CKD** were 142.75$\pm$3.01, 3.57$\pm$0.16 and 105.15$\pm$3.00, in ***E. canis*** were 126.67$\pm$11.03, 5.88$\pm$0.34 and 108.68$\pm$1.52, in ***B. canis*** were 119.88$\pm$5.88, 5.97$\pm$0.09 and 109.87$\pm$1.39 in **pyometra** 170.33$\pm$1.43, 4.70$\pm$0.12 and 106.47$\pm$0.81, respectively.

The analysis of electrolytes in individual anaemic groups showed as follows: The sodium values were found to be significantly decreased in *B. canis*, significantly increased in Pyometra and non-significantly decreased in CKD and *E. canis* anaemic dogs. The potassium values were significantly ($p\leq0.01$) increased in all anaemic groups except a significant decrease in the CKD group. There was a non-significant difference in chloride values in all the anaemic groups except *B. canis* (Significant increase) when compared with healthy control values.

Suggestive reasons for hyponatremia in the CKD group include hypotension, pain and renal injury which activate the sympathetic nervous system, renin-angiotensin-aldosterone system and antidiuretic hormone release (Prowle *et al*., 2010) which results in decreased perfusion, decreased GFR and increased

proximal tubular reabsorption of sodium and water in advanced renal failure (DiBartola, 2006).

4.1.15 Urinary Protein (mg/dl), Urinary Creatinine (mg/dl) and UPC ratio in healthy control and anaemic dogs in different disease conditions:

Table 4.15 depicts the Urinary Protein (mg/dl), Urinary Creatinine (mg/dl) and UPC ratio in healthy control and anaemic dogs. The values of Urinary Protein (mg/dl), Urinary Creatinine (mg/dl) and UPC ratio were (15.08 ± 1.00, 65.50±7.10 and 0.24±0.02) and (41.23 ± 11.76, 76.43 ± 9.61 and 0.64 ± 0.20) in healthy control and anaemic dogs, respectively. Urinary protein was found to be statistically ($p \leq 0.05$) increased while Urinary creatinine and UPC ratio was non significantly increased in anaemic dogs when compared with healthy control dogs.

The values of individual diseased dogs of Urinary Protein (mg/dl), Urinary Creatinine (mg/dl) and UPC ratio are shown in Table 4.16 and the values in the **CKD** group were (116.93 ± 30.33, 77.57 ± 27.98 and 1.85 ± 0.55) in ***E. canis*** group were (11.18 ± 2.69, 67.72 ± 10.68 and 0.17 ± 0.04), in ***B. canis*** were (14.12 ± 4.33, 85.70 ± 26.58 and 0.23 ± 0.06) and in **pyometra** were (22.67 ± 7.38, 74.75 ± 10.77 and 0.29 ± 0.06) respectively. The analysis of Mean ± SE values of Urinary protein, Urinary Creatinine and UPC ratio in individual groups revealed a significant increase in Urinary protein and UPC ratio while a non-significant increase in Urinary Creatinine in the CKD group. statistically non-significant increase was noted in Urinary protein, Urinary creatinine and UPC ratio in the rest of the anaemic dog groups viz. *E. canis*, *B. canis* and pyometra.

4.1.16 Free Hb (g/dl) in healthy control and anaemic dogs in different disease conditions

The values of the Free Haemoglobin (g/dl) in Healthy control dogs and anaemic dogs are presented in Table 4.15. The average value of free haemoglobin in the healthy group (n=6) was 0 (g/dl) while in anaemic dogs (n=24) it was 0.20 ± 0.03 (g/dl) and the significant difference of increment in anaemic dogs was noted when compared with healthy control dogs.

The average value of free haemoglobin in different anaemic dogs was 0.07

± 0.02 (g/dl) in CKD, 0.38± 0.03 (g/dl) in *E. canis*, 0.32± 0.03 (g/dl) in *B. canis* and 0.03± 0.02 (g/dl) in pyometra (Table 4.16). An average higher level of free haemoglobin was recorded in *E. canis* and *B. canis* groups followed by CKD anaemic dogs and a statistically significant difference was noted when anaemic dogs were compared with healthy control dogs. The increment in free haemoglobin in pyometra anaemic dogs was statistically non-significant when compared with healthy control dogs.

Hemolysis can occur either due to internal factors (intrinsic) or external factors (extrinsic). Intrinsic hemolysis is an uncommon condition that arises from an innate metabolic abnormality in red blood cells, such as a deficit of red cell pyruvate in Basenjis (Searcy *et al.,* 1979) and Springer Spaniels (Giger *et al.,* 1985). Extrinsic hemolysis is characterized by the influence of external stimuli such as red cell parasites, medicines, or antibodies that specifically target the red cell membrane. These factors cause the red cell to become aberrant and more prone to being engulfed by phagocytes, leading to a decrease in the lifespan of the red cell.

4.1.17 Erythrocyte Sedimentation Rate (ESR) (mm/hr) in healthy control and anaemic dogs in different disease conditions.

Table 4.15 presents the Mean ± SE values of ESR (mm/hr.) levels in healthy control and anaemic dogs. The Mean ± SE value of ESR (mm/hr.) in healthy dogs was 4.50±0.22 while in the anaemic dog, it was 41.96±2.87 and statistically significant difference of increment was noted in anaemic dogs over healthy control dogs.

The Mean ± SE values of ESR (mm/hr.) in CKD, *E. canis*, *B. canis* and pyometra anaemic groups were 43.00 ± 4.69, 40.00 ±5.64, 43.00 ±7.18 and 41.83 ± 6.72, respectively. A statistically significant ($p \leq 0.01$) increase was observed in all ESR values in all the anaemic dogs when compared with healthy control dogs. The ESR findings of the present study were following Vertimalai (1992) who recorded the ESR in the clinical cases before transfusion as 55.21 ± 8.45. On the third day post-transfusion, the ESR decreased to 47.07 ± 9.36. It further decreased to 40.5 ± 8.74 and 28.14 ± 7.48 on the seventh- and tenth-day post-transfusion

respectively.

Asawapattanakul et al (2021) documented that the ESR level was positively correlated with CRP level in his E canis study which corroborates with the present finding where there was an increase in the ESR level (40.00 ± 5.65mm/hr) in *E. canis* group with increase in the Beta 2 globulin (965.00 ± 63.65) in the present study.

4.1.18 Serum Iron profiling in healthy control and anaemic dogs

Iron profiling helps to evaluate anaemic dog's iron status, including parameters such as serum Iron levels, total iron-binding capacity (TIBC) and transferrin saturation. These parameters provide valuable information about iron availability, transport, and storage in the body, which are essential for erythropoiesis and the management of anaemia.

On perusal of Table 4.15, the Mean ± SE of Serum Iron (ug/dl), TIBC (ug/dl) and percent saturation (%) values in healthy and before-treatment (Anaemic) dogs were (104.12 ± 3.56, 210.58 ± 15.34 and 50.54 ± 3.30) and (98.52 ± 3.15, 233.03 ± 7.95, 42.80 ± 1.57) respectively. There was a nonsignificant decrease in the Serum Iron, a nonsignificant increase and a significant decrease in percent saturation in anaemic dogs over the healthy control dogs. The TIBC values are higher in anaemic dogs compared to healthy dogs as a compensatory mechanism. A significant ($p \leq 0.05$) decrease in percent saturation in anaemic dogs over healthy dogs could be due to the temporary decrease in Iron levels in anaemic dogs due to hemolysis.

Fry and Kirk (2006) reported the values of Serum iron, TIBC and Iron Saturation in chronic or renal disease to be 64.97 ± 23.08 µg/dl, 332.77 ± 51.35 µg/dl and 29.56 ± 14.71 % respectively which are nearly akin with the present findings. The level of these parameters in healthy dogs was 141.83 ± 7.91 µg/dl, 319.33 ± 31.89 µg/dl and 47.53 ± 6.54% respectively, which did not coincide with the findings of the present study. Bohn (2013) suggested that TIBC may increase in the cases of iron deficiency but usually was within range. Devadevi *et al*. (2022) opined that dogs with anaemia of chronic/renal disease, tick-born diseases and inflammatory conditions such as consistent pyometra displayed depletion of

iron stores which could be attributed to decreased erythropoiesis. Chronic inflammation could also contribute to the depletion of iron stores. The serum iron profile level in anaemic patients was in accordance with the findings of Cowgill (1995) who opined that Iron deficiency exists in renal patients. Decreased Iron levels in anaemic dogs are attributed to impaired absorption of Iron, chronic inflammation, blood losses such as gastrointestinal bleeding as well as decreased dietary uptake. Iron studies showed that serum iron levels and transferrin saturation levels decrease in chronic conditions such as renal disease whereas TIBC generally increases in cases of iron deficiency.

4.1.19 Comparison of all study Groups (Anaemic Groups and Healthy Group) in various parameters

Table 4.17, Table 4.18, Table 4.19, Table 4.20 and Table 4.21 are in continuations that describe the comparison amongst all study groups (Anaemic groups and healthy group) in various parameters.

The Hb, erythrocyte and PCV values were significantly decreased ($p \leq 0.01$) in all the anaemic groups [CKD (4.10 ± 0.32), (1.90 ± 0.19), (13.40 ± 1.61), *E. canis* (3.90 ± 1.67), (1.67 ± 0.09), (11.83 ± 0.36), *B. canis* (3.47 ± 0.47), (1.69 ± 0.22), (12.95 ± 1.96) and Pyometra (1.38± 0.25), (2.05 ± 0.19), (13.73 ± 1.12)] when compared with healthy control group. These values were statistically at par with each other amongst all anaemic groups, which revealed a proportionally equal intensity of anaemia in all the diseased groups.

The erythrocytic indices viz. MCV, MCH and MCHC comparisons revealed non-significant differences between each other group while significantly decreased ($p \leq 0.01$) MCHC in the *B. canis* (27.47 ± 1.17) anaemic group. The MCV and MCH values were non-significantly increased but were within the normal range in all anaemic groups except low MCHC in *B. canis* anaemic dogs. The MCHC values were significantly decreased ($p \leq 0.01$) in *B. canis* (27.47 ± 1.17) while the rest of the parameters were non-significant. These findings suggest that affected dogs were suffering from normocytic normochromic in

Table 4.17: Mean ± SE values in different parameters in healthy control group and different anaemic groups (Before Treatment) (1/5)

Anaemic Groups	Parameters							
	Hb (g/dl)	**Erythrocyte (x 10^6/µl)**	**PCV (%)**	**RDW (%)**	**MCV (fl)**	**MCH (pg)**	**MCHC (g/dl)**	**Total WBC (x 10^3/µl)**
CKD (n=6)	4.10±0.32^b	1.90±0.19^b	13.40±1.61^b	15.86±0.84	70.10±2.19	21.88±0.98	31.37±1.68^a	13.94±4.60^{ab}
E. canis (n=6)	3.90±0.16^b	1.67±0.09^b	11.83±0.36^b	23.82±4.16	71.39±2.66	23.43±0.51	32.95±0.82^a	30.59±11.75^a
B. canis (n=6)	3.47±0.47^b	1.69±0.22^b	12.95±1.96^b	19.32±1.45	74.82±3.00	20.39±0.40	27.47±1.17^b	5.78±1.79^b
Pyometra (n=6)	4.38±0.25^b	2.05±0.19^b	13.73±1.12^b	19.48±2.88	67.70±2.49	21.89±1.21	32.28±1.09^a	27.47±5.52^a
Healthy (n=6)	11.80±0.34^a	5.76±0.36^a	36.83±1.61^a	16.27±0.85	64.37±1.36	20.80±1.09	32.23±1.19^a	11.65±1.59^{ab}
F value	119.186	61.232	54.920	1.748	2.655	1.727	3.201	2.903
P value	0.000	0.000	0.000	0.171	0.056	0.175	0.029	0.042
Result	**	**	**	NS	NS	NS	*	*

* - Significant at 0.05% level, ** - Significant at 0.01% level, NS – Non Significant

Table F-value is 2.75 (5 %); 4.17 (1%).

Means bearing different superscripts a, b and c in columns differ significantly.

Table 4.18: Mean ± SE values in different parameters in healthy control group and different anaemic groups (Before Treatment) (2/5)

Anaemic Groups	Parameters							
	Neutrophil (%)	**Eosinophils (%)**	**Lymphocyte (%)**	**Monocyte (%)**	**Basophil (%)**	**Platelet (x 10^3/µl)**	**Free Hb (g/dl)**	**ESR (mm/hr)**
CKD (n=6)	80.00±2.23^{a}	3.83±2.45	14.67±3.76	1.50±0.34^{b}	0.00±0.00	148.17±36.31^{ab}	0.07±0.02^{b}	43.00±4.69^{a}
E. canis (n=6)	81.33±4.43^{a}	3.67±2.50	11.17±3.99	3.83±2.85^{b}	0.00±0.00	112.50±29.90^{bc}	0.38±0.03^{a}	40.00±5.65^{a}
B. canis (n=6)	58.00±3.43^{b}	2.33±0.76	25.83±3.98	13.83±4.87^{a}	0.00±0.00	39.17±6.79^{c}	0.32±0.03^{a}	43.00±7.19^{a}
Pyometra (n=6)	75.66±2.53^{a}	4.67±2.42	17.83±4.49	1.83±0.40^{b}	0.00±0.00	201.75±53.12^{ab}	0.03±0.02^{b}	41.83±6.73^{a}
Healthy (n=6)	74.66±2.03^{a}	2.00±0.00	20.17±1.64	3.17±0.60^{b}	0.00±0.00	238.83±20.05^{a}	0.00±0.00^{b}	4.50±0.22^{b}
F value	9.308	0.328	2.252	4.043	0.000	5.528	56.150	9.354
P value	0.000	0.856	0.092	0.011	0.000	0.002	0.000	0.000
Result	**	NS	NS	*	NS	**	**	**

* - Significant at 0.05% level, ** - Significant at 0.01% level, NS – Non Significant

Table F-value is 2.75 (5 %); 4.17 (1%).

Means bearing different superscripts a, b and c in columns differ significantly.

Table 4.19: Mean ± SE values in different parameters in healthy control group and different anaemic groups (Before Treatment) (3/5)

Anaemic Groups	Parameters							
	Serum BUN (mg/dl)	**Serum Creatinine (mg/dl)**	**Bilirubin Total (mg/dl)**	**Bilirubin Direct (mg/dl)**	**Bilirubin Indirect (mg/dl)**	**SGOT (IU/L)**	**SGPT (IU/L)**	**Alkaline Phosphate (IU/L)**
CKD (n=6)	128.28±13.11^{a}	7.30±0.58^{a}	0.33±0.08^{c}	0.13±0.02^{b}	0.21±0.07^{b}	50.73±10.47	50.10±8.01	65.73±15.77
E. canis (n=6)	41.39±11.79^{bc}	4.59±0.26^{b}	4.90±2.13^{a}	2.60±1.16^{a}	2.30±1.02^{a}	288.00±145.81	209.67±159.90	226.00±82.09
B. canis (n=6)	56.03±9.39^{b}	1.87±0.40^{cd}	3.85±1.05^{ab}	1.98±0.59^{ab}	1.87±0.51^{a}	142.29±33.74	112.23±30.89	186.55±30.87
Pyometra (n=6)	44.07±11.71^{bc}	3.28±1.36^{bc}	0.76±0.49^{bc}	0.52±0.38^{b}	0.24±0.11^{b}	73.94±31.17	186.68±62.47	252.67±114.96
Healthy (n=6)	21.50±1.63^{c}	1.05±0.15^{d}	0.53±0.07^{bc}	0.32±0.05^{b}	0.22±0.05^{b}	57.67±7.65	55.33±4.67	111.67±18.74
F value	15.651	13.134	3.874	3.334	4.012	2.097	0.884	1.424
P value	0.000	0.000	0.013	0.025	0.011	0.111	0.487	0.255
Result	**	**	*	*	*	NS	NS	NS

* - Significant at 0.05% level, ** - Significant at 0.01% level, NS – Non Significant

Table F-value is 2.75 (5 %); 4.17 (1%).

Means bearing different superscripts a, b and c in columns differ significantly.

Table 4.20: Mean ± SE values in different parameters in healthy control group and different anaemic groups (Before Treatment) (4/5)

Anaemic Groups	Parameters							
	Total Proteins (g/dl)	**Albumin (g/dl)**	**Globulin (g/dl)**	**A:G Ratio**	**Lactate (mmol/L)**	**Reticulocyte (%)**	**Absolute Reticulocyte (thousand cells/µl)**	**RPI**
CKD (n=6)	7.57 ± 0.76^{a}	1.95 ± 0.15^{b}	5.51 ± 0.70^{a}	0.36 ± 0.03^{b}	6.12 ± 1.05^{a}	0.84 ± 0.07^{b}	15.77 ± 2.02^{b}	0.13 ± 0.02^{b}
E. canis (n=6)	7.58 ± 0.51^{a}	2.19 ± 0.16^{b}	5.39 ± 0.57^{ab}	0.43 ± 0.05^{b}	4.85 ± 0.21^{ab}	2.33 ± 1.49^{b}	38.28 ± 24.57^{b}	0.32 ± 0.20^{b}
B. canis (n=6)	5.89 ± 0.09^{b}	1.74 ± 0.07^{b}	4.16 ± 0.04^{bc}	0.42 ± 0.02^{b}	4.56 ± 0.15^{b}	14.49 ± 2.07^{a}	224.34 ± 7.75^{a}	2.02 ± 0.11^{a}
Pyometra (n=6)	6.37 ± 0.39^{ab}	2.01 ± 0.31^{b}	4.36 ± 0.20^{abc}	0.46 ± 0.08^{b}	3.67 ± 0.08^{b}	0.99 ± 0.13^{b}	19.92 ± 2.93^{b}	0.16 ± 0.02^{b}
Healthy (n=6)	6.41 ± 0.05^{ab}	3.19 ± 0.03^{a}	3.22 ± 0.05^{c}	0.99 ± 0.02^{a}	0.87 ± 0.14^{c}	0.75 ± 0.11^{b}	44.86 ± 9.36^{b}	0.34 ± 0.06^{b}
F value	2.897	10.980	5.225	33.290	16.142	26.966	50.536	55.167
P value	0.042	0.000	0.003	0.000	0.000	0.000	0.000	0.000
Result	*	**	**	**	**	**	**	**

* - Significant at 0.05% level, ** - Significant at 0.01% level, NS – Non Significant

Table F-value is 2.75 (5 %); 4.17 (1%).

Means bearing different superscripts a, b and c in columns differ significantly.

Table 4.21: Mean ± SE values in different parameters in healthy control group and different anaemic groups (Before Treatment) (5/5)

Anaemic Groups	Parameters					
	Sodium (mEq/L)	**Potassium (mEq/L)**	**Chloride (mEq/L)**	**Urinary Protein (mg/dl)**	**Urinary Creatinine (mg/dl)**	**UPC Ratio**
CKD (n=6)	142.75 ± 3.01^{bc}	3.57 ± 0.16^{c}	105.15 ± 3.01	116.93 ± 30.33^{a}	77.57 ± 26.98	1.85 ± 0.55^{a}
E. canis (n=6)	126.67 ± 11.03^{cd}	5.88 ± 0.34^{a}	108.68 ± 1.53	11.18 ± 2.69^{b}	67.72 ± 10.69	0.18 ± 0.05^{b}
B. canis (n=6)	119.88 ± 5.88^{d}	5.97 ± 0.09^{a}	109.87 ± 1.39	14.12 ± 4.34^{b}	85.70 ± 26.58	0.24 ± 0.06^{b}
Pyometra (n=6)	170.33 ± 1.43^{a}	4.70 ± 0.13^{b}	106.47 ± 0.82	22.67 ± 7.38^{b}	74.75 ± 10.77	0.29 ± 0.07^{b}
Healthy (n=6)	147.67 ± 2.15^{b}	4.23 ± 0.09^{b}	106.50 ± 0.56	15.08 ± 1.00^{b}	65.50 ± 7.11	0.24 ± 0.03^{b}
F value	11.316	30.587	1.265	10.310	0.191	8.213
P value	0.000	0.000	0.310	0.000	0.941	0.000
Result	**	**	NS	**	NS	**

* - Significant at 0.05% level, ** - Significant at 0.01% level, NS – Non Significant

Table F-value is 2.75 (5 %); 4.17 (1%).

Means bearing different superscripts a, b and c in columns differ significantly.

CKD, *E. canis* and pyometra anaemic dogs while Macrocytic hypochromic anaemia was noted in *B. canis* infection.

Contrary to the present findings Sumit *et al.,* (2018) noted microcytic hypochromic anaemia in CKD dogs and mentioned the possibility of various pathogenesis involved such as shortened survival period and haemolysis of red blood cells due to uremia, loss of blood in the gastrointestinal tract as melena and haematemesis, loss from urinary tract in haematuria due to poor platelet production and due to deficiency of erythropoietin production by the diseased kidneys. Results of PCV were in accordance with Couto (2009) who documented significantly decreased PCV in CRF dogs. In chronic renal failure, impaired production of erythropoietin due to loss of renal parenchyma, increased haemolysis, and suppression of bone marrow erythropoiesis were associated with anaemia.

Anaemia, usually non-regenerative normocytic and normochromic, is the common finding in *E. canis* (Baneth, 2010) which corroborates with the current findings. Eichenberger *et al.,* (2016) observed mild to moderate normocytic normochromic non-regenerative anaemia in *B. canis* infection which was not similar to the present findings of Macrocytic hypochromic anaemia. Santos marina *et al.*, (2021) reported anaemia in 72 % of pyometra cases wherein 65 % cases were of normocytic normochromic which is akin to the present findings where all cases of pyometra were documented as the same type of anaemia. Feldman and Nelson, 2004 documented that anaemia in pyometra-affected dogs may occur due to the migration of RBCs into the uterus by diapedesis in a chronic process due to bone marrow suppression as a result of endotoxins from the systemic bacterial proliferation, which also causes the leuckocytosis.

The clinical manifestations of acute babesiosis are not always proportional to the degree of anaemia and are not correlated with the level of parasitemia which often remains below 1 % (Eichenberger R. M. *et al.*,2016). Hence besides mechanical erythrocyte damage, other pathophysiologic mechanisms have been proposed to contribute to hemolysis such as toxic hemolytic factors and immune-mediated destruction of erythrocytes. Several variables impact the clinical course

of a *B. canis* infection, and the basic pathophysiologic processes of babesiosis in dogs are still not well understood. Canine *B. rossi* infections have a negative outcome and high death rate due to the presence of high levels of lactate in the blood, weakened circulation, a large number of parasites, elevated levels of cortisol in the blood, and symptoms of coagulation disorder. In cases of *B. canis* infections, a heightened inflammatory reaction has been seen, characterised by elevated levels of fibrinogen, C-reactive protein (CRP), and secreted intracellular adhesion molecule-1 (sICAM-1) from erythrocytes, as well as thrombocytopenia. This inflammatory response has been linked to a worse prognosis. In addition, there is evidence linking an elevation in lipid mediators to the occurrence of serious consequences, such as the onset of systemic inflammatory response syndrome (SIRS) and malfunction of various organs.

The leucocytic count revealed at par values of marked leucocytosis in *E. canis* (30.59 ± 11.75), pyometra (27.47 ± 5.52) and CKD (13.94 ± 4.60) anaemic dogs and the increase was statistically significant ($p \leq 0.01$) over the healthy control group. A statistically significant ($p \leq 0.01$) decrease (leukopenia) was noted in *B. canis* (5.78 ± 1.79) anaemic dogs. The *B. canis* and CKD anaemic dogs were statistically par with each other but statistically ($p \leq 0.01$) different from the rest of the other two anaemic groups. Robinson *et al.,* (1989) reported higher TLC in dogs with CRF owing to primary inflammatory diseases of the urinary system and engrossment of other body systems and tissues which corroborates with the present study findings. The severe complications in *E. canis* are associated with various inflammatory mediators such as cytokines, acute phase protein, nitric oxide and oxidative stress, resulting in a systemic inflammatory response syndrome (Asawapattanakul, *et al.*,2021) and hence marked leucocytosis in the present study have been noted. Eichenberger *et al.,* (2016) mentioned that WBC counts for *B. canis* in all of the survivors' dogs were within the normal reference range which is contradictory to the present findings where leukopenia (5.78 ± 1.79 $x10^3/\mu l$) was noted. Santos marina *et al.,* (2021) observed leucocytosis in pyometra anaemic dogs with a frequency higher than 70%. Left shift was found in 60.58 % of all cases of which 93.65 % had regenerative left shifts. He observed the averages of leukocyte counts above 30,000 leukocytes/ µl in pyometra

anaemic dogs. These findings are akin with the present study findings. Feldman and Nelson, 2004 recorded that left-shift leukocytosis is a reaction that reflects the infectious inflammatory process as well as the installed septicaemia in pyometra anaemic dogs.

Statistically significant ($p \leq 0.01$) neutropenia and monocytosis and at par values of neutrophil and monocyte in CKD, *E. canis*, pyometra and healthy dogs were noted in the present study. The eosinophil, lymphocyte and basophil counts were non-significant in all the anaemic and healthy control dogs. The important cellular findings in *E. canis* were lymphocytosis and monocytosis (Asawapattanakul *et al.,* 2021) which was contrary to the present findings where the normal range monocytes (3.83 ± 2.85) and lower margin lymphocytes (11.17 ± 3.99) were noted. This might be due to the strain-specific response or duration of infection or exposure of dogs to the concurrent infection of *B. canis* infection in earlier times. Eichenberger *et al.,* (2016) mentioned inconsistent leukocyte abnormalities such as leukocytosis, leukopenia, neutrophilia, neutropenia, and eosinophilia in *B. canis* infection. Wherein the present study documented non-significant leukopenia, neutropenia, lymphocytosis and monocytosis and normal Eosinophilia in the *B. canis* anaemic group. Leukopenia was reported in 60% of mild cases of acute canine babesiosis (Mathe *et al.,* 2006). Several affected dogs had mild to moderate neutropenia, with an overall degenerative tendency and lacking a left shift. Furthermore, lymphopenia seems to be a hallmark of acute canine babesiosis Eichenberger *et al.,* (2016) which was not reported in the present study.

Statistically significant ($p \leq 0.01$) thrombocytopenia in hemoprotozoan infections i.e in *E. canis* and *B. canis* anaemic dogs while comparatively less thrombocytopenia but at par with the healthy control values was noted in CKD and pyometra anaemic dogs. Lower mean values of platelet count in CKD corroborate the findings of Gafter *et al.,* (1987) who opined insufficient thrombopoietic activity in CKD while Benjamin (1985) reasoned the uremic intoxication.

There are likely several reasons why platelets drop during canine

ehrlichiosis. Thrombophagocytosis with immunological destruction, platelet sequestration and pooling in the spleen, increased consumption with vascular endothelial changes, a decrease in the half-life of circulating platelets possibly due to opsonization with antibodies, and production impairment from bone marrow destruction and hypocellularity are some of these mechanisms. Apart from the reduction in the quantity of circulating platelets, thrombocytopathy, or platelet malfunction, has also been identified as a contributing cause to the absence of platelet functionality in canine monocytic ehrlichiosis.

Multiple mechanisms contribute to the development of thrombocytopenia in canine babesiosis, including heightened platelet activation and depletion due to systemic inflammatory response syndrome (SIRS), enhanced platelet sequestration and clumping, and reduced platelet generation. In cases of *B. rossi* infections, a negative result was linked to a consumptive coagulopathy. However, even in cases of severe thrombocytopenia, there was no evidence of bleeding diathesis or haemorrhage (Goddard *et al.,* 2013).

Haemolysis was marked in *E. canis* and *B. canis* anaemic dogs where Free Hb was statistically increased ($p \leq 0.01$) while there was a rise in Free Hb in CKD and pyometra-affected dogs but the increase was at par with the healthy control dogs. Eichenberger *et al.,* (2016) emphasized that disease severity can not be readily explained as a consequence of hemolysis alone which often is mild to moderate in acute infections. Along the hemolysis, other factors such as septic shock, acute renal failure, and multiple organ dysfunction syndrome also contribute to the Babesia infection.

The Erythrocytic sedimentation rate was found to increase in all the anaemic dogs in all the groups. Statistically significant ($p \leq 0.01$) difference was noted between all anaemic groups and healthy control group. Asawapattanakul *et al.,* (2121) documented that the ESR level was positively correlated with CRP level in his *E. canis* study which corroborates with the present finding where there was an increase in the ESR level (40.00 ± 5.65mm/hr) in *E. canis* group with an increase in the Beta 2 globulin (965.00 ± 63.65) in the present study.

In the present study, the Serum BUN values were significantly ($p \leq 0.01$)

increased in CKD (128.28 ± 13.11) and *B. canis* (56.03 ± 9.39) anaemic dogs while in *E. canis* and pyometra anaemic dogs, though there was an increase in the BUN, the values were at par with the healthy control dogs. Increased ureas and creatinine levels in renal failure might be due to a marked reduction in glomerular filtration rate (GFR), diminished renal excretion, enhanced tubular absorption of urea and impaired ability of kidneys to excrete proteinaceous catabolites (Osborne *et al.*, 1972). Similarly, findings of increased mean value of BUN and creatinine in dogs with CRF have been recorded by other workers (Kumar, 2013; Pradhan and Roy,2012). The serum creatinine values were statistically increased ($p \leq 0.01$) in all the anaemic dogs except in *B. canis* when compared with healthy control dogs. The highest creatinine was noted in CKD (7.30 ± 0.58), followed by *E. canis* (4.59 ± 0.26), pyometra (3.28 ± 1.36) and *B. canis* (1.87 ± 0.40). De loor *et al.,* (2013) observed higher levels of creatinine and urea in pyometra anaemic dogs which were also observed in the present study. It is recognised that urea levels can also rise due to non-renal factors. Acute kidney injury is not an independent occurrence but rather leads to dysfunction in distant organs such as the lungs, heart, liver, intestines, and brain. This is facilitated by a pro-inflammatory process that involves the migration of neutrophil cells, the expression of cytokines, and heightened oxidative stress (Makris and Spanou, 2016). Elevated levels of urea and creatinine in the bloodstream can be attributed to the accumulation of immunocomplexes in the glomeruli. This deposition is a consequence of endotoxemia triggered by bacterial infection with Escherichia coli (Feldman and Nelson, 2004). Similar to what was observed in the present study, urea and creatinine levels were elevated, possibly due to the presence of *E. coli* in the uterine fluids. Significant bilirubinaemia ($p \leq 0.01$) was noticed in *E. canis* followed by *B. canis* while the total bilirubin was at par increased in CKD and pyometra anaemic dogs but statistically at par with the healthy control group. Hyperbilirubinemia in *E. canis* might have arised from extravascular haemolysis as compared to intravascular haemolysis. Direct bilirubin was significantly increased ($p \leq 0.01$) in *E. canis* (2.60 ± 1.16) while Indirect bilirubin was significantly increased ($p \leq 0.01$) in *E. canis* (2.30 ± 1.02) and *B. canis* (1.87 ± 0.51) anaemic dogs. Sumit *et al.,* (2018) documented similar findings of normal

range total and direct bilirubin. Beckel *et al.,* (2005) observed elevated total bilirubin in dogs suffering from renal failure due to leptospirosis. Mild to moderate bilirubin in the urine of dogs with CRF has been observed by Srivastava *et al.,* (2012) which may be due low threshold for bilirubin due to concurrent polysystemic etiology of renal dysfunction including liver and bile duct.

There was a rise in enzymatic concentrations i.e. in SGOT, SGPT and alkaline phosphate concentrations in all anaemic dogs except CKD, where mild decrease but within normal range values were noted. However, the difference amongst these values is statistically non-significant. Similar findings were noted by Sumit *et al.,* (2018) who noted normal mean values of ALT/SGPT and mild elevated AST/ SGOT in dogs with CKD. Contrary to the present findings Carrero *et al.,* (2016) mentioned that loss of the skeletal muscle during CKD might be the reason for the increased values of AST observed in CKD patients. The opposite finding of elevated serum ALP was reported by Puri (2015) and suggested the reason for secondary renal hyperparathyroidism. Santos marina *et al.,* (2021) observed increased ALP in 52.2 % while ALT in 3.3 % pyometra pyometra-affected females, wherein contradictory findings of increased ALP, AST and alkaline phosphatase were noted in all the pyometra cases in the present study. In fact ALP is less specific than ALT in detecting liver inflammation. It is often used the ALP/ALT proportion of 2:1 signifying hepatic injury.

Serum protein values were found to be significantly increased in CKD (7.57 ± 0.76) and *E. canis* (7.58 ± 0.51), Significantly decreased in *B. canis* (5.89 ± 0.09) and at par in pyometra (6.37 ± 0.39) anaemic dogs when compared with the healthy control dogs. The albumin concentrations were significantly decreased ($p \leq 0.01$) in all anaemic dogs when compared with healthy dogs and the order of decrement was *B. canis* (1.74 ± 0.07), CKD (1.95 ± 0.15), pyometra (2.01 ± 0.31) and *E. canis* (2.19 ± 0.16) respectively. The globulin protein was also statistically increased ($p \leq 0.01$) in all the anaemic dogs and the order of increment was CKD (5.51 ± 0.70), *E. canis* (5.39 ± 0.57), pyometra (4.36 ± 0.20) and *B. canis* (4.16 ± 0.04) respectively. A: G ratio was found to be statistically decreased ($p \leq 0.01$) in all the anaemic dogs when compared with healthy control dogs.

Contrary to the present findings Pradhan and Roy (2012) reported hypoproteinaemia (3.26 ± 1.84 g/dl) but the similar finding of hypoalbuminemia (1.38 ± 1.28 g/dl) in dogs with chronic kidney disease. Hypoalbuminemia in the present study might be due to the gastrointestinal or renal protein loss of albumin. The UPC in CKD dogs in present study was 1.84 ± 0.55. Kaneko *et al.,* 2008 mentioned that the progress of an infection is usually associated with marked changes in the serum proteins. Production of acute phase proteins (APP's) by the liver and most of the APP's are globulins. Thereby the concurrent infection of CKD, *E. canis*, *B. canis* and Pyometra in the present study might be the reason for increased globulin and total protein levels. Feldman and Nelson (2004) noted hyperproteinaemia in the pyometra-affected dogs and reasoned that pyometra may be due to dehydration and/or chronic antigenic stimulation of the immune system as the production of immunoglobulins and complementary proteins may be increased in these cases. Total blood protein levels with no difference in animals with open and closed pyometra were also reported in the study by Gupta *et al.,* (2013). Statistically increased ($p \leq 0.01$) blood lactate was noted in all the anaemic dogs and the order of increment of lactate was CKD (6.12 ± 1.05), *E. canis* (4.85 ± 0.21), *B. canis* (4.56 ± 0.15) and pyometra (3.67 ± 0.08). Elevated lactate levels might have caused due to hypoxic anoxia in anaemic conditions. Eichenberger *et al.,* (2016) highlighted that lactate concentrations and WBC counts are the best prognostic sensitivity and specificity to differentiate between survivors and non-survivors in *B. canis* infections. All non-survivor had moderate to severe hyperlactatemia (8.35 mmol/L) whereas most survivors had concentrations within the reference range (1.6 mmol/L) where in 4.56 ± 0.15 mmol/L of lactate level was measured in *B. canis* in the present study and all were survivors. Survivors had much-reduced lactate concentrations, which were distinctly different from those of the non-survivors. This discovery is comparable to the observations made in dogs infected with *Babesia rossi*, the causative agent of severe canine babesiosis in South Africa. In these cases, serum lactate concentration is utilised to assess the progress of therapy. It has been found that elevated blood lactate levels are associated with worse outcomes. However, the exact origin of hyperlactatemia in dogs with acute babesiosis is not well understood. It is unlikely

to be due to hypoxia resulting from anaemia, which is generally mild to severe in most dogs infected with *B. canis*. Therefore, hypoxia in canine babesiosis may result from changes in the macro- and micro-circulation caused by protozoal sepsis, hypotension, disseminated intravascular coagulation (DIC), and systemic inflammatory response syndrome (SIRS), all of which are commonly observed in *B. canis* infections. Undoubtedly, elevated levels of lactate have predictive significance in systemic inflammatory response syndrome (SIRS) resulting from several aetiologies.

A statistically significant increase in reticulocyte % (14.49 ± 2.07), absolute reticulocyte count (224.34 ± 7.75) (thousand cells/µl) and RPI (2.02± 0.11) was noted in *B. canis* anaemic dogs while the rise of these parameters in other anaemic dogs was statistically non-significant when compared with healthy control dogs.

The electrolyte analysis in anaemic dogs revealed significant ($p \leq 0.01$) hypernatremia in pyometra (170.33 ± 1.43) while significant hyponatremia in *B. canis* (119.88 ± 5.88) and *E. canis* (126.67 ± 11.03) dogs while significant hyperkalaemia in *B. canis* (5.97 ± 0.09), *E. canis* (5.88 ± 0.34), significant hypokalaemia in CKD (3.57 ± 0.16) and at par in pyometra (4.70 ± 0.13) when compared with healthy control dogs. The chloride values were non-significant in all the anaemic groups when compared with healthy dogs. Sumit *et al.*, (2018) observed serum potassium levels towards the higher side of the reference range in affected dogs which is contrary to the present findings where hypokalemia was reported in the CKD-affected dogs. Prowle *et al.*,(2010) suggested the reasons for hyponatremia which include hypotension, pain and renal injury which activate the angiotensin-aldosterone system and antidiuretic hormone release which results in decreased GFR and increased proximal tubular reabsorption of sodium and water in advanced renal failure.

Statistically significant ($p \leq 0.01$) increased UPC ratio was observed in CKD dogs (1.84 ± 0.55) while at par values with healthy control were noted in *E. canis, B. canis* and pyometra anaemic dogs. Proteinuria is more common than azotemia in dogs with pyometra. This is because to focal glomerulosclerosis,

which causes protein to flow into the urine. Monitoring is necessary following ovariohysterectomy, according to Maddens *et al.*(2011)

4.2 Evaluation of Therapeutic Efficacy of Anaemic Dogs :

Anaemic dogs under study were confirmed for their etiologies and tailor-made treatment was adopted containing the medications mentioned in Table 3.2 which varied individually. The blood transfusion was performed to every anaemic dog @ 300ml per dog except 2 cases (# Case no 14 and 21, being low weight) where calculated blood volume was administered. Tapering doses of steroids (Inj Prednisolone) were given to selective cases showing high severity of infection, thrombocytopenia and positive agglutination. Iron Injections were advised to Iron deficient, and marginally low Iron dogs.

E. canis and *B. canis* dogs were treated with oral Doxycycline, platelet enhancers, hematinics parenteral fluid therapy, vitamins, amino acid supplementations etc. The CKD dogs were maintained on fluid therapy, vitamins, amino acid supplementations, oral hematinics, phosphorus binders etc. Inj. Darbopoetin was used as erythropoietin stimulants and subsequent proliferation and maturation of RBCs. The pyometra dogs underwent ovariohysterectomy along with routine anaemia treatment.

Every individual dog having the same eitology of anaemia may differ in clinical manifestation, haematological and biochemical alterations with varied disease severity. Hence base value and percent improvement/decrement based on the base value of every parameter [Table 4.22 (Overall % improvement) and Table 4.23 (Disease-wise % improvement)] were considered for therapeutic evaluation of anaemic groups and discussed in length in respective parameters.

The treatment evaluation was done based on overall clinical improvement, the extent of normalcy of haematological, biochemical parameters, urine analysis and reduction in disease severity on different day intervals i.e. Day 0, 3, 7, 15, 28 and 35. (Day 0 is the first day of treatment). Despite clinical improvement and presumable clearance of the infection, bone marrow regeneration may require up

Table 4.22: Overall Percent Improvement from respective baes value in all the Parameters on different day intervals (n=24)

Sr. No	Parameter	% Improvement on Day 0	% Improvement on Day 3	% Improvement on Day 7	% Improvement on Day 15	% Improvement on Day 28	% Improvement on Day 35
1	Hb (g/dl)	**3.99**	**15.45**	**27.65**	**47.74**	**64.57**	**80.86**
2	Erythrocyte (x 10^6/µl)	**8.66**	**14.50**	**24.80**	**53.75**	**63.31**	**75.69**
3	PCV (%)	**1.35**	**11.26**	**24.05**	**47.89**	**52.68**	**63.23**
4	MCV (fl)	**-5.69**	**1.80**	**2.03**	**-2.96**	**-5.50**	**-6.71**
5	MCH (pg)	**-3.60**	**5.97**	**3.90**	**-3.49**	**1.47**	**2.10**
6	MCHC (g/dl)	**2.67**	**4.10**	**1.90**	**-0.27**	**7.24**	**8.94**
7	Total WBC	**7.35**	**24.51**	**-19.86**	**-20.63**	**-27.43**	**-48.16**
8	Neutrophil (%)	**3.22**	**1.98**	**3.56**	**3.50**	**1.75**	**3.45**
9	Eosinophil (%)	**-10.34**	**-14.94**	**-50.58**	**-31.03**	**-13.79**	**6.90**
10	Lymphocyte (%)	**-21.82**	**-6.96**	**-8.63**	**-5.52**	**-10.55**	**-6.95**
11	Monocyte (%)	**34.13**	**5.56**	**5.56**	**-9.52**	**-3.97**	**-30.15**
12	Platelet (x 10^3/µl)	**17.30**	**28.08**	**51.82**	**62.67**	**106.37**	**106.63**
13	Free Hb (g/dl)	**-16.63**	**-31.25**	**-37.50**	**-50.00**	**-75.00**	**-85.38**
14	ESR (mm/hr)	**-25.22**	**-38.63**	**-42.80**	**-59.88**	**-67.92**	**-78.85**
15	Serum BUN (mg/dl)	**34.16**	**12.11**	**5.44**	**-28.18**	**-51.76**	**-54.76**
16	Serum Creatinine (mg/dl)	**21.08**	**11.02**	**25.42**	**-9.34**	**-31.46**	**-37.76**
17	Bilirubin total (mg/dl)	**6.02**	**-9.63**	**-11.10**	**-18.77**	**-33.49**	**-65.12**
18	Bilirubin Direct (mg/dl)	**2.76**	**-15.08**	**-12.61**	**-22.07**	**-36.00**	**-71.67**
19	Bilirubin Indirect (mg/dl)	**9.74**	**-3.44**	**-9.44**	**-15.02**	**-30.63**	**-57.35**
20	SGOT (IU/L)	**3.93**	**-31.40**	**-47.11**	**-49.19**	**-50.75**	**-61.26**
21	SGPT (IU/L)	**35.05**	**-32.46**	**-39.44**	**-43.57**	**-45.90**	**-57.13**

Sr. No	Parameter	% Improvement on Day 0	% Improvement on Day 3	% Improvement on Day 7	% Improvement on Day 15	% Improvement on Day 28	% Improvement on Day 35
22	Alkaline Phosphatase (IU/L)	**-20.50**	**-11.04**	**48.12**	**-4.95**	**-33.20**	**-39.44**
23	Total Proteins (g/dl)	**2.69**	**-4.08**	**-4.35**	**-7.41**	**-10.40**	**-10.27**
24	Albumin (g/dl)	**9.29**	**12.34**	**13.99**	**15.27**	**25.83**	**28.12**
25	Globulin (g/dl)	**0.67**	**-10.16**	**-11.21**	**-16.06**	**-24.34**	**-25.26**
26	A: G Ratio	**10.78**	**24.31**	**31.86**	**38.20**	**67.78**	**75.99**
27	Lactate (mmol/L)	**-8.19**	**-14.50**	**-20.20**	**-28.74**	**-37.96**	**-51.44**
28	Reticulocyte (%)	**-6.34**	**31.08**	**-7.30**	**-21.24**	**-10.14**	**-7.44**
29	Absolute Reticulocyte (thousand cells/µl)	**20.71**	**69.65**	**51.69**	**45.28**	**68.66**	**79.57**
30	RPI	**13.76**	**68.53**	**49.33**	**48.57**	**58.87**	**63.70**
31	Sodium (mEq/L)	**-1.61**	**-0.44**	**-1.21**	**2.56**	**3.62**	**6.89**
32	Potassium (mEq/L)	**-1.33**	**23.61**	**-0.42**	**-4.64**	**-2.57**	**-1.91**
33	Chloride (mEq/L)	**-0.13**	**0.18**	**0.83**	**1.17**	**1.75**	**3.40**
34	Urinary Protein (mg/dl)	**43.99**	**74.53**	**29.64**	**59.18**	**-4.76**	**-0.32**
35	Urinary Creatinine (mg/dl)	**35.25**	**23.65**	**24.60**	**27.16**	**30.89**	**45.02**
36	UPC Ratio	**7.32**	**65.10**	**6.81**	**53.33**	**-21.83**	**-29.15**

Table 4.23 : Percent Improvement from respective base value in Haematological and Biochemical Parameters on different day intervals in Anaemic dogs (n=24)

a) Hamatological Parameters

Name of Parameter	Disease	% Impro. on Day 0	% Impro. on Day 3	% Impro. on Day 7	% Impro. on Day 15	% Impro. on Day 28	% Impro. on Day 35
Hb (g/dl)	**CKD**	2.44	2.44	15.85	42.68	56.51	72.76
	E. canis	-3.00	8.54	30.33	47.44	65.82	80.77
	B. canis	24.98	43.73	67.29	73.55	95.64	115.37
	Pyometra	-4.93	11.41	4.95	32.33	46.41	61.24
	Healthy	3.11	0.42	0.70	4.81	1.13	2.69
Name of Parameter	**Disease**	**% Impro. on Day 0**	**% Impro. on Day 3**	**% Impro. on Day 7**	**% Impro. on Day 15**	**% Impro. on Day 28**	**% Impro. on Day 35**
RBC (x 10^6/µl)	**CKD**	-2.90	-2.64	8.18	36.78	47.12	68.76
	E. canis	1.68	-8.68	17.84	55.21	68.08	86.65
	B. canis	26.10	50.38	66.00	76.90	85.92	90.93
	Pyometra	10.60	19.53	11.72	49.07	55.66	60.55
	Healthy	4.05	13.34	13.41	15.76	15.50	13.24
Name of Parameter	**Disease**	**% Impro. on Day 0**	**% Impro. on Day 3**	**% Impro. on Day 7**	**% Impro. on Day 15**	**% Impro. on Day 28**	**% Impro. on Day 35**
PCV (%)	**CKD**	-3.84	-5.11	4.98	26.96	36.36	59.38
	E. canis	-3.38	5.07	28.03	51.27	64.30	75.21
	B. canis	17.89	37.43	59.75	81.71	71.57	66.69
	Pyometra	-5.10	7.89	5.59	33.50	40.78	53.40
	Healthy	4.85	11.86	8.73	0.73	8.58	10.37

Name of Parameter	Disease	% Impro. on Day 0	% Impro. on Day 3	% Impro. on Day 7	% Impro. on Day 15	% Impro. on Day 28	% Impro. on Day 35
MCV (fl)	**CKD**	0.01	-1.76	-2.21	-5.53	-6.85	-4.25
	E. canis	-3.87	25.62	13.41	-2.54	-2.50	-6.81
	B. canis	-6.67	-7.86	-1.73	5.06	-5.12	-9.77
	Pyometra	-12.44	-8.96	-1.44	-9.59	-7.68	-5.78
	Healthy	0.88	0.78	-2.42	-11.74	-5.75	-2.81
Name of Parameter	**Case No**	**% Impro. on Day 0**	**% Impro. on Day 3**	**% Impro. on Day 7**	**% Impro. on Day 15**	**% Impro. on Day 28**	**% Impro. on Day 35**
MCH (pg)	**CKD**	3.75	2.81	3.24	4.41	5.39	1.79
	E. canis	-2.56	29.56	14.26	-4.83	-1.72	-3.31
	B. canis	-1.32	-5.08	1.58	-1.31	6.84	13.59
	Pyometra	-14.15	-5.82	-4.36	-11.99	-4.04	-2.50
	Healthy	-0.65	-10.09	-9.31	-8.96	-12.62	-10.19
Name of Parameter	**Disease**	**% Impro. on Day 0**	**% Impro. on Day 3**	**% Impro. on Day 7**	**% Impro. on Day 15**	**% Impro. on Day 28**	**% Impro. on Day 35**
MCHC (g/dl)	**CKD**	3.27	4.09	5.09	10.02	12.79	5.86
	E. canis	1.78	4.56	1.37	-2.57	0.80	3.35
	B. canis	7.74	4.50	3.72	-6.77	12.41	25.32
	Pyometra	-1.31	3.28	-2.21	-2.39	4.02	3.70
	Healthy	-1.19	-9.69	-6.17	3.46	-6.73	-6.28
Name of Parameter	**Disease**	**% Impro. on Day 0**	**% Impro. on Day 3**	**% Impro. on Day 7**	**% Impro. on Day 15**	**% Impro. on Day 28**	**% Impro. on Day 35**
Total WBC ($x10^3$/µl)	**CKD**	27.02	44.13	22.98	43.76	13.18	-18.57
	E. canis	27.20	36.22	-46.14	-58.50	-74.74	-70.64
	B. canis	-24.69	-20.20	1.59	3.35	18.76	41.71

	Pyometra	-17.99	10.93	-16.85	-16.17	-5.09	-57.08
	Healthy	7.99	13.40	24.44	7.21	11.94	11.03
Name of Parameter	**Case No**	**% Impro. on Day 0**	**% Impro. on Day 3**	**% Impro. on Day 7**	**% Impro. on Day 15**	**% Impro. on Day 28**	**% Impro. on Day 35**
Neutrophil (%)	**CKD**	0.63	-1.04	0.21	1.25	-4.38	0.00
	E. canis	-2.87	-4.71	-4.92	-5.53	-7.79	-12.91
	B. canis	7.47	7.18	17.53	16.67	21.84	27.59
	Pyometra	9.25	8.37	5.51	5.51	3.08	6.17
	Healthy	1.79	3.57	-1.79	-3.13	-4.24	-1.56
Name of Parameter	**Disease**	**% Impro. on Day 0**	**% Impro. on Day 3**	**% Impro. on Day 7**	**% Impro. on Day 15**	**% Impro. on Day 28**	**% Impro. on Day 35**
Eosinophil (%)	**CKD**	-73.91	-65.22	-73.91	-56.51	-43.46	-39.13
	E. canis	-40.91	-31.82	-50.01	-45.46	49.99	122.72
	B. canis	92.88	21.43	21.43	50.02	42.86	50.02
	Pyometra	14.27	21.43	-67.86	-39.30	-67.86	-67.86
	Healthy	16.65	-8.35	25.00	16.65	-8.35	16.65
Name of Parameter	**Disease**	**% Impro. on Day 0**	**% Impro. on Day 3**	**% Impro. on Day 7**	**% Impro. on Day 15**	**% Impro. on Day 28**	**% Impro. on Day 35**
Lymphocyte (%)	**CKD**	18.18	26.13	19.32	11.36	35.22	13.64
	E. canis	-32.84	14.92	0.00	46.26	7.46	41.78
	B. canis	-23.87	-9.68	-23.87	-32.90	-37.42	-41.29
	Pyometra	-44.86	-43.92	-14.95	-12.15	-20.56	-4.67
	Healthy	-3.31	-9.92	7.44	14.05	19.01	8.26
Name of Parameter	**Disease**	**% Impro. on Day 0**	**% Impro. on Day 3**	**% Impro. on Day 7**	**% Impro. on Day 15**	**% Impro. on Day 28**	**% Impro. on Day 35**
Monocyte (%)	**CKD**	-22.20	-33.33	-11.13	-33.33	0.00	-33.33
	E. canis	195.67	86.98	152.20	26.09	95.67	34.80

	B. canis	-2.41	-15.66	-32.53	-16.87	-28.92	-46.99
	Pyometra	18.22	27.28	0.00	-9.06	-27.28	-36.33
	Healthy	-31.58	-15.79	-21.06	-26.33	-15.79	-26.33
Name of Parameter	**Case No**	**% Impro. on Day 0**	**% Impro. on Day 3**	**% Impro. on Day 7**	**% Impro. on Day 15**	**% Impro. on Day 28**	**% Impro. on Day 35**
Platelet ($x10^3$/µl)	**CKD**	-1.48	2.55	30.88	54.90	88.99	84.81
	E. canis	21.39	17.54	45.82	89.19	137.75	119.25
	B. canis	57.45	116.94	205.19	284.97	390.42	529.78
	Pyometra	21.02	35.46	40.78	10.42	46.47	33.46
	Healthy	32.17	19.84	32.33	40.68	40.54	61.34
Name of Parameter	**Disease**	**% Impro. on Day 0**	**% Impro. on Day 3**	**% Impro. on Day 7**	**% Impro. on Day 15**	**% Impro. on Day 28**	**% Impro. on Day 35**
Free Hb (g/dl)	**CKD**	0.00	-100.00	-74.63	-74.63	-100.00	-100.00
	E. canis	-21.67	-17.23	-34.73	-52.22	-69.45	-82.51
	B. canis	-10.73	-26.50	-26.50	-36.91	-73.82	-84.23
	Pyometra	-48.48	-100.00	-100.00	-100.00	-100.00	-100.00
	Healthy	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Name of Parameter	**Disease**	**% Impro. on Day 0**	**% Impro. on Day 3**	**% Impro. on Day 7**	**% Impro. on Day 15**	**% Impro. on Day 28**	**% Impro. on Day 35**
ESR (mm/hr)	**CKD**	-27.52	-41.09	-27.91	-63.57	-69.38	-78.30
	E. canis	-17.50	-34.58	-43.33	-57.50	-67.92	-79.17
	B. canis	-31.40	-41.47	-53.10	-60.08	-69.38	-79.84
	Pyometra	-23.90	-37.05	-47.01	-58.17	-64.94	-78.09
	Healthy	18.51	18.51	3.71	-3.71	25.93	-3.71

b) Biochemical Parameters

Name of Parameter	Disease	% Impro. on Day 0	% Impro. on Day 3	% Impro. on Day 7	% Impro. on Day 15	% Impro. on Day 28	% Impro. on Day 35
Serum BUN (mg/dl)	**CKD**	23.06	-0.45	-20.32	-49.31	-65.17	-73.50
	E. canis	35.36	17.77	-4.01	-25.72	-26.64	-21.77
	B. canis	16.90	-11.99	-38.40	-27.42	-44.97	-63.12
	Pyometra	87.32	74.01	145.06	30.06	-44.94	-20.58
	Healthy	11.63	17.06	33.33	31.01	13.18	17.83
Name of Parameter	**Disease**	**% Impro. on Day 0**	**% Impro. on Day 3**	**% Impro. on Day 7**	**% Impro. on Day 15**	**% Impro. on Day 28**	**% Impro. on Day 35**
Creatinine (mg/dl)	**CKD**	11.41	8.67	-1.14	-15.52	-32.42	-45.21
	E. canis	57.74	26.42	10.06	1.70	-11.82	-7.74
	B. canis	47.45	8.15	-23.16	0.11	-16.89	-37.43
	Pyometra	9.82	10.43	119.65	-6.28	-47.13	-35.94
	Healthy	3.14	12.67	39.71	39.71	30.19	14.29
Name of Parameter	**Disease**	**% Impro. on Day 0**	**% Impro. on Day 3**	**% Impro. on Day 7**	**% Impro. on Day 15**	**% Impro. on Day 28**	**% Impro. on Day 35**
Bilirubin Total (mg/dl)	**CKD**	-34.83	-15.02	-24.92	-15.02	-24.92	-34.83
	E. canis	8.22	-12.59	-20.06	-22.45	-34.76	-71.08
	B. canis	18.69	0.91	-26.79	-5.59	-28.09	-62.76
	Pyometra	-54.13	-41.42	131.59	-63.30	-56.36	-51.90
	Healthy	-28.14	-31.14	-9.38	6.38	0.00	-12.38
Name of	**Disease**	**% Impro. on**	**% Impro. on**	**% Impro. on**	**% Impro. on**	**% Impro. on**	**% Impro. on**

Parameter		Day 0	Day 3	Day 7	Day 15	Day 28	Day 35
Bilirubin Direct (mg/dl)	**CKD**	-21.88	3.91	3.91	30.47	3.91	-21.88
	E. canis	7.69	-17.37	-18.02	-24.40	-40.16	-79.63
	B. canis	14.78	-2.94	-27.44	-9.72	-25.72	-67.09
	Pyometra	-61.54	-54.42	66.73	-70.19	-64.04	-61.54
	Healthy	-36.91	-21.14	-5.36	15.77	5.05	-31.55
Name of Parameter	**Disease**	**% Impro. on Day 0**	**% Impro. on Day 3**	**% Impro. on Day 7**	**% Impro. on Day 15**	**% Impro. on Day 28**	**% Impro. on Day 35**
Bilirubin Indirect (mg/dl)	**CKD**	-42.93	-26.83	-42.93	-42.93	-42.93	-42.93
	E. canis	8.88	-7.18	-22.41	-20.23	-28.63	-60.70
	B. canis	22.80	5.02	-26.16	-1.23	-30.59	-58.20
	Pyometra	-38.27	-13.58	270.37	-48.56	-39.51	-31.28
	Healthy	-15.67	-46.08	-15.67	-7.83	-7.83	15.21
Name of Parameter	**Disease**	**% Impro. on Day 0**	**% Impro. on Day 3**	**% Impro. on Day 7**	**% Impro. on Day 15**	**% Impro. on Day 28**	**% Impro. on Day 35**
SGOT (IU/L)	**CKD**	8.09	13.68	38.98	14.59	-4.72	-7.02
	E. canis	33.33	-28.13	-67.19	-65.91	-75.00	-83.91
	B. canis	-28.60	-46.24	-49.16	-43.89	-35.81	-57.97
	Pyometra	-50.86	-46.53	-24.04	-38.01	-16.60	-16.60
	Healthy	4.62	-0.29	11.27	1.73	0.87	-1.16
Name of Parameter	**Disease**	**% Impro. on Day 0**	**% Impro. on Day 3**	**% Impro. on Day 7**	**% Impro. on Day 15**	**% Impro. on Day 28**	**% Impro. on Day 35**
SGPT (IU/L)	**CKD**	-13.52	-13.52	9.44	-11.04	-1.21	-39.13
	E. canis	111.76	-46.58	-52.23	-53.26	-68.07	-76.60
	B. canis	11.36	-6.14	-15.94	-19.81	-21.29	-18.81

	Pyometra	-23.84	-37.51	-52.32	-55.72	-47.77	-63.13
	Healthy	14.76	10.24	3.92	15.36	12.65	12.95
Name of Parameter	**Disease**	**% Impro. on Day 0**	**% Impro. on Day 3**	**% Impro. on Day 7**	**% Impro. on Day 15**	**% Impro. on Day 28**	**% Impro. on Day 35**
Alkaline Phosphatase (IU/L)	**CKD**	-1.12	-7.20	10.29	30.02	35.40	12.32
	E. canis	21.98	19.25	46.17	76.77	-17.47	-38.94
	B. canis	-19.73	16.86	66.09	-54.35	-55.02	-51.31
	Pyometra	-64.12	-59.72	46.44	-50.66	-49.01	-44.59
	Healthy	8.36	7.01	8.36	11.94	14.92	-4.93
Name of Parameter	**Disease**	**% Impro. on Day 0**	**% Impro. on Day 3**	**% Impro. on Day 7**	**% Impro. on Day 15**	**% Impro. on Day 28**	**% Impro. on Day 35**
Total Protein (g/dl)	**CKD**	-4.43	-15.43	-15.00	-20.36	-20.94	-16.53
	E. canis	8.51	-1.49	-0.80	-6.08	-10.46	-13.47
	B. canis	7.97	9.38	3.44	6.88	9.67	10.02
	Pyometra	-0.66	-6.11	-3.14	-6.82	-16.37	-17.81
	Healthy	0.28	0.59	-0.84	-0.89	-0.61	-0.94
Name of Parameter	**Disease**	**% Impro. on Day 0**	**% Impro. on Day 3**	**% Impro. on Day 7**	**% Impro. on Day 15**	**% Impro. on Day 28**	**% Impro. on Day 35**
Albumin (g/dl)	**CKD**	7.69	5.64	5.64	-0.51	9.23	32.31
	E. canis	2.29	10.09	11.01	11.93	24.77	5.96
	B. canis	30.06	30.06	34.68	52.60	66.47	78.03
	Pyometra	0.50	6.00	7.50	2.00	8.00	5.00
	Healthy	2.51	3.13	-2.82	-1.25	-0.94	-0.94
Name of	**Disease**	**% Impro. on**	**% Impro. on**	**% Impro. on**	**% Impro. on**	**% Impro. on**	**% Impro. on**

Parameter		Day 0	Day 3	Day 7	Day 15	Day 28	Day 35
Globulin (g/dl)	**CKD**	-6.89	-21.40	-20.79	-25.87	-30.20	-32.29
	E. canis	11.09	-6.12	-5.53	-13.30	-24.58	-21.24
	B. canis	-1.03	0.96	-9.45	-12.09	-13.05	-18.15
	Pyometra	-1.03	-11.54	-7.82	-10.85	-27.42	-28.11
	Healthy	-2.05	-1.96	1.06	-0.65	-0.25	-1.09
Name of Parameter	**Disease**	**% Impro. on Day 0**	**% Impro. on Day 3**	**% Impro. on Day 7**	**% Impro. on Day 15**	**% Impro. on Day 28**	**% Impro. on Day 35**
A:G ratio	**CKD**	18.89	35.28	35.56	34.72	56.94	99.44
	E. canis	-8.14	14.65	16.98	26.28	58.60	27.91
	B. canis	33.57	29.02	56.59	77.94	96.16	123.74
	Pyometra	1.51	20.52	20.52	16.20	59.18	59.40
	Healthy	4.72	5.03	-3.82	-0.50	-0.80	0.00
Name of Parameter	**Disease**	**% Impro. on Day 0**	**% Impro. on Day 3**	**% Impro. on Day 7**	**% Impro. on Day 15**	**% Impro. on Day 28**	**% Impro. on Day 35**
Blood lactate (mmol/L)	**CKD**	-8.50	-10.33	-8.45	-16.35	-24.75	-30.90
	E. canis	0.43	-13.33	-24.61	-45.01	-47.30	-56.72
	B. canis	-8.95	-18.64	-25.11	-31.64	-40.02	-68.60
	Pyometra	-18.13	-17.83	-27.85	-24.26	-45.06	-57.39
	Healthy	36.45	82.58	78.78	76.82	71.05	46.14
Name of Parameter	**Disease**	**% Impro. on Day 0**	**% Impro. on Day 3**	**% Impro. on Day 7**	**% Impro. on Day 15**	**% Impro. on Day 28**	**% Impro. on Day 35**
Reticulocyte (%)	**CKD**	30.47	46.71	12.66	7.89	40.02	67.86
	E. canis	33.86	285.26	13.80	-60.65	-6.86	-2.61
	B. canis	-16.06	-11.17	-15.45	-21.18	-19.84	-20.06
	Pyometra	10.02	37.35	45.45	46.26	81.88	102.43

	Healthy	15.60	2.27	20.00	24.40	24.40	37.73
Name of Parameter	**Disease**	**% Impro. on Day 0**	**% Impro. on Day 3**	**% Impro. on Day 7**	**% Impro. on Day 15**	**% Impro. on Day 28**	**% Impro. on Day 35**
Absolute Reticulocyte (thou/µl)	**CKD**	21.14	43.71	23.10	45.99	112.43	186.92
	E. canis	48.00	214.16	40.08	-39.08	44.44	69.06
	B. canis	15.69	46.79	53.89	51.19	56.92	58.29
	Pyometra	24.40	69.98	71.86	140.29	212.74	254.48
	Healthy	19.53	8.67	22.83	40.82	41.90	50.83
Name of Parameter	**Disease**	**% Impro. on Day 0**	**% Impro. on Day 3**	**% Impro. on Day 7**	**% Impro. on Day 15**	**% Impro. on Day 28**	**% Impro. on Day 35**
RPI	**CKD**	18.80	40.60	20.30	36.84	99.25	174.44
	E. canis	40.25	284.83	40.87	-40.25	41.49	62.54
	B. canis	9.61	36.62	51.24	58.42	48.56	42.32
	Pyometra	8.28	57.32	66.88	114.65	192.99	247.13
	Healthy	20.76	11.11	20.47	21.93	30.12	46.20
Name of Parameter	**Disease**	**% Impro. on Day 0**	**% Impro. on Day 3**	**% Impro. on Day 7**	**% Impro. on Day 15**	**% Impro. on Day 28**	**% Impro. on Day 35**
Sodium (mEq/L)	**CKD**	0.23	0.97	-0.82	1.44	-0.61	0.15
	E. canis	-8.49	-1.37	0.26	4.71	12.63	21.75
	B. canis	5.26	4.85	9.00	14.29	18.17	23.79
	Pyometra	-2.86	-4.67	-9.82	-6.34	-9.77	-10.39
	Healthy	5.19	2.26	0.34	2.71	-0.90	6.77
Name of Parameter	**Disease**	**% Impro. on Day 0**	**% Impro. on Day 3**	**% Impro. on Day 7**	**% Impro. on Day 15**	**% Impro. on Day 28**	**% Impro. on Day 35**
Potassium	**CKD**	-0.95	165.41	12.14	16.82	28.03	29.89

(mEq/L)							
	E. canis	1.99	-4.52	-0.27	-9.91	-9.62	-16.71
	B. canis	-4.76	-7.83	-9.79	-12.85	-13.98	-14.25
	Pyometra	-1.43	-8.87	1.77	-3.89	-2.49	8.15
	Healthy	10.25	16.16	15.76	16.54	14.17	9.45
Name of Parameter	**Disease**	**% Impro. on Day 0**	**% Impro. on Day 3**	**% Impro. on Day 7**	**% Impro. on Day 15**	**% Impro. on Day 28**	**% Impro. on Day 35**
Chloride (mEq/L)	**CKD**	-0.95	-1.20	-0.27	1.76	0.98	2.46
	E. canis	1.04	-0.11	1.37	1.67	2.04	3.71
	B. canis	-0.36	0.18	0.71	0.20	0.20	0.50
	Pyometra	-0.27	1.85	1.50	1.08	3.80	7.00
	Healthy	3.60	3.76	14.08	9.55	3.91	10.33

c) Urine Examination:

Name of Parameter	Disease	% Impro. on Day 0	% Impro. on Day 3	% Impro. on Day 7	% Impro. on Day 15	% Impro. on Day 28	% Impro. on Day 35
Urinary Protein (mg/dl)	**CKD**	51.10	51.30	25.37	24.70	-7.34	-0.47
	E. canis	-13.11	90.76	-17.73	277.96	-1.48	-13.56
	B. canis	80.87	108.03	60.44	64.46	29.99	54.54
	Pyometra	12.50	165.51	55.88	125.80	-14.71	-27.21
	Healthy	3.31	1.77	-0.33	-4.75	-2.87	-4.20
Name of Parameter	**Disease**	**% Impro. on Day 0**	**% Impro. on Day 3**	**% Impro. on Day 7**	**% Impro. on Day 15**	**% Impro. on Day 28**	**% Impro. on Day 35**
Urinary Creatinine (mg/dl)	**CKD**	27.35	32.49	39.07	44.34	94.97	119.40
	E. canis	-7.16	0.91	-6.05	-0.62	-6.89	-21.33
	B. canis	88.66	66.14	50.19	60.23	18.34	17.23
	Pyometra	20.62	-13.62	8.03	-3.43	13.00	59.82
	Healthy	-1.83	4.10	14.99	5.70	10.05	10.15

Name of Parameter	Disease	% Impro. on Day 0	% Impro. on Day 3	% Impro. on Day 7	% Impro. on Day 15	% Impro. on Day 28	% Impro. on Day 35
UPC Ratio	**CKD**	12.55	32.14	-4.60	1.19	-24.89	-33.17
	E. canis	-4.57	101.71	-13.14	402.86	4.57	1.14
	B. canis	-9.17	-9.17	-9.58	-11.67	-15.83	7.08
	Pyometra	-5.12	311.95	104.10	226.62	-23.21	-51.54
	Healthy	0.82	-6.17	-16.87	-11.52	-13.58	-12.76

to 120 days following treatment (Breitschwerdt, 2011).

4.2.1 Haemoglobin (g/dl) values in healthy control group and different anaemic groups at all the time intervals:

Table 4.24 (Figure 4.9) indicates haemoglobin (g/dl) values (Mean ± SE) of dogs in different anaemic groups as well as healthy control group at all the time intervals. The overall haemoglobin (g/dl) values before treatment, Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35 were 3.96 ± 0.16, 4.12 ± 0.22, 4.58 ± 0.25, 5.06 ± 0.33, 5.85 ± 0.25, 6.52 ± 0.28 and 7.17 ± 0.30, respectively with percent increment in haemoglobin as 3.99, 15.45, 27.65, 47.74, 64.57 and 80.86 on Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35, respectively (Table 4.22).

Comparative analysis of the haemoglobin values between CKD, *E. canis*, *B. canis* and pyometra groups on BT, Day 0, 3, 7,15, 28 and 35 revealed significant ($p \leq 0.01$) differences when compared individually with the healthy control groups on respective days. This showed that the Healthy control group had a significantly higher haemoglobin value than all the anaemic groups.

The haemoglobin values in CKD, *E. canis*, *B. canis* and pyometra anaemic groups on before treatment were (4.10 ± 0.31, 3.90 ± 0.60, 3.46 ± 0.46 and 4.38 ± 0.25) and on Day 35 (7.08 ± 0.25, 7.05 ± 0.85, 7.46 ± 0.58 and 7.06 ± 0.79), respectively having overall percent improvement as 77.19, 82.52, 137.70 and 64.71 in respective anaemic groups (Table 4.23). The highest percentage of improvement in Haemoglobin was noted in *B. canis* (137.70) followed by *E. canis* (82.52), CKD (77.19) and pyometra (64.71) anaemic dogs.

There was statistically significant increase in the Haemoglobin values of CKD, *E. canis*, *B. canis* and pyometra anaemic groups when Day 35 values of respective diseases were compared with before-treatment values.

Conclusively, a post-treatment haemoglobin study revealed that since there was a significant increase in the haemoglobin values between before treatment and Day 35 in CKD, *E. canis*, *B. canis* and pyometra groups, it showed that the whole blood transfusion had a beneficial impact on haemoglobin, resulting in a substantial and radical improvement in the clinical condition of the patient.

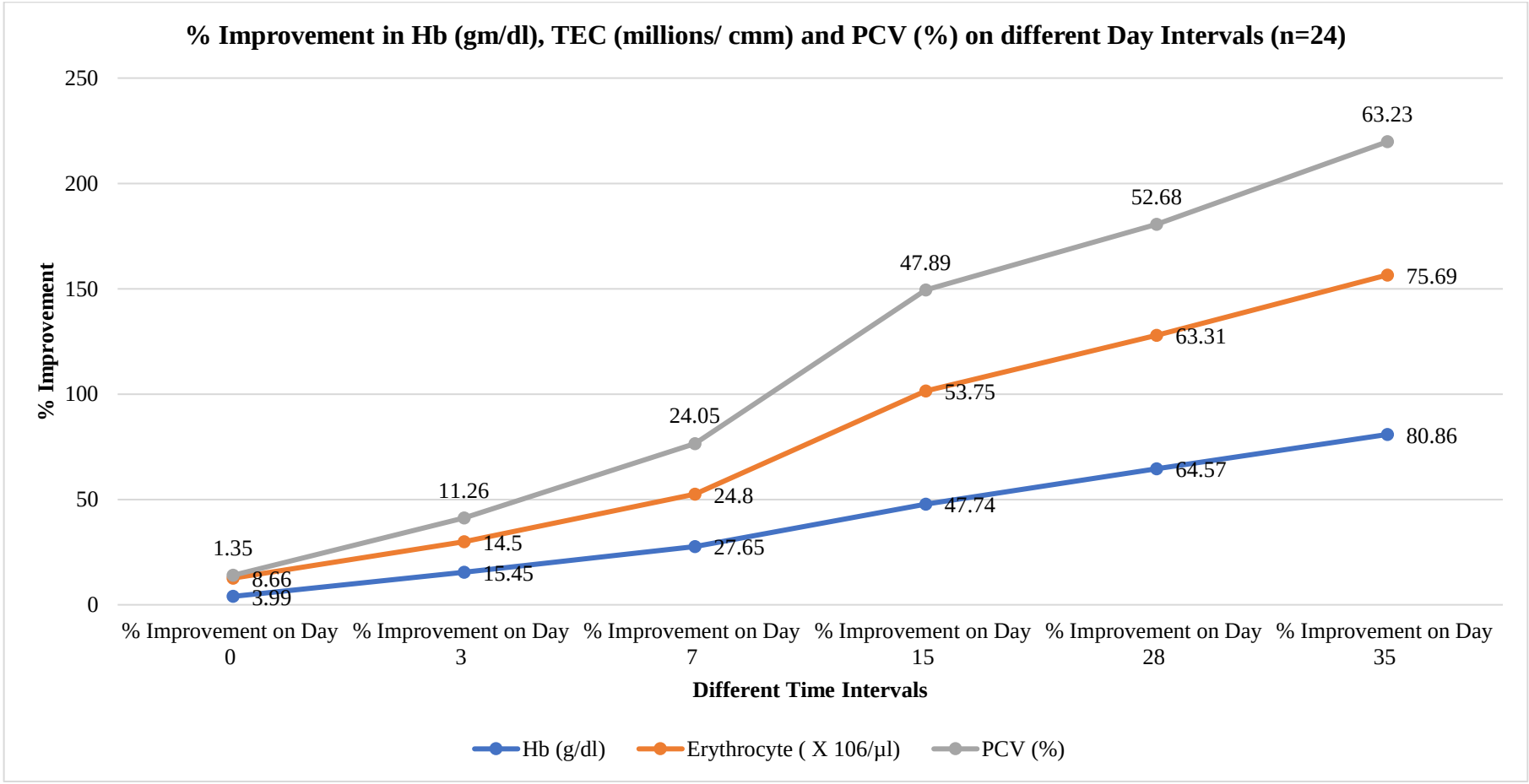


Figure 4.9 Percent Improvement in Hb (gm/dl), TEC (millions/ cmm) and PCV (%) in Anaemic Dogs on different Day Intervals (n=24)

Table 4.24: Mean ± SE Haemoglobin (g/dl) in the healthy control group and different anaemic groups at all the time intervals

Time Intervals	CKD (a) (n=6)	*E. canis (b)* (n=6)	*B. canis (c)* (n=6)	Pyometra(d) (n=6)	Healthy(e) (n=6)	Overall (a+b+c+d) (n=24)	F value	P value	S / NS
BT	4.10 ± 0.31^{cpq}	3.90 ± 0.16^{cq}	3.46 ± 0.46^{cq}	4.38 ± 0.25^{bcq}	11.80 ± 0.33^{p}	3.96 ± 0.16	119.18	0.000	**
Day 0	4.20 ± 0.49^{cq}	3.78 ± 0.34^{cq}	4.33 ± 0.55^{bcq}	4.16 ± 0.43^{cq}	12.16 ± 0.23^{p}	4.12 ± 0.22	70.72	0.000	**
Day 3	4.20 ± 0.53^{cq}	4.23 ± 0.48^{cq}	4.98 ± 0.56^{bcq}	4.88 ± 0.45^{bcq}	11.85 ± 0.38^{p}	4.58 ± 0.25	45.01	0.000	**
Day 7	4.75 ± 0.70^{bcq}	5.08 ± 0.41^{bcq}	5.80 ± 0.60^{abq}	4.60 ± 0.87^{bcq}	11.88 ± 0.30^{p}	5.06 ± 0.33	25.14	0.000	**
Day 15	5.85 ± 0.32^{abq}	5.75 ± 0.46^{abq}	6.01 ± 0.50^{abq}	5.80 ± 0.75^{abcq}	12.36 ± 0.26^{p}	5.85 ± 0.25	35.06	0.000	**
Day 28	6.41 ± 0.31^{aq}	6.46 ± 0.49^{abq}	6.78 ± 0.58^{aq}	6.41 ± 0.88^{abq}	11.93 ± 0.23^{p}	6.52 ± 0.28	19.35	0.000	**
Day 35	7.08 ± 0.25^{aq}	7.05 ± 0.85^{aq}	7.46 ± 0.58^{aq}	7.06 ± 0.70^{aq}	12.11 ± 0.21^{p}	7.17 ± 0.30	14.64	0.000	**
Overall	5.23 ± 0.24	5.18 ± 0.25	5.55 ± 0.28	5.33 ± 0.28	12.02 ± 0.10				
F value	7.35	6.71	6.29	2.81	0.514				
P value	0.000	0.000	0.000	0.024	0.793				
S / NS	**	**	**	**	NS				

Table F-value for Days is 2.37 (5 %) and 2.75 (1 %); Table F value for Groups is 3.36 (5 %) and 4.17 (1 %).
* $p\leq0.05$; ** $p\leq0.01$ and NS – Non Significant
Means bearing superscripts p,q,r,s in rows show statistical significance between the groups while means bearing superscripts a,b,c,d in columns show the statistical significance within the groups.

4.2.2 Erythrocyte Count (x 10^6/µl) in healthy control group and different anaemic groups at all the time intervals:

Table 4.25 (Figure 4.9) indicates the erythrocyte count (X 10^6/µl) of dogs in different anaemic groups as well as healthy control group at all the time intervals. The overall erythrocyte count in before treatment, Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35 were 1.83 ± 0.09, 1.99 ± 0.12, 2.09 ± 0.15, 2.28 ± 0.18, 2.81 ± 0.15, 2.98 ± 0.16 and 3.21 ± 0.13 respectively with percent increment in erythrocyte count as 8.66, 14.50, 24.80, 53.75,63.31 and 75.69 on Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35, respectively (Table 4.22).

Comparative analysis of the erythrocyte count between CKD, *E. canis*, *B. canis* and pyometra groups on BT, Day 0, 3, 7,15, 28 and 35 revealed significant ($p \leq 0.01$) difference when compared individually with the healthy control groups on respective days. This showed that there was a significant ($p \leq 0.01$) decrease in the erythrocyte count in all the anaemic groups than the Healthy control group (Table 4.25)

The erythrocyte count in CKD, *E. canis*, *B. canis* and pyometra anaemic groups on before treatment were (1.89 ± 0.18, 1.67 ± 0.08, 1.69 ± 0.21 and 2.04 ± 0.19) and on Day 35 (3.19 ± 0.17, 3.11 ± 0.36, 3.24 ± 0.28 and 3.28 ± 0.29), respectively having overall percent improvement as 68.76, 86.65, 90.93 and 60.55 in respective anaemic groups (Table 4.23), The highest percentage of improvement in erythrocyte count was noted in *B. canis* (90.93) followed by *E. canis* (86.65), CKD (68.76) and pyometra (60.55) anaemic dogs.

There was a statistically significant increase in the erythrocyte counts of CKD, *E. canis* and *B. canis* anaemic groups while non-significant increase in pyometra group when Day 35 values of respective disease were compared with before treatment values (Table 4.25)

The primary cause of anaemia in renal failure is insufficient synthesis of erythropoietin, a glycoprotein hormone that plays a crucial role in the proliferation and survival of erythroid progenitor cells. The advent of recombinant human erythropoietin has significantly transformed the management of anaemia in individuals with chronic renal failure (Santoro, 2022).

Table 4.25: Mean ± SE Erythrocyte count (x 10^6/µl) in healthy control group and different anaemic groups at all the time intervals

Time Intervals	CKD (a) (n=6)	*E. canis (b)* (n=6)	*B. canis (c)* (n=6)	Pyometra(d) (n=6)	Healthy(e) (n=6)	Overall (a+b+c+d) (n=24)	F value	P value	S / NS
BT	1.89±0.18^{cq}	1.67±0.08^{cq}	1.69±0.21^{cq}	2.04±0.19^{q}	5.75±0.35^{p}	1.83±0.09	61.23	0.000	**
Day 0	1.84±0.19^{cq}	1.69±0.21^{cq}	2.14±0.19^{bcq}	2.26±0.29^{q}	5.98±0.38^{p}	1.99±0.12	44.85	0.000	**
Day 3	1.84±0.20^{cq}	1.52±0.26^{cq}	2.55±0.22^{abq}	2.44±0.32^{q}	6.52±0.55^{p}	2.09±0.15	35.21	0.000	**
Day 7	2.05±0.26^{bcq}	1.96±0.20^{baq}	2.81±0.31^{abq}	2.28±0.51^{q}	6.52±0.65^{p}	2.28±0.18	20.42	0.000	**
Day 15	2.59±0.21^{abq}	2.59±0.22^{abq}	3.00±0.27^{aq}	3.05±0.43^{q}	6.66±0.40^{p}	2.81±0.15	28.58	0.000	**
Day 28	2.78±0.15^{aq}	2.80±0.19^{aq}	3.15±0.31^{aq}	3.18±0.55^{q}	6.64±0.34^{p}	2.98±0.16	23.25	0.000	**
Day 35	3.19±0.17^{aq}	3.11±0.36^{aq}	3.24±0.28^{aq}	3.28±0.29^{q}	6.51±0.18^{p}	3.21±0.13	30.32	0.000	**
Overall	2.32±0.11	2.20±0.12	2.66±0.12	2.65±0.16	6.37±0.16				
F value	7.29	7.19	4.53	1.67	0.65				
P value	0.000	0.000	0.001	0.156	0.682				
S / NS	**	**	**	NS	NS				

Table F-value for Days is 2.37 (5 %) and 2.75 (1 %); Table F value for Groups is 3.36 (5 %) and 4.17 (1 %).

* $p \leq 0.05$; ** $p \leq 0.01$ and NS – Non Significant

Means bearing superscripts p,q,r,s in rows show statistical significance between the groups while means bearing superscripts a,b,c,d in columns show the statistical significance within the groups.

In conclusion, the post-treatment analysis of red blood cells demonstrated a significant rise in erythrocyte count between the pre-treatment stage and Day 35 in the CKD, *E. canis*, *B. canis*, and pyometra groups. This indicates that both, the administration of whole blood transfusion and disease-specific treatment have a highly advantageous effect on erythrocytes, leading to a profound enhancement in the patient's clinical condition.

4.2.3 Packed Cell Volume (PCV) (%) in healthy control group and different anaemic groups at all the time intervals:

Table 4.26 (Figure 4.9) indicates PCV % (Mean± SE) of dogs in different anaemic groups as well as healthy control group at all the time intervals. The overall PCV percentage before treatment, Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35 were 12.98 ± 0.67, 13.15 ± 0.80, 14.44 ± 0.93, 16.10 ± 1.11, 19.19 ± 0.96, 19.82 ± 0.97 and 21.19 ± 0.85, respectively with percent increment in PCV as 1.35, 11.26, 24.05, 47.89, 52.68 and 63.23 on Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35, respectively (Table 4.22).

Comparative analysis of the PCV values between CKD, *E. canis*, *B. canis* and pyometra groups on BT, Day 0, 3, 7,15, 28 and 35 revealed significant ($p \leq 0.01$) differences when compared individually with the healthy control groups on respective days. This showed that the PCV values of the healthy control group were significantly higher than those of the anaemic groups.

The PCV values in CKD, *E. canis*, *B. canis* and pyometra anaemic groups on before treatment were (13.40 ± 1.60, 11.83 ± 0.36, 12.95 ± 1.95 and 13.73 ± 1.12) and on Day 35 (21.35 ± 0.84, 20.73 ± 2.53, 21.58 ± 1.34 and 21.06 ± 2.08), respectively having overall percent improvement as 59.38, 75.21, 66.69 and 53.40 in respective anaemic groups (Table 4.23). The highest percentage of improvement in PCV was noted in *E. canis* (75.21) followed by *B. canis* (66.69), CKD (59.38) and pyometra (10.37) anaemic dogs.

There was a statistically significant increase in the PCV values of CKD, *E. canis* and *B. canis* anaemic groups while non-significant increase in the pyometra group when Day 35 values of respective diseases were compared with before-treatment values (Table 4.26).

Table 4.26: Mean ± SE PCV (%) values in healthy control group and different anaemic groups at all the time intervals

Time Intervals	CKD (a) (n=6)	*E. canis (b)* (n=6)	*B. canis (c)* (n=6)	Pyometra(d) (n=6)	Healthy(e) (n=6)	Overall (a+b+c+d) (n=24)	F value	P value	S / NS
BT	13.40±1.60^{cq}	11.83±0.36^{cq}	12.95±1.95^{cq}	13.73±1.12^{q}	36.83±1.61^{p}	12.98±0.67	54.92	0.000	**
Day 0	12.88±1.40^{cq}	11.43±1.20^{cq}	15.26±2.32^{bcq}	13.03±1.22^{q}	38.62±1.94^{p}	13.15±0.80	46.72	0.000	**
Day 3	12.71±1.44^{cq}	12.43±1.30^{cq}	17.79±2.43^{abcq}	14.81±1.58^{q}	41.20±2.06^{p}	14.44±0.93	44.94	0.000	**
Day 7	14.06±1.83^{cq}	15.15±1.01^{bcq}	20.68±2.38^{abq}	14.50±2.63^{q}	40.05±2.97^{p}	16.10±1.11	23.50	0.000	**
Day 15	17.01±1.08^{bcq}	17.90±1.28^{abq}	23.53±2.02^{aq}	18.33±2.22^{q}	37.10±0.86^{p}	19.19±0.96	27.96	0.000	**
Day 28	18.27±1.22^{abq}	19.44±1.38^{abq}	22.21±2.00^{aq}	19.33±2.88^{q}	39.99±1.61^{p}	19.82±0.97	22.77	0.000	**
Day 35	21.35±0.84^{aq}	20.73±2.53^{aq}	21.58±1.34^{abq}	21.06±2.08^{q}	40.65±2.06^{p}	21.19±0.85	21.59	0.000	**
Overall	15.67±0.68	15.56±0.75	19.15±0.93	16.40±0.85	39.21±0.73				
F value	5.66	7.20	3.50	2.29	0.76				
P value	0.000	0.000	0.008	0.056	0.601				
S / NS	**	**	**	NS	NS				

Table F-value for Days is 2.37 (5 %) and 2.75 (1 %); Table F value for Groups is 3.36 (5 %) and 4.17 (1 %).
* $p \leq 0.05$; ** $p \leq 0.01$ and NS – Non Significant
Means bearing superscripts p,q,r,s in rows show statistical significance between the groups while means bearing superscripts a,b,c,d in columns show the statistical significance within the groups.

Whole blood transfusion has supplied coagulation factors, plasma proteins, a portion of white cells, and platelets. Dogs who get transfusions experience increased packed cell volumes (PCVs) and improved oxygen-carrying capacity, which helps them overcome the underlying condition.

The results of this study align with the findings of Sukullaya and Anuchai (2006) and Chiaramonte (2004), who saw a comparable rise in packed cell volume (PCV) following the transfusion of anaemic dogs at doses of 29 ml/kg and 20 ml/kg, respectively. After transfusing 7.5 mL/kg whole blood, Ognean *et al.* (2015) observed a 10.43 percent increase in PCV, Chiaramonte (2004) observed a 10% increase, and Assarasakorn & Niwetpathomwat (2006) observed a 15.2% increase. According to Callan *et al.* (1996), a bigger mean volume of 33mL/kg Bwt. of whole blood given had equal oxygen-carrying capability to 19mL/kg Bwt. of pRBCs, since WB PCV is 45-50% and pRBCs 75-80%.

The research done after treatment showed a significant rise in PCV levels from the pre-treatment stage to Day 35 in the groups with chronic kidney disease (CKD), Ehrlichia canis infection, Babesia canis infection, and pyometra. These findings indicate that both the administration of whole blood transfusion and targeted therapy for the specific illness have a substantial and profound beneficial effect on PCV values, leading to a considerable improvement in the patient's overall condition.

4.2.4 MCV (fl) values in healthy control group and different anaemic groups at all the time intervals:

Table 4.27 (Figure 4.10) indicates MCV (fl) values (Mean± SE) of dogs in different anaemic groups as well as healthy control groups at all the time intervals. The overall MCV values in before treatment, Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35 were 71.00 ± 1.33, 66.96 ± 2.30, 72.28 ± 3.80, 72.44 ± 2.82, 68.91 ± 1.52, 67.10 ± 1.43 and 66.24 ± 0.90, respectively.

There was a decrease of 5.69 % on Day 0, an increase of 1.80 %, 2.03 % on Day 3 and Day 7 respectively and a decrease of 2.96 %, 5.50% and 6.71 % on Day 15, Day 28 and Day 35 respectively compared to MCV values before transfusion (Table 4.22).

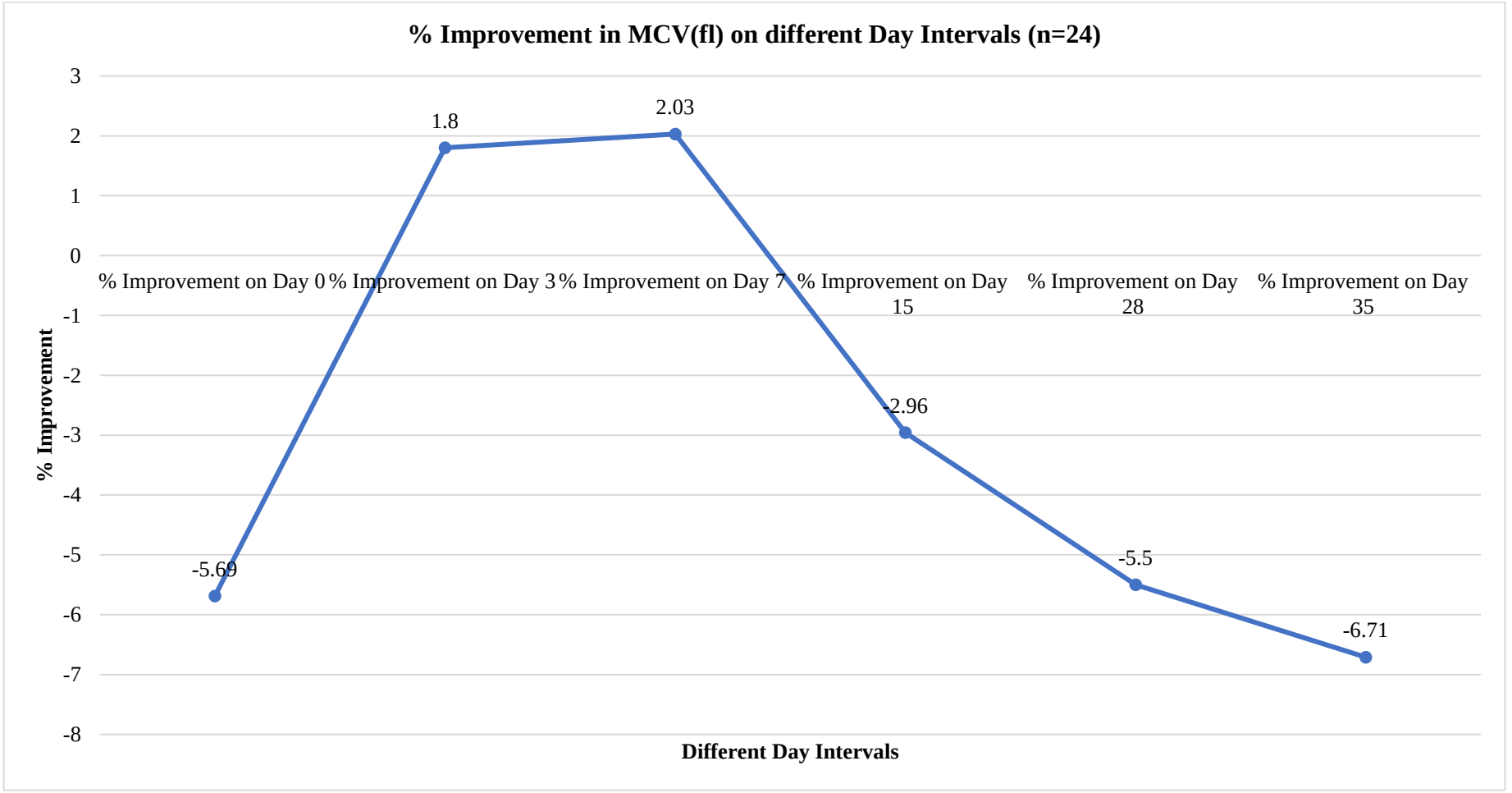


Figure 4.10 Percent Improvement in MCV (fl) in Anaemic Dogs on different Day Intervals (n=24)

Table 4.27: Mean ± SE MCV (fl) values in healthy control group and different anaemic groups at all the time intervals

Time Intervals	CKD (a) (n=6)	*E. canis (b)* (n=6)	*B. canis (c)* (n=6)	Pyometra(d) (n=6)	Healthy(e) (n=6)	Overall (a+b+c+d) (n=24)	F value	P value	S / NS
BT	70.10±2.19	71.39±2.66	74.81±3.00	67.70±2.49	64.36±1.36	71.00±1.33	2.65	0.056	NS
Day 0	70.10±1.91	68.63±4.56	69.82±6.93	59.28±2.90	64.93±2.26	66.96±2.30	1.20	0.332	NS
Day 3	68.87±1.23^{q}	89.69±11.64^{p}	68.93±5.71^{q}	61.63±2.80^{q}	64.86±4.68^{q}	72.28±3.80	3.02	0.036	*
Day 7	68.55±1.95	80.97±9.56	73.52±2.87	66.73±4.31	62.80±3.96	72.44±2.82	1.77	0.165	NS
Day 15	66.22±1.67^{qr}	69.58±1.43^{q}	78.60±0.63^{p}	61.21±2.29^{rs}	56.80±3.77^{s}	68.91±1.52	14.02	0.000	**
Day 28	65.30±0.93^{pqr}	69.61±3.62^{pq}	70.98±2.71^{p}	62.50±2.63^{qr}	60.66±2.71^{r}	67.10±1.43	2.76	0.049	*
Day 35	67.12±1.76	66.53±1.74	67.50±2.44	63.78±1.11	62.55±3.21	66.24±0.90	1.01	0.416	NS
Overall	68.04±0.66	73.78±2.50	72.03±1.50	63.27±1.07	62.43±1.23				
F value	1.197	1.81	0.93	1.18	0.77				
P value	0.330	0.125	0.485	0.338	0.595				
S / NS	NS	NS	NS	NS	NS				

Table F-value for Days is 2.37 (5 %) and 2.75 (1 %); Table F value for Groups is 3.36 (5 %) and 4.17 (1 %).
* $p \leq 0.05$; ** $p \leq 0.01$ and NS – Non Significant
Means bearing superscripts p,q,r,s in rows show statistical significance between the groups while means bearing superscripts a,b,c,d in columns show the statistical significance within the groups.

Comparative analysis of the MCV values between CKD, *E. canis*, *B. canis* and pyometra groups on Day 3, Day 15 and Day 28 revealed significant ($p \leq 0.01$) differences while non-significant differences in before treatment, Day 0, Day 7 and Day 35 when compared individually with the healthy control groups on respective days.

The MCV values in CKD, *E. canis*, *B. canis* and pyometra anaemic groups on before treatment was (70.10 ± 2.19, 71.39 ± 2.66, 74.81 ± 3.00 and 67.70 ± 2.49) and on Day 35 (67.12 ± 1.76, 66.53 ± 1.74, 67.50 ± 2.44 and 63.78 ± 1.11), respectively (Table 4.27) having overall percent decrement as 4.25, 6.81, 9.77 and 2.81 in respective anaemic groups. The highest percentage of difference in MCV was noted in *B. canis* (9.77) followed by *E. canis* (6.81), pyometra (5.78) and CKD (4.25) anaemic dogs (Table 4.23).

There was statistically non-significant decrease in the MCV values of CKD, *E. canis B. canis* and pyometra anaemic groups when Day 35 values of respective diseases were compared with before-treatment values.

Upon blood transfusion on Day 0, there was an increase in the MCV values indicating macrocytosis to compensate for anaemia. From Day 3 onwards till day Day 7, there was no further macrocytosis and the MCV graph is almost stable indicating that the regeneration of the RBC has been initiated. From Day 7 onwards since the haemopoietic system could have been geared up and hence due to active bone marrow there is a normocytic blood picture which revealed the progression in reducing anaemia.

Correlation between RDW and MCV:

The RDW in normal healthy dogs is 12-16 %. RDW along with mean corpuscular volume (MCV) is helpful in narrowing the cause of anemia. Table 4.15 represents RDW and MCV values in healthy control and anemic dogs. In the present study, the RDW in all the anemic dogs (n=24) was 19.62 ± 1.38 % against the healthy control dogs (n=6) value of 16.26 ± 0.85 % which implies increased RDW in anaemic dogs over healthy control dogs however the increase was statistically non-significant. The MCV in anaemic dogs (n=24) was 71.00 ± 1.33 (fl) against the healthy control dog value of 64.36 ± 1.36 (fl) and the increase was

statistically ($p \leq 0.01$) significant. High RDW can also occur with iron deficiency anemia however in those cases, the MCV is often low. Neiger *et al*., 2012 observed similar findings were noted in the present study where the Iron value was found to be decreased (98.52 ± 3.15 µg/dl)) over the healthy control group (104.12 ± 3.56) however the decrease was statistically insignificant.

Table 4.30 and Table 4.27 indicate RDW and MCV values in the healthy control group and different anaemic groups at all the time intervals. The highest RDW was noted in *E. canis* (23.81 ± 4.16) followed by *B. canis* (19.32 ± 1.45), pyometra (19.48 ± 2.88) and CKD (15.86 ± 0.83) anaemic dogs in before treatment group.

In *E. canis*, out of 6 anaemic dogs, 5 dogs had high RDW (%) and normal MCV (fl) while one dog (# Case no 3) had normal RDW and high MCV. The overall Serum Iron (µg/dl) in *E. canis* anaemic dogs was 97.40 ± 6.09 which was at the lower side of the normal range (94-122 µg/dl). The high RDW and normal MCV is associated with Early Iron, Vitamin B_{12} deficiency or folate deficiency and chronic liver diseases. The Iron content of *E. canis* anaemic dogs were within the lower normal range.

In *B. canis* out of 6 anaemic dogs, 5 had increased RDW and increased MCV while one dog had normal RDW and normal MCV. The overall Serum Iron (µg/dl) in *B. canis* anaemic dogs was 102.92 ± 2.87 µg/dl. The high RDW and high MCV in present study could be associated with immune mediated hemoyitc anaemia which have contributed with hypergammaglobulinemia.

In Pyometra, out of 6 anaemic dogs, 2 dogs had increased RDW and increased MCV. 2 dogs had increased RDW and normal MCV, while one dog had Normal RDW and Normal MCV; and one dog had Normal RDW and decreased MCV. The overall Serum Iron (µg/dl) in pyometra anaemic dogs was 91.50 ± 9.51 µg/dl. Since there was normal RDW and normal MCV; normal RDW and low MCV the anaemia of pyometra was of chronic nature where hemolysis is noted. The haematological picture of High RDW and high MCV is indicative of immune hemolytic anemia and toxicities. However, increased RDW and decreased MCV can be good indicators of hemorrhagic diseases (Neiger *et al*.,

2012) which aligns with the findings in overall pyometra anemic dogs in the present study where overall RDW was 19.48 ± 2.88 % while MCV was 67.70 ± 2.49 (fl).

In CKD, out of 6 dogs 3 had increased RDW and Normal MCV; 2 dogs had normal RDW and increased MCV while one dog had increased RDW and increased MCV. The overall Serum Iron (µg/dl) in CKD anaemic dogs was 100.84 ± 7.37 µg/dl which is associated with the high RDW and normal MCV. The other contributory factors listed were deficiency of vitamin B_{12} or folate or iron deficiency and in turn maturation of RBC.

At the end of the study, i.e. on Day 35 the overall RDW was decreased to 16.69 ± 0.45 % from before treatment value i.e. 19.62 ± 1.38 %. Similarly, the overall MCV value was decreased to 66.24 ± 0.90 from before the treatment value of 71.00 ± 1.33. Both the overall values i.e. RDW and MCV were found to be decreased at the end of the study from their before-treatment values.

4.2.5 MCH (pg) values in healthy control group and different anaemic groups at all the time intervals:

Table 4.28 indicates MCH (pg) values (Mean± SE) of dogs in different anaemic groups as well as healthy control groups at all the time intervals. The overall MCH values in before treatment, Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35 were 21.90 ± 0.45, 21.12 ± 0.68, 23.21 ± 1.32, 22.76 ± 0.86, 21.14 ± 0.41, 22.23 ± 0.45 and 22.36 ± 0.29, respectively.

There was an overall decrement of 3.60%, an increment of 5.97 %, an increment of 3.90 %, a decrement of 3.49 %, an increment of 1.47 % and an increment of 2.10 % respectively on Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35 respectively (Table 4.23).

Table 4.28: Mean ± SE MCH (pg) values in healthy control group and different anaemic groups at all the time intervals

Time Intervals	CKD (a) (n=6)	*E. canis (b)* (n=6)	*B. canis (c)* (n=6)	Pyometra(d) (n=6)	Healthy(e) (n=6)	Overall (a+b+c+d) (n=24)	F value	P value	S / NS
BT	21.88±0.98	23.43±0.51^{b}	20.39±0.40	21.89±1.21	20.80±1.09	21.90±0.45	1.72	0.175	NS
Day 0	22.70±0.94	22.83±1.13^{b}	20.12±1.81	18.79±0.85	20.66±1.15	21.12±0.68	1.99	0.126	NS
Day 3	22.49±0.68^{q}	30.36±3.77^{ap}	19.35±0.92^{q}	20.62±1.31^{q}	18.70±1.39^{q}	23.21±1.32	5.81	0.001	**
Day 7	22.59±1.11^{pq}	26.78±2.56^{abp}	20.71±0.49^{q}	20.94±0.98^{q}	18.86±1.35^{q}	22.76±0.86	4.11	0.010	*
Day 15	22.84±0.67^{p}	22.30±0.46^{bpq}	20.12±0.37^{qr}	19.27±0.75^{r}	18.94±1.26^{r}	21.14±0.41	5.32	0.003	**
Day 28	23.06±0.26^{p}	23.03±0.87^{bp}	21.79±0.96^{p}	21.01±1.15^{p}	18.17±0.92^{q}	22.23±0.45	5.11	0.003	**
Day 35	22.27±0.51^{p}	22.66±0.67^{bp}	23.16±0.49^{p}	21.35±0.43^{p}	18.68±0.71^{q}	22.36±0.29	9.45	0.000	**
Overall	22.55±0.28	24.49±0.78	20.81±0.37	20.56±0.38	19.26±0.43				
F value	0.24	2.63	1.94	1.25	0.81				
P value	0.960	0.032	0.101	0.302	0.568				
S / NS	NS	*	NS	NS	NS				

Table F-value for Days is 2.37 (5 %) and 2.75 (1 %); Table F value for Groups is 3.36 (5 %) and 4.17 (1 %).
* $p \leq 0.05$; ** $p \leq 0.01$ and NS – Non Significant
Means bearing superscripts p,q,r,s in rows show statistical significance between the groups while means bearing superscripts a,b,c,d in columns show the statistical significance within the groups.

Comparative analysis of the MCH values between CKD, *E. canis*, *B. canis* and pyometra groups on Day 3, Day 7, Day 15, Day 28 and Day 35 revealed significant ($p \leq 0.01$) differences while non-significant differences in before treatment and Day 0 when compared individually with the healthy control groups on respective days.

The MCH values in CKD, *E. canis*, *B. canis* and pyometra anaemic groups on before treatment were (21.88 ± 0.98, 23.43 ± 0.51, 20.39 ± 0.40 and 21.89 ± 1.21) and on Day 35 (22.27 ± 0.51, 22.66 ± 0.67, 23.16 ± 0.49 and 21.35 ± 0.43), respectively having overall percent increment as 1.79, percent decrement 3.31, percent increment 13.59 and percent decrement 2.50 in respective anaemic groups (Table 4.23).

There was statistically non-significant difference in the MCH values of CKD, *B. canis* and pyometra anaemic groups while significant ($p \leq 0.05$) increase in the *E. canis* group when Day 35 values of respective diseases were compared with before treatment values.

4.2.6 MCHC (g/dl) in healthy control group and different anaemic groups at all the time intervals:

Table 4.29 (Figure 4.11) indicates MCHC (g/dl) values (Mean± SE) of dogs in different anaemic groups as well as healthy control group at all the time intervals. The overall MCHC values in before treatment, Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35 were 31.02 ± 0.73, 31.85 ± 0.78, 32.29 ± 0.89, 31.61 ± 0.70, 30.93 ± 0.75, 33.27 ± 0.61 and 33.79 ± 0.25, respectively with percent increment in MCHC as 2.67, 4.10, 1.90, (Decrement 0.27),7.24 and 8.94 on Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35, respectively (Table 4.22).

Comparative analysis of the MCHC values between CKD, *E. canis*, *B. canis* and pyometra groups on before treatment, Day 15, Day 28 and Day 35 revealed significant ($p \leq 0.01$) differences while non-significant differences in Day 0, Day 3 and Day 7 when compared individually with the healthy control groups on respective days.

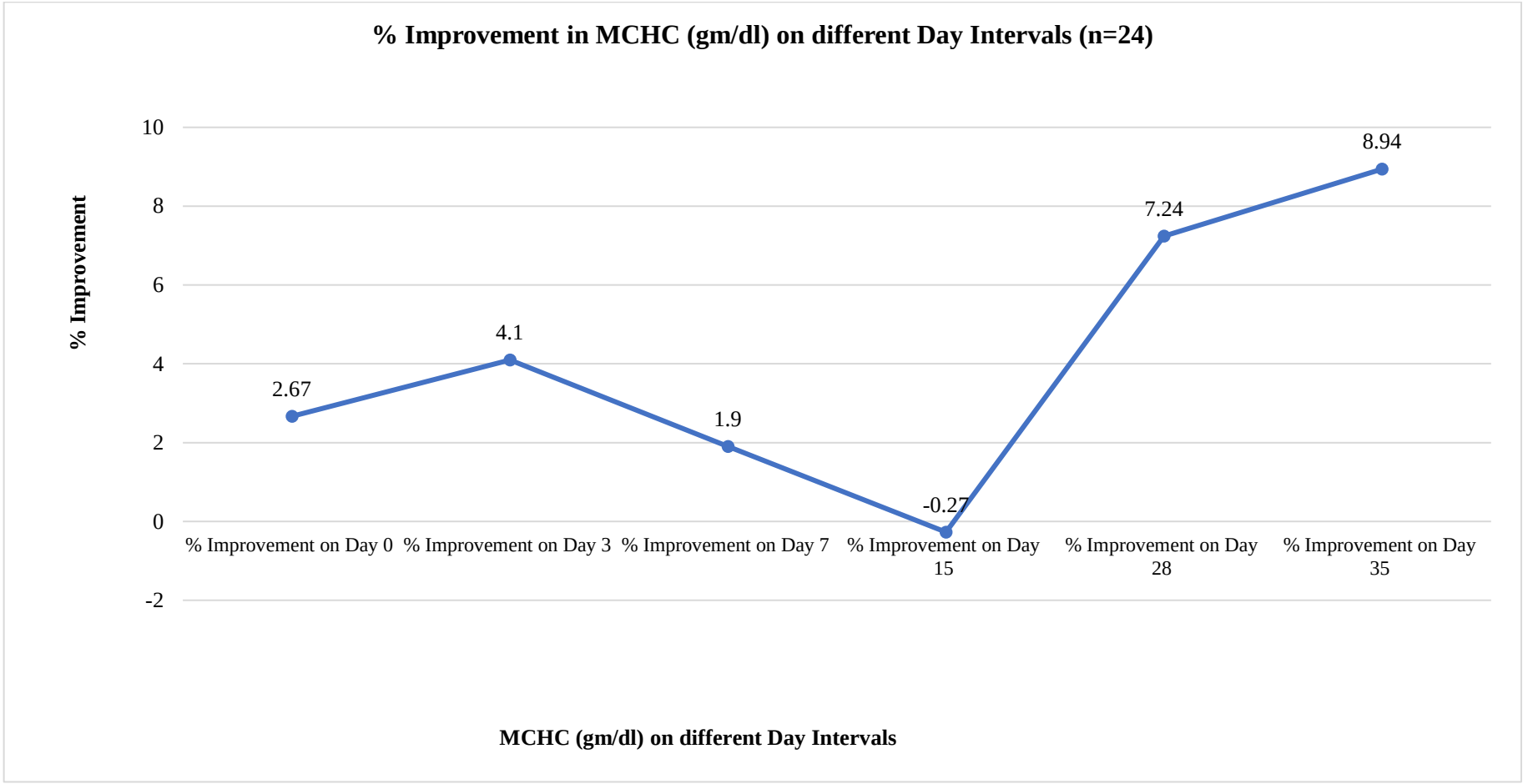


Figure 4.11 Percent Improvement in MCHC (gm/dl) in Anaemic dogs on different Day Intervals (n=24)

Table 4.29: Mean ± SE MCHC (g/dl) values in healthy control group and different anaemic groups at all the time intervals

Time Intervals	CKD (a) (n=6)	*E. canis (b)* (n=6)	*B. canis (c)* (n=6)	Pyometra(d) (n=6)	Healthy(e) (n=6)	Overall (a+b+c+d) (n=24)	F value	P value	S / NS
BT	31.37±1.68^{p}	32.95±0.81^{p}	27.47±1.17^{bcq}	32.28±1.09^{p}	32.23±1.19^{p}	31.02±0.73	3.20	0.029	*
Day 0	32.39±1.08	33.53±1.38	29.59±2.21^{bc}	31.86±1.29	31.85±1.52	31.85±0.78	0.85	0.503	NS
Day 3	32.65±0.71	34.45±2.63	28.70±1.64^{bc}	33.34±0.87	29.11±1.72	32.29±0.89	2.41	0.075	NS
Day 7	32.97±1.44	33.40±0.78	28.49±1.69^{bc}	31.56±0.77	30.24±1.67	31.61±0.70	2.26	0.090	NS
Day 15	34.51±0.72^{p}	32.10±0.78^{q}	25.61±0.41^{cr}	31.51±0.67^{q}	33.35±0.40^{pq}	30.93±0.75	30.96	0.000	**
Day 28	35.38±0.91^{p}	33.21±0.66^{pqr}	30.88±1.56^{abqr}	33.58±1.02^{pq}	30.06±1.23^{r}	33.27±0.61	3.67	0.017	*
Day 35	33.21±0.41^{p}	34.05±0.29^{p}	34.42±0.70^{ap}	33.47±0.47^{p}	30.21±1.62^{q}	33.79±0.25	3.84	0.014	*
Overall	33.22±0.42	33.39±0.46	29.31±0.65	32.52±0.35	31.01±0.54				
F value	1.55	0.35	3.65	1.00	1.12				
P value	0.189	0.899	0.006	0.365	0.365				
S / NS	NS	NS	**	NS	NS				

Table F-value for Days is 2.37 (5 %) and 2.75 (1 %); Table F value for Groups is 3.36 (5 %) and 4.17 (1 %).
* $p \leq 0.05$; ** $p \leq 0.01$ and NS – Non Significant
Means bearing superscripts p,q,r,s in rows show statistical significance between the groups while means bearing superscripts a,b,c,d in columns show the statistical significance within the groups.

Table 4.30 : Mean ± SE RDW (%) in the healthy control group and different anaemic groups at all the time intervals

Time Intervals	CKD (a) (n=6)	*E. canis (b)* (n=6)	*B. canis (c)* (n=6)	Pyometra(d) (n=6)	Healthy(e) (n=6)	Overall (a+b+c+d) (n=24)	F value	P value	S / NS
BT	15.86±0.83	23.81±4.16	19.32±1.45	19.48±2.88	16.26±0.85	19.62±1.38	1.74	0.171	NS
0 Day	15.28±0.86^{r}	20.55±1.28^{p}	19.26±1.48^{pq}	16.13±0.64^{pr}	15.70±0.95^{r}	17.81±0.69	4.71	0.005	**
3rd Days	15.25±0.82^{q}	24.56±2.66^{p}	17.81±0.75^{q}	17.44±1.44^{q}	15.08±1.12^{q}	18.77±1.05	6.34	0.001	**
7th Days	16.36±1.22	18.61±0.74	18.93±1.16	17.38±1.18	15.70±1.13	17.83±0.55	1.59	0.206	NS
15th Days	15.51±0.91	16.63±0.55	18.26±1.66	15.95±0.70	18.66±1.74	16.59±0.54	1.31	0.291	NS
28th Days	15.90±0.92	19.86±2.27	17.60±1.23	16.73±0.91	14.88±0.60	17.53±0.74	2.04	0.118	NS
35th Days	16.25±1.33	17.16±0.37	16.56±1.08	16.78±0.69	15.73±0.67	16.69±0.45	0.36	0.831	NS
Overall	15.78±0.36	20.17±0.87	18.25±0.48	17.13±0.52	16.00±0.42				
F value	0.19	2.05	0.60	0.69	1.38				
P value	0.976	0.084	0.724	0.656	0.248				
Result	NS	NS	NS	NS	NS				

Table F-value for Days is 2.37 (5 %) and 2.75 (1 %); Table F value for Groups is 3.36 (5 %) and 4.17 (1 %).

* $p \leq 0.05$; ** $p \leq 0.01$ and NS – Non Significant

Means bearing superscripts p,q,r,s in rows show statistical significance between the groups while means bearing superscripts a,b,c,d in columns show the statistical significance within the groups.

The MCHC values in CKD, *E. canis*, *B. canis* and pyometra anaemic groups on before treatment were (31.37 ± 1.68, 32.95 ± 0.81, 27.47 ± 1.17 and 32.28 ± 1.09) and on Day 35 (33.21 ± 0.41, 34.05 ± 0.29, 34.42 ± 0.70 and 33.47 ± 0.47), respectively having overall percent improvement as 5.86, 3.35, 25.32 and 3.70 in respective anaemic groups. The highest percentage of improvement in MCHC was noted in *B. canis* (25.32) followed by CKD (5.86), pyometra (3.70) and *E. canis* (3.35) anaemic dogs.

There was a non-significant increase in the MCHC values of CKD, *E. canis* and pyometra anaemic groups while significant ($p \leq 0.05$) increase in *B. canis* group when Day 35 values of respective diseases were compared with before treatment values.

The blood transfusion on Day 0 could have shown an immediate effect on the MCHC values till Day 3. From day 3 onwards the MCHC values started declining till Day 15 might be due to the complete utilization of transfereed red cells. However, from Day 15 onwards the the MCHC showed a gradual increase till Day 35 of the study. This revealed that the dog's body has geared up due to and activation of bone marrow and also the replenishment of Iron stores due to Iron injections.

Vetrimalai (1992) studied that the mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration values did not alter significantly after blood transfusion. The mean corpuscular volume and mean corpuscular haemoglobin values observed in the present study were in agreement with the findings of Vetrimalai (1992) and Parthasarathy *et al.* (1985). It is possible since both the denominator and numerator (Haemoglobin, packed Cell Volume and red blood cell) are increasing after blood transfusion, the values of erythrocyte indices do not differ significantly.

4.2.7 Total Leucocyte Count (TLC) ($x10^3$/µl) in healthy control group and different anaemic groups at all the time intervals:

Table 4.31 indicates the Total Leucocyte Count (Mean± SE) ($x10^3$/µl) of dogs in different anaemic groups as well as healthy control group at all the time intervals.

Table 4.31 : Mean ± SE Total WBC (x 10^3/µl) count in healthy control group and different anaemic groups at all the time intervals

Time Intervals	CKD (a) (n=6)	*E. canis (b)* (n=6)	*B. canis (c)* (n=6)	Pyometra(d) (n=6)	Healthy(e) (n=6)	Overall (a+b+c+d) (n=24)	F value	P value	S / NS
BT	13.94±4.60^{pq}	30.59±11.75^{p}	5.78±1.79^{abcq}	27.47±5.52^{p}	11.65±1.59^{pq}	19.45±3.86	2.90	0.042	*
Day 0	17.70±3.86	38.91±18.74	4.35±0.12^{c}	22.53±5.30	12.58±1.23	20.88±5.30	2.10	0.110	NS
Day 3	20.09±3.08^{pqr}	41.67±16.48^{p}	4.61±0.12^{bcr}	30.47±5.20^{pq}	13.21±2.02^{qr}	24.22±4.98	3.37	0.024	*
Day 7	17.14±2.33	16.47±8.49	5.87±0.75^{abc}	22.84±1.36	14.50±1.54	15.59±2.45	2.29	0.087	NS
Day 15	20.04±0.66^{pq}	12.69±4.93^{qr}	5.97±0.20^{abcr}	23.03±5.20^{p}	12.49±1.82^{qr}	15.44±2.18	4.13	0.010	*
Day 28	15.77±2.19^{q}	7.72±0.30^{r}	6.86±0.16^{abr}	26.07±1.93^{p}	13.04±1.37^{q}	14.11±1.75	28.34	0.000	**
Day 35	11.35±0.82	8.98±1.41	8.19±0.43^{a}	11.79±1.63	12.93±1.46	10.08±0.64	2.56	0.063	NS
Overall	16.58±1.10	22.44±4.39	5.95±0.33	23.46±1.69	12.92±0.57				
F value	1.24	1.67	2.94	2.02	0.29				
P value	0.307	0.155	0.019	0.088	0.936				
S / NS	NS	NS	**	NS	NS				

Table F-value for Days is 2.37 (5 %) and 2.75 (1 %); Table F value for Groups is 3.36 (5 %) and 4.17 (1 %).

* $p \leq 0.05$; ** $p \leq 0.01$ and NS – Non Significant

Means bearing superscripts p,q,r,s in rows show statistical significance between the groups while means bearing superscripts a,b,c,d in columns show the statistical significance within the groups.

The overall total leucocyte count in before treatment, Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35 were 19.45 ± 3.86, 20.88 ± 5.30, 24.22 ± 4.98, 15.59 ± 2.45, 15.44 ± 2.18, 14.11 ± 1.75 and 10.08 ± 0.64, respectively with percent increment in total leucocyte as 7.35, 54.51 on Day 0 and Day 3 respectively and later on percent decrement as 19.86, 20.63, 27.43 and 48.16 on Day 7, Day 15, Day 21 and Day 35 respectively (Table12).

Comparative analysis of the total leucocyte count between CKD, *E. canis*, *B. canis* and pyometra groups on before treatment, Day 3, Day 15 and Day 28 revealed significant ($p \leq 0.05$) differences while the non-significant difference on Day 0, Day 7 and Day 35 when compared individually with the healthy control groups on respective days.

The total leucocyte count in CKD, *E. canis*, *B. canis* and pyometra anaemic groups on before treatment was (13.94 ± 4.60, 30.59 ± 11.75, 5.78 ± 1.79 and 27.47 ± 5.52) and on Day 35 (11.35 ± 0.82, 8.98 ± 1.41, 8.19 ± 0.43 and 11.79 ± 1.63), respectively having overall percent difference as 18.57 (decrement), 70.64 (decrement), 47.71 (increment) and 57.08 (decrement) in respective anaemic groups. The highest percentage of improvement (decrease) in total leucocyte was noted in *E. canis* (-70.64), followed by pyometra (-57.08), CKD (-18.57) and *B. canis* (41.71) anaemic dogs.

There was a statistically non-significant difference in the Total leucocyte values of CKD, *E. canis* and pyometra anaemic groups while a significant ($p \leq 0.05$) increase in *B. canis* group when Day 35 values of respective diseases were compared with before treatment values.

Rajan and Nagarajan (1980) examined the rise in white blood cell count following blood transfusion. On the third day following a blood transfusion, Vetrimalai (1992) observed an increase in the number of white blood cells and suggested that this was caused by a defence mechanism. Following a blood transfusion, Keskar *et al.* (1985) observed a drop in the white blood cell count. According to Coles (1986), receiving antibiotics causes a drop in leukocyte counts.

In the present study, on the third day following transfusion, a slight non-significant increase in white blood cell level was seen, but no such substantial increase in white blood cell count was observed in subsequent treatment days which could be due to the administration of antibiotics.

4.2.8 Leucogram in healthy control group and different anaemic groups at all the time intervals:

Table 4.32, Table 4.33, Table 4.34 and Table 4.35 indicate Leucogram containing neutrophil (%), eosinophil (%), lymphocyte (%) and monocyte (%) (Mean ± SE) of dogs in different anaemic groups as well as healthy control group at all the time intervals.

Since there were frequent deviations of leucocytic cells during the study the exact percentage of increment or decrement could not be determined. However, the overall percent change in neutrophil count in all anaemic dogs was 3.22,1.98, 3.56, 3.50, 1.75 and 3.45 on Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35, respectively (Table 4.22).

Comparative analysis between CKD, *E. canis*, *B. canis* and pyometra groups on different day intervals revealed as follows: For neutrophil count significant difference in before treatment, Day 0 and Day 3 values while non-significant difference on Day 7, Day 15, Day 28 and Day 35 when compared individually with the healthy control groups on respective days (Table 4.32)

For eosinophils, non-significant difference throughout all the days (Tabel 22). For lymphocytes, significant difference on Day 0, Day 3 and Day 28 while non-significant difference on before treatment, Day 7, Day 15 and Day 35 (Table 4.34). For monocytes, statistically significant difference throughout all the days when compared individually with the healthy control groups on respective days (Table 4.35). No, basophil was detected throughout the study intervals.

There was a statistically non-significant difference in the whole leucogram, i.e. neutrophil, eosinophil, lymphocyte and monocyte values of CKD, *E. canis* and pyometra anaemic groups except statistically significant neutrophilia in the *B. canis* group when Day 35 values of respective disease were compared with before treatment values.

Table 4.32: Mean ± SE Neutrophil (%) count in healthy control group and different anaemic groups at all the time intervals

Time Intervals	CKD (a) (n=6)	*E. canis (b)* (n=6)	*B. canis (c)* (n=6)	Pyometra(d) (n=6)	Healthy(e) (n=6)	Overall (a+b+c+d) (n=24)	F value	P value	S / NS
BT	80.00±2.23^{p}	81.33±4.42^{p}	58.00±3.43^{cq}	75.66±2.52^{p}	74.66±2.02^{p}	73.75±2.47	9.30	0.000	**
Day 0	80.50±4.08^{p}	79.00±5.10^{p}	62.33±5.35^{bcq}	82.66±2.90^{p}	76.00±1.77^{p}	76.13±2.68	3.91	0.013	*
Day 3	79.16±3.43^{p}	77.50±4.38^{p}	62.16±3.61^{bcq}	82.00±2.87^{p}	77.33±1.87^{p}	75.21±2.33	5.37	0.002	*
Day 7	80.16±2.33	77.33±4.41	68.16±4.37^{abc}	79.83±2.73	73.33±3.02	76.38±1.96	2.09	0.111	NS
Day 15	81.00±3.20	76.83±3.26	67.66±2.48^{abc}	79.83±2.27	72.33±5.55	76.33±1.71	2.42	0.074	NS
Day 28	76.50±2.55	75.00±2.51	70.66±1.70^{ab}	78.00±2.55	71.50±3.84	75.04±1.24	1.35	0.279	NS
Day 35	80.00±2.39	70.83±3.63	74.00±1.12^{a}	80.33±2.31	73.50±2.86	76.29±1.45	2.64	0.057	NS
Overall	79.62±1.06	76.83±1.49	66.14±1.45	79.76±0.97	74.10±1.17				
F value	0.25	0.66	2.60	0.82	0.39				
P value	0.955	0.680	0.034	0.559	0.874				
S / NS	NS	NS	*	NS	NS				

Table F-value for Days is 2.37 (5 %) and 2.75 (1 %); Table F value for Groups is 3.36 (5 %) and 4.17 (1 %).
* $p \leq 0.05$; ** $p \leq 0.01$ and NS – Non Significant
Means bearing superscripts p,q,r,s in rows show statistical significance between the groups while means bearing superscripts a,b,c,d in columns show the statistical significance within the groups.

Table 4.33: Mean ± SE Eosinophil (%) count in the healthy control group and different anaemic groups at all the time intervals

Time Intervals	CKD (a) (n=6)	*E. canis (b)* (n=6)	*B. canis (c)* (n=6)	Pyometra(d) (n=6)	Healthy(e) (n=6)	Overall (a+b+c+d) (n=24)	F value	P value	S / NS
BT	3.83±2.45	3.66±2.49	2.33±0.76	4.66±2.41	2.00±0.00	3.63±1.02	0.32	0.856	NS
Day 0	1.00±0.25	2.16±0.30	4.50±1.05	5.33±2.94	2.33±0.42	3.25±0.82	1.58	0.209	NS
Day 3	1.33±0.42	2.50±1.17	2.83±0.70	5.66±3.93	1.83±0.16	3.08±1.03	0.80	0.531	NS
Day 7	1.00±0.44	1.83±0.65	2.83±0.60	1.50±0.56	2.50±0.34	1.79±0.30	1.93	0.136	NS
Day 15	1.66±0.42	2.00±0.63	3.50±0.22	2.83±1.10	2.33±0.33	2.50±0.35	1.32	0.289	NS
Day 28	2.16±0.47	5.50±3.53	3.33±0.42	1.50±0.71	1.83±0.16	3.13±0.91	0.98	0.435	NS
Day 35	2.33±0.71	8.16±5.61	3.50±0.22	1.50±0.61	2.33±0.42	3.88±1.43	1.08	0.383	NS
Overall	1.90±0.39	3.69±1.02	3.26±0.24	3.29±0.80	2.17±0.11				
F value	0.94	0.73	1.18	0.77	0.81				
P value	0.475	0.621	0.335	0.595	0.568				
S / NS	NS	NS	NS	NS	NS				

Table F-value for Days is 2.37 (5 %) and 2.75 (1 %); Table F value for Groups is 3.36 (5 %) and 4.17 (1 %).
* $p \leq 0.05$; ** $p \leq 0.01$ and NS – Non Significant
Means bearing superscripts p,q,r,s in rows show statistical significance between the groups while means bearing superscripts a,b,c,d in columns show the statistical significance within the groups.

Table 4.34: Mean ± SE Lymphocyte (%) count in the healthy control group and different anaemic groups at all the time intervals

Time Intervals	CKD (a) (n=6)	*E. canis (b)* (n=6)	*B. canis (c)* (n=6)	Pyometra(d) (n=6)	Healthy(e) (n=6)	Overall (a+b+c+d) (n=24)	F value	P value	S / NS
BT	14.66±3.75	11.16±3.99	25.83±3.97	17.83±4.49	20.16±1.64	17.38±2.21	2.25	0.092	NS
Day 0	17.33±4.03^{p}	7.50±1.83^{q}	19.66±4.12^{p}	9.83±3.48^{pq}	19.50±1.23^{p}	13.58±1.94	3.23	0.028	*
Day 3	18.50±3.34^{pq}	12.83±4.16^{q}	23.33±1.28^{p}	10.00±2.63^{q}	18.16±1.62^{pq}	16.17±1.78	3.43	0.022	*
Day 7	17.50±2.48	11.16±2.16	19.66±2.24	15.16±3.42	21.66±2.39	15.88±1.39	2.49	0.068	NS
Day 15	16.33±2.90	16.33±1.45	17.33±1.90	15.66±2.99	23.00±5.68	16.42±1.13	0.81	0.528	NS
Day 28	19.83±2.24^{pq}	12.00±2.40^{q}	16.16±1.53^{pq}	14.16±3.14^{q}	24.00±3.58^{p}	15.54±1.27	3.14	0.031	*
Day 35	16.66±1.72	15.83±3.79	15.16±1.07	17.00±2.56	21.83±2.62	16.27±1.18	1.08	0.385	NS
Overall	17.26±1.08	12.40±1.14	19.60±1.06	14.24±1.24	21.19±1.10				
F value	0.29	1.00	2.23	0.92	0.44				
P value	0.935	0.439	0.062	0.486	0.840				
S / NS	NS	NS	NS	NS	NS				

Table F-value for Days is 2.37 (5 %) and 2.75 (1 %); Table F value for Groups is 3.36 (5 %) and 4.17 (1 %).
* $p \leq 0.05$; ** $p \leq 0.01$ and NS – Non Significant
Means bearing superscripts p,q,r,s in rows show statistical significance between the groups while means bearing superscripts a,b,c,d in columns show the statistical significance within the groups.

Table 4.35 : Mean ± SE Monocyte (%) count in healthy control group and different anaemic groups at all the time intervals

Time Intervals	CKD (a) (n=6)	*E. canis (b)* (n=6)	*B. canis (c)* (n=6)	Pyometra(d) (n=6)	Healthy(e) (n=6)	Overall (a+b+c+d) (n=24)	F value	P value	S / NS
BT	1.50±0.34^{q}	3.83±2.84^{q}	13.83±4.86^{p}	1.83±0.40^{q}	3.16±0.60^{q}	5.25±1.69	4.04	0.011	*
Day 0	1.16±0.16^{q}	11.33±4.44^{p}	13.50±2.12^{p}	2.16±0.79^{q}	2.16±0.47^{q}	7.04±1.62	6.84	0.000	**
Day 3	1.00±0.00^{q}	7.16±4.07^{pq}	11.66±2.15^{p}	2.33±0.61^{q}	2.66±0.42^{q}	5.54±1.39	4.46	0.007	**
Day 7	1.33±0.21^{q}	9.66±2.78^{p}	9.33±2.71^{p}	1.83±0.65^{q}	2.50±0.76^{q}	5.54±1.24	5.41	0.002	**
Day 15	1.00±0.00^{r}	4.83±2.13^{q}	11.50±0.71^{p}	1.66±0.33^{r}	2.33±0.33^{qr}	4.75±1.02	17.40	0.000	**
Day 28	1.50±0.34^{q}	7.50±1.76^{p}	9.83±0.47^{p}	1.33±0.55^{q}	2.66±0.42^{q}	5.04±0.90	18.92	0.000	**
Day 35	1.00±0.00^{q}	5.16±1.74^{p}	7.33±0.33^{p}	1.16±0.16^{q}	2.33±0.21^{q}	3.67±0.70	11.86	0.000	**
Overall	1.21±0.08	7.07±1.12	11.00±0.91	1.76±0.20	2.55±0.18				
F value	1.21	0.82	0.92	0.59	0.45				
P value	0.323	0.561	0.489	0.729	0.839				
S / NS	NS	NS	NS	NS	NS				

Table F-value for Days is 2.37 (5 %) and 2.75 (1 %); Table F value for Groups is 3.36 (5 %) and 4.17 (1 %).
* $p \leq 0.05$; ** $p \leq 0.01$ and NS – Non Significant
Means bearing superscripts p,q,r,s in rows show statistical significance between the groups while means bearing superscripts a,b,c,d in columns show the statistical significance within the groups.

Before treatment values in *B. canis* (13.83 ± 4.86) group, revealed marked monocytosis which on blood transfusion and disease-specific treatment decreased to 7.33±0.33 on Day 35 however which was also not at par with the healthy control group 2.33 ±0.21 (Table 4.35).

4.2.9 Platelet (x 10^3/µl) in healthy control group and different anaemic groups at all the time intervals:

Table 4.36 (Figure 4.12) indicates platelet values (Mean± SE) of dogs in different anaemic groups as well as healthy control group at all the time intervals. The overall platelet values in before treatment, Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35 were 125.42 ± 20.68, 147.12 ± 22.56, 160.64 ± 20.19, 190.42 ± 21.03, 204.03 ± 13.62, 258.83 ± 14.08 and 259.15 ± 11.66, respectively with percent increment in platelet as 17.30, 28.08, 51.82, 62.67, 106.37 and 106.63 on Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35, respectively (Table 4.22).

Comparative analysis of the platelet values between CKD, *E. canis*, *B. canis* and pyometra groups on BT, Day 0, 3, 7,15, 28 and 35 revealed significant ($p \leq 0.05$) differences when compared individually with the healthy control groups on respective days. This showed that there was a significant ($p \leq 0.01$) decrease in the platelet value in all the anaemic groups than the Healthy control group.

The platelet values in CKD, *E. canis*, *B. canis* and pyometra anaemic groups on before treatment was (148.18 ± 36.30, 112.58 ± 29.86, 39.16 ± 6.79 and 201.75 ± 53.11) and on Day 35 (273.85 ± 16.32, 246.83 ± 30.89, 246.66 ± 16.37 and 269.25 ±29.81), respectively having overall percent improvement (Increase) as 84.81, 19.25, 529.78 and 33.46 in respective anaemic groups. Marked thrombocytopenia was noticed in *B. canis* followed by *E. canis* and CKD and anaemic groups while moderate thrombocytopenia was recorded in the pyometra group in the before-treatment group. The highest percentage of improvement (Increase) in platelets was noted in *B. canis* (529.78) followed by *E. canis* (119.25), CKD (84.81) and pyometra (33.46) anaemic dogs (Table 4.23)

There was statistically significant increase in the platelet count of CKD, *E. canis* and *B. canis* anaemic groups while non-significant increase in the pyometra group when Day 35 values of respective disease were compared with before

Table 4.36 : Mean ± SE Platelet (x 10^3/µl) count in healthy control group and different anaemic groups at all the time intervals

Time Intervals	CKD (a) (n=6)	*E. canis (b)* (n=6)	*B. canis (c)* (n=6)	Pyometra(d) (n=6)	Healthy(e) (n=6)	Overall (a+b+c+d) (n=24)	F value	P value	S / NS
BT	148.18±36.30^{cpq}	112.58±29.86^{dqr}	39.16±6.79^{fr}	201.75±53.11^{pq}	238.83±20.05^{p}	125.42±20.68	5.52	0.002	**
Day 0	145.98±23.82^{cqr}	136.66±38.52^{cdqr}	61.67±7.02e^{fr}	244.16±62.42^{pq}	315.66±38.15^{p}	147.12±22.56	6.63	0.000	**
Day 3	151.96±21.03^{cq}	132.33±34.87^{cdq}	84.96±12.36^{deq}	273.28±42.81^{p}	286.21±31.84^{p}	160.64±20.19	8.56	0.000	**
Day 7	193.93±26.53^{bcqr}	164.16±14.27^{bcdr}	119.53±17.32^{cdr}	284.03±63.49^{pq}	316.05±36.99^{p}	190.42±21.03	5.12	0.003	**
Day 15	229.54±22.88^{abq}	213.00±31.33^{abcq}	150.78±21.43^{cq}	222.77±25.76^{q}	336.00±32.67^{p}	204.03±13.62	6.03	0.001	**
Day 28	280.05±10.33^{ap}	267.66±28.16^{apq}	192.08±5.13^{bq}	295.50±38.14^{p}	335.66±33.27^{p}	258.83±14.08	3.96	0.012	*
Day 35	273.85±16.32^{aq}	246.83±30.89^{abq}	246.66±16.37^{aq}	269.25±29.81^{q}	385.33±22.25^{p}	259.15±11.66	5.81	0.001	**
Overall	203.36±11.84	181.89±13.82	127.84±11.72	255.82±17.21	316.25±12.80				
F value	6.11	3.96	29.48	0.52	2.09				
P value	0.000	0.003	0.000	0.785	0.078				
S / NS	**	**	**	NS	NS				

Table F-value for Days is 2.37 (5 %) and 2.75 (1 %); Table F value for Groups is 3.36 (5 %) and 4.17 (1 %).
* $p \leq 0.05$; ** $p \leq 0.01$ and NS – Non Significant
Means bearing superscripts p,q,r,s in rows show statistical significance between the groups while means bearing superscripts a,b,c,d in columns show the statistical significance within the groups.

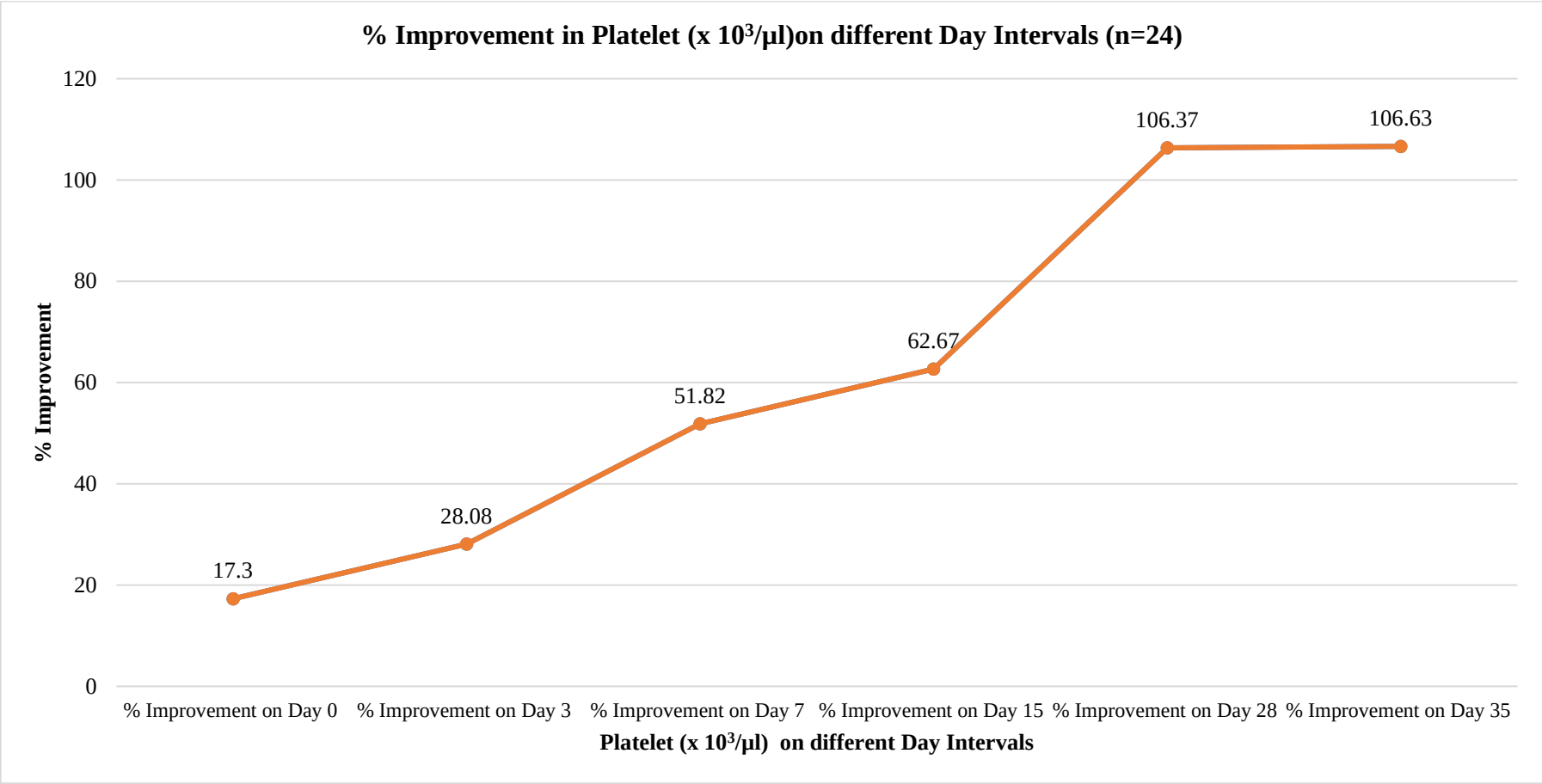


Figure 4.12 Percent Improvement in Platelet (x 10^3/µl) in anaemic dogs on different Day Intervals (n=24)

treatment values.

In summary, the analysis revealed a significant increase in platelet counts from the beginning to Day 35 in the groups of patients with chronic renal disease, *Ehrlichia canis* infection, *Babesia canis* infection, and pyometra. The results indicate that both the administration of whole blood transfusion, injections of corticosteroids at tapering doses and the use of targeted medicine, had a highly favorable effect on platelet levels, leading to a substantial and meaningful enhancement in the patient's clinical condition.

4.2.10 Erythrocyte Sedimentation Rate (ESR) (mm/hr) values in healthy control group and different anaemic groups at all the time intervals

Table 4.37 (Figure 4.13) indicates ESR values (Mean± SE) of dogs in different anaemic groups as well as healthy control groups at all the time intervals. The overall ESR values in before treatment, Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35 were 41.96 ± 2.87, 31.38 ± 2.21, 25.75 ± 1.71, 24.00 ± 3.07, 16.83 ± 1.18, 13.46 ± 1.00 and 8.88 ± 0.76, respectively with percent decrement in ESR as 25.22, 38.63, 42.80, 59.88, 67.92 and 78.85, respectively (Table 4.22).

Comparative analysis of the ESR values between CKD, *E. canis*, *B. canis* and pyometra groups on BT, Day 0, 3, 7,15, 28 and 35 revealed significant ($p \leq 0.05$) differences when compared individually with the healthy control groups on respective days, except Day 35 where the non-significant difference was noted. This showed that the anaemic dogs had a significantly higher ESR value than all the anaemic groups on all the day intervals except on Day 35. This also implies that on Day 35 the ESR values were at par with the healthy control group values indicating successful treatment efficacy.

The ESR values in CKD, *E. canis*, *B. canis* and pyometra anaemic groups on before treatment were (43.00 ± 4.69, 40.00 ± 5.64, 43.00 ± 7.18 and 41.83 ± 6.72) and on Day 35 (9.33 ± 1.85, 8.33 ± 1.33, 8.66 ± 1.17 and 9.16 ± 1.95), respectively having overall percent decrement as 78.30, 79.17, 79.84 and 78.09 in respective anaemic groups. The highest percentage of improvement i.e. decrease in ESR was noted in *B. canis* (79.84) followed by *E. canis* (79.17), CKD (78.30) and pyometra (78.09) in anaemic dogs.

Table 4.37: Mean ± SE ESR (mm/ hr.) in the healthy control group and different anaemic groups at all the time intervals

Time Intervals	CKD (a) (n=6)	*E. canis (b)* (n=6)	*B. canis (c)* (n=6)	Pyometra(d) (n=6)	Healthy(e) (n=6)	Overall (a+b+c+d) (n=24)	F value	P value	S / NS
BT	43.00±4.69^{ap}	40.00±5.64^{ap}	43.00±7.18^{ap}	41.83±6.72^{ap}	4.50±0.22^{q}	41.96±2.87	9.35	0.000	**
Day 0	31.16±3.76^{abp}	33.00±4.88^{abp}	29.50±4.35^{bp}	31.83±5.61^{abp}	5.33±0.33^{q}	31.38±2.21	7.73	0.000	**
Day 3	25.33±2.61^{bcp}	26.16±3.69^{bcp}	25.16±2.35^{bcp}	26.33±5.22^{bcp}	5.33±0.55^{q}	25.75±1.71	7.79	0.000	**
Day 7	31.00±10.87^{abp}	22.66±3.33^{bcdp}	20.16±2.02^{bcdp}	22.16±5.06^{bcdp}	4.66±0.21^{q}	24.00±3.07	2.88	0.042	*
Day 15	15.66±1.94^{cdp}	17.00±2.68^{cdep}	17.16±2.12^{cdep}	17.50±3.09^{bcdp}	4.33±0.33^{q}	16.83±1.18	6.30	0.001	**
Day 28	13.16±2.05^{cdp}	12.83±1.49^{dep}	13.16±1.55^{dep}	14.66±3.04^{cdp}	5.66±0.55^{q}	13.46±1.00	3.42	0.022	*
Day 35	9.33±1.85^{dp}	8.33±1.33^{ep}	8.66±1.17e^{p}	9.16±1.95^{dp}	4.33±0.21^{q}	8.88±0.76	2.04	0.118	NS
Overall	24.10±2.45	22.86±2.05	22.40±2.07	23.36±2.28	4.88±0.15				
F value	5.84	9.58	10.38	5.62	2.15				
P value	0.000	0.000	0.000	0.000	0.071				
S / NS	**	**	**	**	NS				

Table F-value for Days is 2.37 (5 %) and 2.75 (1 %); Table F value for Groups is 3.36 (5 %) and 4.17 (1 %).
* $p \leq 0.05$; ** $p \leq 0.01$ and NS – Non Significant
Means bearing superscripts p,q,r,s in rows show statistical significance between the groups while means bearing superscripts a,b,c,d in columns show the statistical significance within the groups.

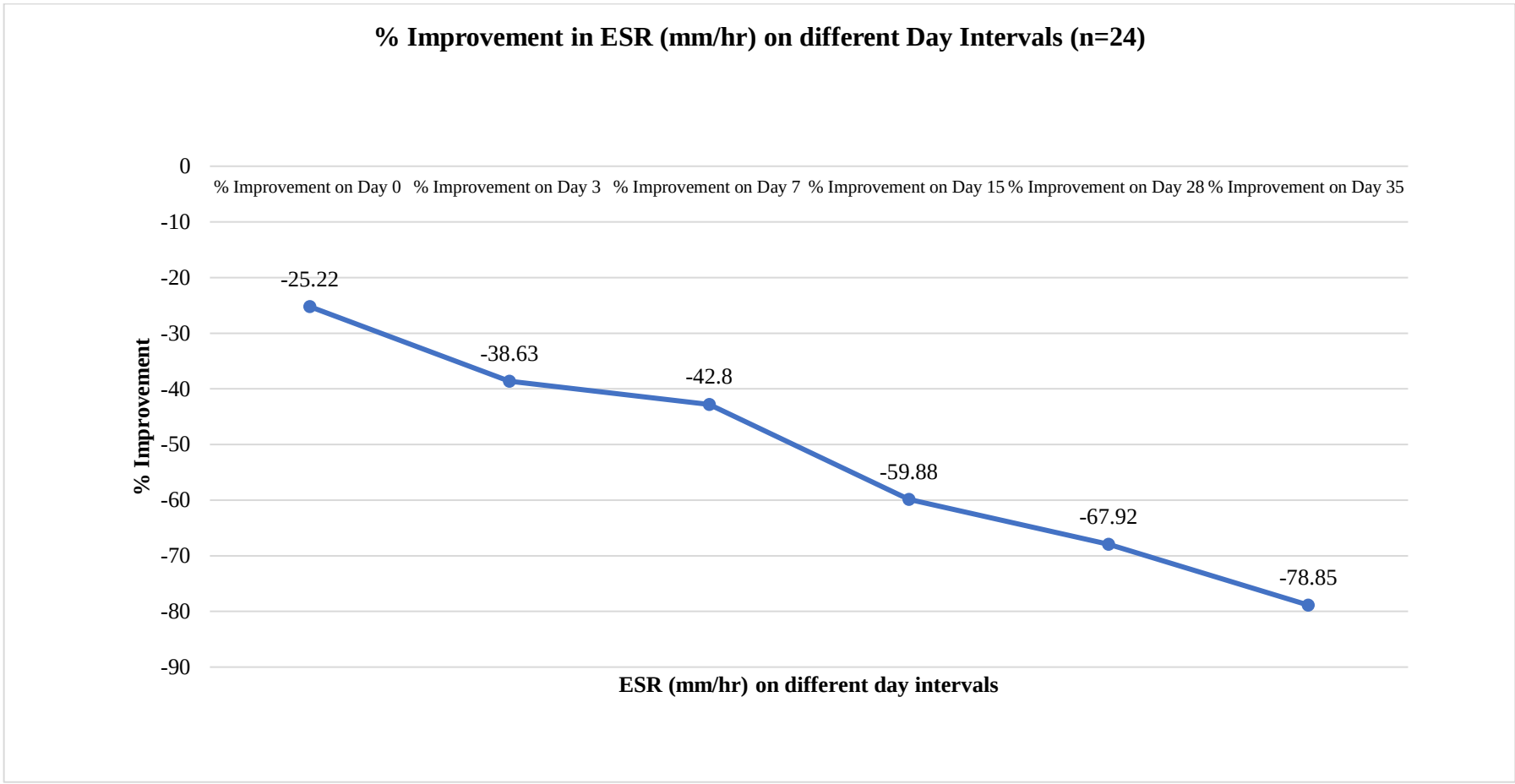


Figure 4.13 % Improvement in ESR (mm/hr) in Anaemic dogs on different Day Intervals (n=24)

There was statistically significant decrease in the ESR values of CKD, *E. canis*, *B. canis* and pyometra anaemic groups on Day 35 values of respective diseases when compared with before-treatment values.

Anaemia and macrocytosis elevate the erythrocyte sedimentation rate (ESR). In cases of anaemia, where the hematocrit is decreased, the speed at which plasma flows upwards is changed, causing red blood cell clusters to descend more rapidly. Macrocytic red blood cells, which have a reduced surface-to-volume ratio, have a higher sedimentation rate.

The ESR findings of the present study were following Vertimalai (1992) who recorded the ESR in the clinical cases before transfusion as 55.21 ± 8.45. On the third day post-transfusion, the ESR decreased to 47.07 ± 9.36. It further decreased to 40.5 ± 8.74 and 28.14 ± 7.48 on the seventh- and tenth-day post-transfusion respectively.

Conclusively, post treatment ESR study revealed that since there is a significant decrease in the ESR values on Day 35 than before treatment values in CKD, *E. canis*, *B. canis* and pyometra groups, it showed that the whole blood transfusion and disease-specific treatment has triggeed the anaemia and hence increased the Hb values which intrun caused a beneficial impact on ESR leading to improvement in the clinical condition of the patient.

4.2.11 Blood Urea Nitrogen (BUN) (mg/dl) values in healthy control group and different anaemic groups at all the time intervals:

Table 4.38 (Figure 4.14) indicates serum BUN (mg/dl) (Mean± SE) of dogs in different anaemic groups as well as healthy control group at all the time intervals. The overall serum BUN values in before treatment, Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35 were 67.45 ± 9.17, 90.49 ± 12.92, 75.61 ± 12.18, 71. 12 ± 11.16, 48.44 ± 6.20, 32.54 ± 2.46 and 30.51 ± 3.20, respectively with percent change in serum BUN as 34.16, 12.11, 5.44, -28.18, -51.76 and -54.76 on Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35, respectively (Table 4.22).

Comparative analysis of the serum BUN values between CKD, *E. canis*, *B. canis* and pyometra groups on Day 3, Day 7, Day 15, Day 28 and Day 35 revealed significant ($p \leq 0.01$) differences in before treatment, Day 0, Day 3, Day 7

Table 4.38: Mean ± SE Serum BUN (mg/dl) in healthy control group and different anaemic groups at all the time intervals

Time Intervals	CKD (a) (n=6)	*E. canis (b)* (n=6)	*B. canis (c)* (n=6)	Pyometra(d) (n=6)	Healthy(e) (n=6)	Overall (a+b+c+d) (n=24)	F value	P value	S / NS
BT	128.28±13.10^{abp}	41.39±11.79^{qr}	56.03±9.38^{abq}	44.07±11.71^{bcqr}	21.50±1.62^{r}	67.45±9.17	15.65	0.000	**
Day 0	157.86±32.84^{ap}	56.03±16.69^{q}	65.50±2.21^{aq}	82.55±20.60^{abq}	24.00±1.91^{q}	90.49±12.92	6.94	0.000	**
Day 3	127.70±36.23^{abp}	48.75±13.44^{q}	49.31±8.35^{abcq}	76.68±18.45^{abcpq}	25.16±2.12^{q}	75.61±12.18	4.03	0.011	*
Day 7	102.21±20.75^{abcp}	39.73±6.61^{q}	34.51±8.72^{cdq}	108.00±28.49^{ap}	28.66±3.86^{q}	71.12±11.16	5.52	0.002	**
Day 15	65.03±15.47^{bcd}	30.75±6.30	40.66±2.67^{bc}	57.31±16.57^{abc}	28.16±5.72	48.44±6.20	2.22	0.095	NS
Day 28	44.68±5.07^{cdp}	30.36±4.57^{q}	30.83±2.12^{cdq}	24.26±3.97^{cq}	24.33±1.45^{q}	32.54±2.46	5.01	0.004	**
Day 35	34.00±2.59^{d}	32.38±7.82	20.66±1.68^{d}	35.00±9.51^{bc}	25.33±1.25	30.51±3.20	1.18	0.342	NS
Overall	94.25±10.17	39.92±3.91	42.50±3.07	61.13±7.40	25.31±1.10				
F value	4.76	0.87	6.62	2.92	0.68				
P value	0.001	0.052	0.000	0.020	0.660				
S / NS	**	NS	**	**	NS				

Table F-value for Days is 2.37 (5 %) and 2.75 (1 %); Table F value for Groups is 3.36 (5 %) and 4.17 (1 %).
* $p \leq 0.05$; ** $p \leq 0.01$ and NS – Non Significant
Means bearing superscripts p,q,r,s in rows show statistical significance between the groups while means bearing superscripts a,b,c,d in columns show the statistical significance within the groups.

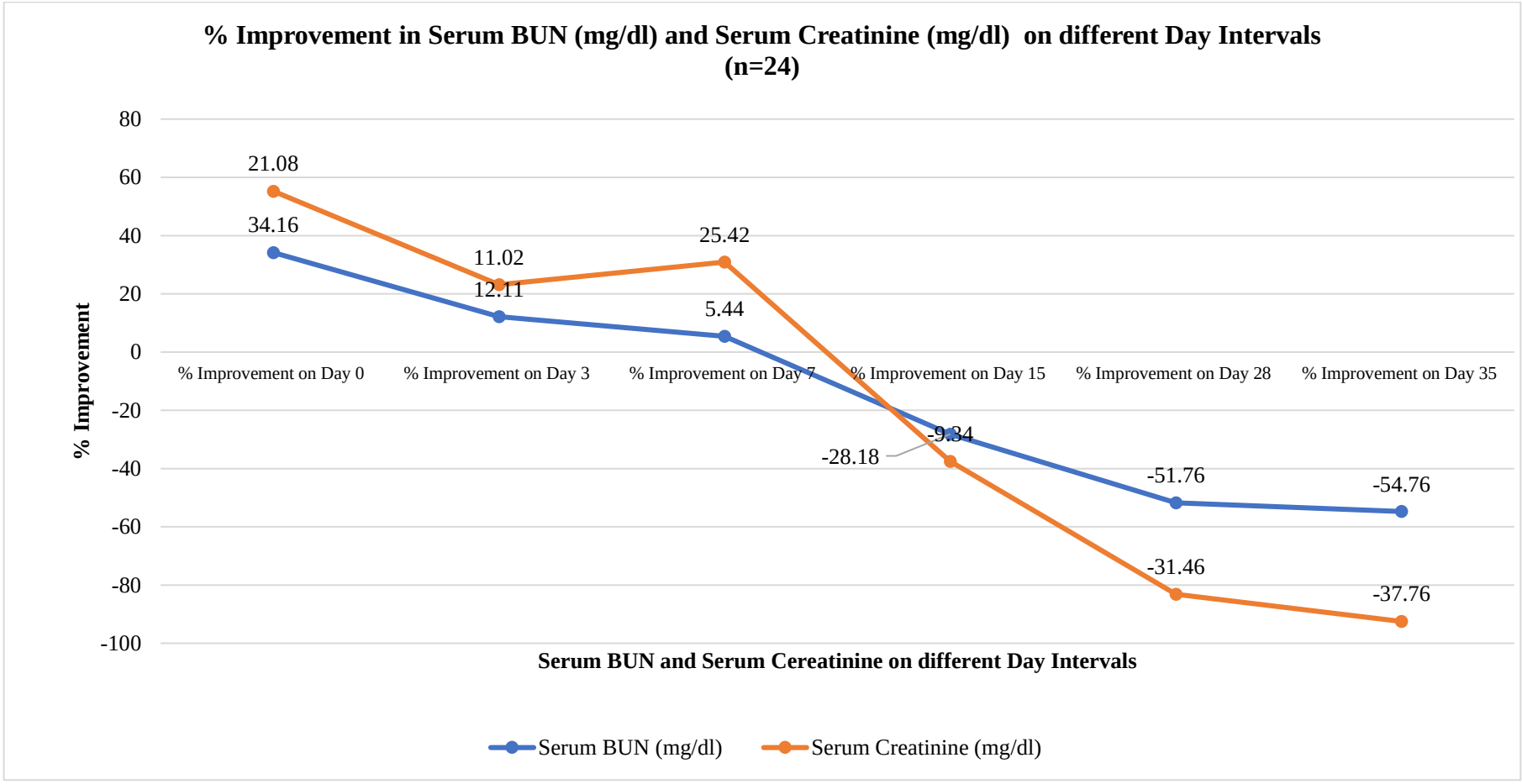


Figure 4.14 Percent Improvement in Serum BUN (mg/dl) and Serum Creatinine (mg/dl) on different Day Intervals (n=24)

and Day 28 while non-significant difference in Day 15 and Day 35 when compared individually with the healthy control groups on respective days.

The serum BUN values in CKD, *E. canis*, *B. canis* and pyometra anaemic groups on before treatment was (128.28 ± 13.10, 41.39 ± 11.79, 56.03 ± 9.38 and 44.07 ± 11.71) and on Day 35 (34.00 ± 2.59, 32.38 ± 7.82, 20.66 ± 1.68 and 35.00 ± 9.51) respectively having overall percent decrement as 73.50, 21.77, 63.12 and 20.58 in respective anaemic groups. The highest percentage of improvement i.e decrease in serum BUN was noted in CKD (73.50), followed by *B. canis* (63.12), *E. canis* (21.77) and pyometra (20.58) respectively in anaemic dogs (Table 4.23).

There was statistically significant decrease in the serum BUN values of CKD, *B. canis* and pyometra anaemic groups while non-significant ($p \leq 0.05$) decrease in *E. canis* group on Day 35 when respective diseases were compared with before-treatment values.

The decreased BUN level in the present study might be due to the treatment with proper fluid therapy, good urine output and prerenal azotemia which helps to correct dehydration, improve GFR and improve kidney perfusion.

Conclusively, post-treatment BUN study revealed that since there is significant decrease in the BUN values on Day 35 than before treatment values in CKD, *E. canis*, *B. canis* and pyometra groups, it showed that the fluid therapy, and disease-specific treatment has a beneficial impact on reducing the azotemia and the clinical condition of the patient and the treatment was beneficial in progression of the health of the animal.

4.2.12 Serum Creatinine (mg/dl) values in healthy control group and different anaemic groups at all the time intervals:

Table 4.39 (Figure 4.14) indicates serum creatinine (mg/dl) values (Mean± SE) of dogs in different anaemic groups as well as healthy control group at all the time intervals. The overall serum creatinine values in before treatment, Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35 were 3.51 ± 0.60, 4.25 ± 0.57, 3.90 ± 0.64, 4.40 ± 0.84, 3.18 ± 0.50, 2.40 ± 0.37 and 2.18 ± 0.42 mg/dl, respectively with percent increment of 21.08, 11.02, 25.42, and decrement of 9.34, 31.46 and 37.76 in serum creatinine on Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35,

Table 4.39: Mean ± SE Serum Creatinine (mg/dl) in healthy control group and different anaemic groups at all the time intervals

Time Intervals	CKD (a) (n=6)	*E. canis (b)* (n=6)	*B. canis (c)* (n=6)	Pyometra(d) (n=6)	Healthy(e) (n=6)	Overall (a+b+c+d) (n=24)	F value	P value	S / NS
BT	7.30 ± 0.57^{abp}	1.59 ± 0.25^{qr}	1.86 ± 0.40^{abqr}	3.27 ± 1.36^{q}	1.05 ± 0.15^{r}	3.51 ± 0.60	13.13	0.000	**
Day 0	8.13 ± 0.74^{ap}	2.50 ± 0.53^{qr}	2.75 ± 0.35^{aqr}	3.60 ± 0.96^{q}	1.08 ± 0.09^{r}	4.25 ± 0.57	18.99	0.000	**
Day 3	7.93 ± 1.14^{ap}	2.01 ± 0.47^{qr}	2.01 ± 0.17^{abqr}	3.62 ± 1.11^{q}	1.18 ± 0.15^{r}	3.90 ± 0.64	12.96	0.000	**
Day 7	7.21 ± 0.63^{abp}	1.75 ± 0.19^{q}	1.43 ± 0.22^{bq}	7.20 ± 2.49^{p}	1.46 ± 0.16^{q}	4.40 ± 0.84	7.12	0.000	**
Day 15	6.16 ± 0.53^{abcp}	1.61 ± 0.25^{q}	1.86 ± 0.31^{abq}	3.07 ± 1.21^{q}	1.46 ± 0.21^{q}	3.18 ± 0.50	9.79	0.000	**
Day 28	4.93 ± 0.54^{bcp}	1.40 ± 0.29^{q}	1.55 ± 0.30^{bq}	1.73 ± 0.60^{q}	1.36 ± 0.17^{q}	2.40 ± 0.37	13.36	0.000	**
Day 35	4.00 ± 1.02^{cp}	1.46 ± 0.33^{q}	1.16 ± 0.31^{bq}	2.10 ± 1.03^{pq}	1.20 ± 0.15^{q}	2.18 ± 0.42	2.99	0.037	*
Overall	6.53 ± 0.35	1.76 ± 0.14	1.81 ± 0.13	3.51 ± 0.54	1.26 ± 0.06				
F value	4.04	1.17	2.78	1.68	1.15				
P value	0.0030	0.343	0.25	0.153	0.351				
S / NS	**	NS	**	NS	NS				

Table F-value for Days is 2.37 (5 %) and 2.75 (1 %); Table F value for Groups is 3.36 (5 %) and 4.17 (1 %).
* $p \leq 0.05$; ** $p \leq 0.01$ and NS – Non Significant
Means bearing superscripts p,q,r,s in rows show statistical significance between the groups while means bearing superscripts a,b,c,d in columns show the statistical significance within the groups.

respectively (Table 4.22).

Comparative analysis of the serum creatinine values between CKD, *E. canis*, *B. canis* and pyometra groups on Day 3, Day 7, Day 15, Day 28 and Day 35 revealed significant ($p \leq 0.01$) differences in all the days when compared individually with the healthy control groups on respective days. The serum creatinine values in CKD, *E. canis*, *B. canis* and pyometra anaemic groups before treatment were (7.30 ± 0.57, 1.59 ± 0.25, 1.86 ± 0.40 and 3.27 ± 1.36) and on Day 35 (6.53 ± 0.35, 1.76 ± 0.14, 1.81 ± 0.13, 3.51 ± 0.54 and 1.26 ± 0.06) respectively having percent decrement as 45.21, 7.74, 37.43 and 35.94 in respective anaemic groups. The highest percentage of improvement i.e. decrease in serum creatinine was noted in CKD (45.21) followed by *B. canis* (37.43), pyometra (35.94) and *E. canis* (7.74), respectively in anaemic dogs. There was statistically significant ($p \leq 0.05$) decrease in the serum creatinine values of CKD and *B. canis* values groups while a non-significant ($p \leq 0.05$) decrease in *E. canis* and pyometra groups on Day 35 when respective diseases were compared with before treatment values.

Conclusively, a post-treatment creatinine study revealed that since there is significant decrease in the creatinine values on Day 35 than before treatment values in CKD, *E. canis*, *B. canis* and pyometra groups, it showed that fluid administration had helped in creatinine clearance and disease-specific treatment had a beneficial impact on improving the renal health and clinical condition of the patient.

4.2.13 Total Bilirubin (mg/dl), Direct Bilirubin (mg/dl) and Indirect Bilirubin (mg/dl) in healthy control group and different anaemic groups at all the time intervals:

Total Bilirubin (mg/dl), Direct Bilirubin (mg/dl) and Indirect Bilirubin (mg/dl) are depicted in Table 4.40 (Figure 4.15), Table 4.41 (Figure 4.16) and Table 4.42 (Figure 4.17) respectively in different anaemic and healthy groups on different day intervals.

Table 4.40: Mean ± SE Total Bilirubin (mg/dl) in healthy control group and different anaemic groups at all the time intervals

Time Intervals	CKD (a) (n=6)	*E. canis (b)* (n=6)	*B. canis (c)* (n=6)	Pyometra(d) (n=6)	Healthy(e) (n=6)	Overall (a+b+c+d) (n=24)	F value	P value	S / NS
BT	0.33±0.08^{r}	4.90±2.13^{p}	3.84±1.04^{pq}	0.76±0.48^{qr}	0.53±0.07^{qr}	2.46±0.70	3.87	0.013	*
Day 0	0.21±0.01^{q}	5.30±1.90^{p}	4.56±0.63^{p}	0.35±0.13^{q}	0.38±0.05^{q}	2.61±0.68	7.99	0.000	**
Day 3	0.28±0.06^{q}	4.28±1.52^{p}	3.88±0.78^{p}	0.44±0.10^{q}	0.36±0.06^{q}	2.22±0.56	7.03	0.000	**
Day 7	0.25±0.05	3.91±1.31	2.81±0.84	1.76±1.54	0.48±0.04	2.19±0.58	2.49	0.068	NS
Day 15	0.28±0.03^{q}	3.80±1.09^{p}	3.63±0.45^{p}	0.28±0.10^{q}	0.56±0.10^{q}	2.00±0.45	11.82	0.000	**
Day 28	0.25±0.03^{p}	3.19±1.12^{p}	2.76±0.43^{p}	0.33±0.06^{q}	0.53±0.13^{q}	1.64±0.40	6.99	0.000	**
Day 35	0.21±0.01^{q}	1.41±0.74^{p}	1.43±0.17^{p}	0.36±0.09^{pq}	0.46±0.09^{pq}	0.86±0.22	2.90	0.041	*
Overall	0.26±0.02	3.83±0.55	3.28±0.28	0.62±0.23	0.48±0.03				
F value	0.73	0.74	2.27	0.73	0.77				
P value	0.627	0.615	0.058	0.621	0.595				
S / NS	NS	NS	NS	NS	NS				

Table F-value for Days is 2.37 (5 %) and 2.75 (1 %); Table F value for Groups is 3.36 (5 %) and 4.17 (1 %).

* $p \leq 0.05$; ** $p \leq 0.01$ and NS – Non Significant

Means bearing superscripts p,q,r,s in rows show statistical significance between the groups while means bearing superscripts a,b,c,d in columns show the statistical significance within the groups.

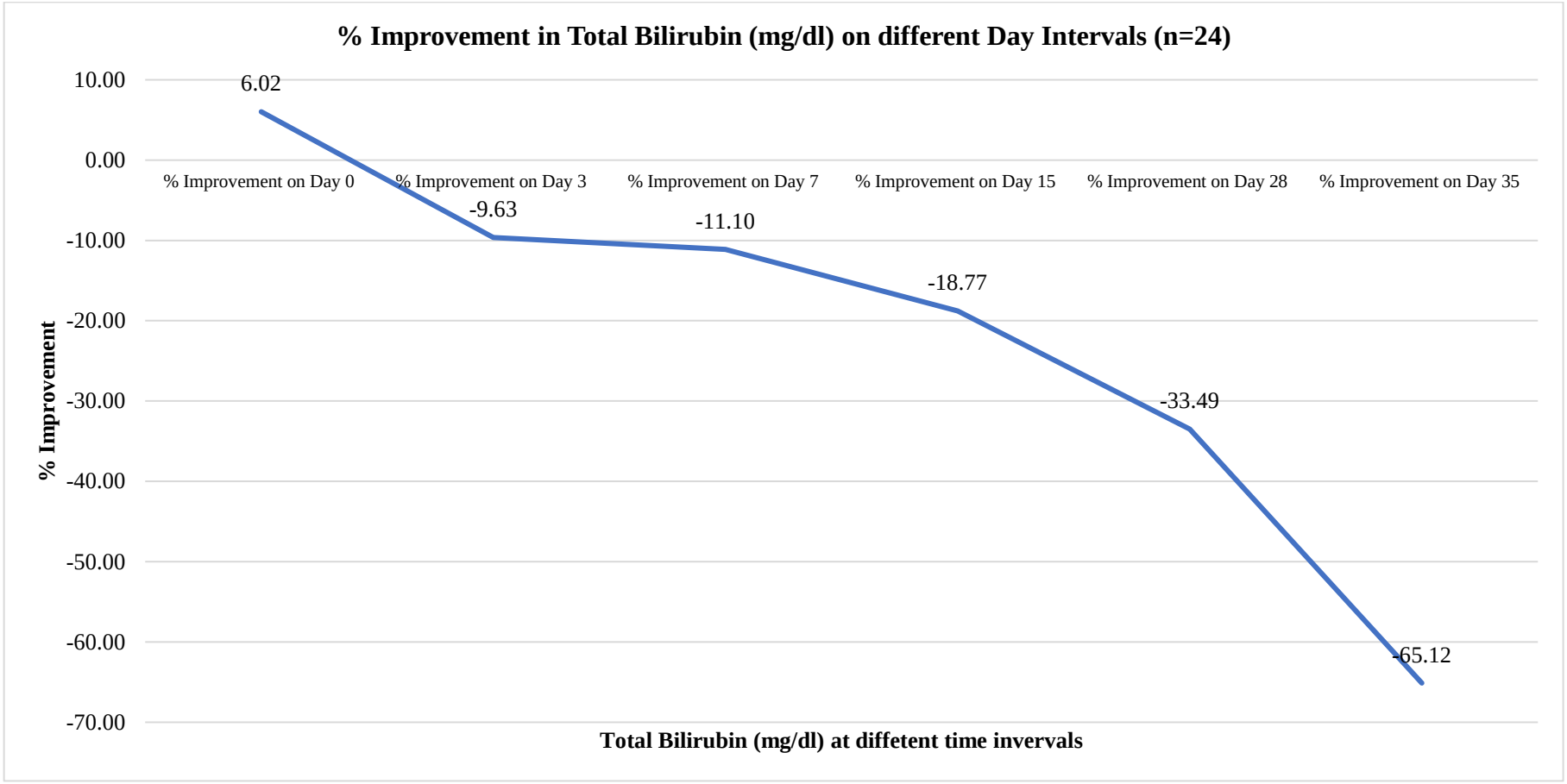


Figure 4.15 Percent Improvement in Total Bilirubin (mg/dl) in Anaemic dogs on different Day Intervals (n=24)

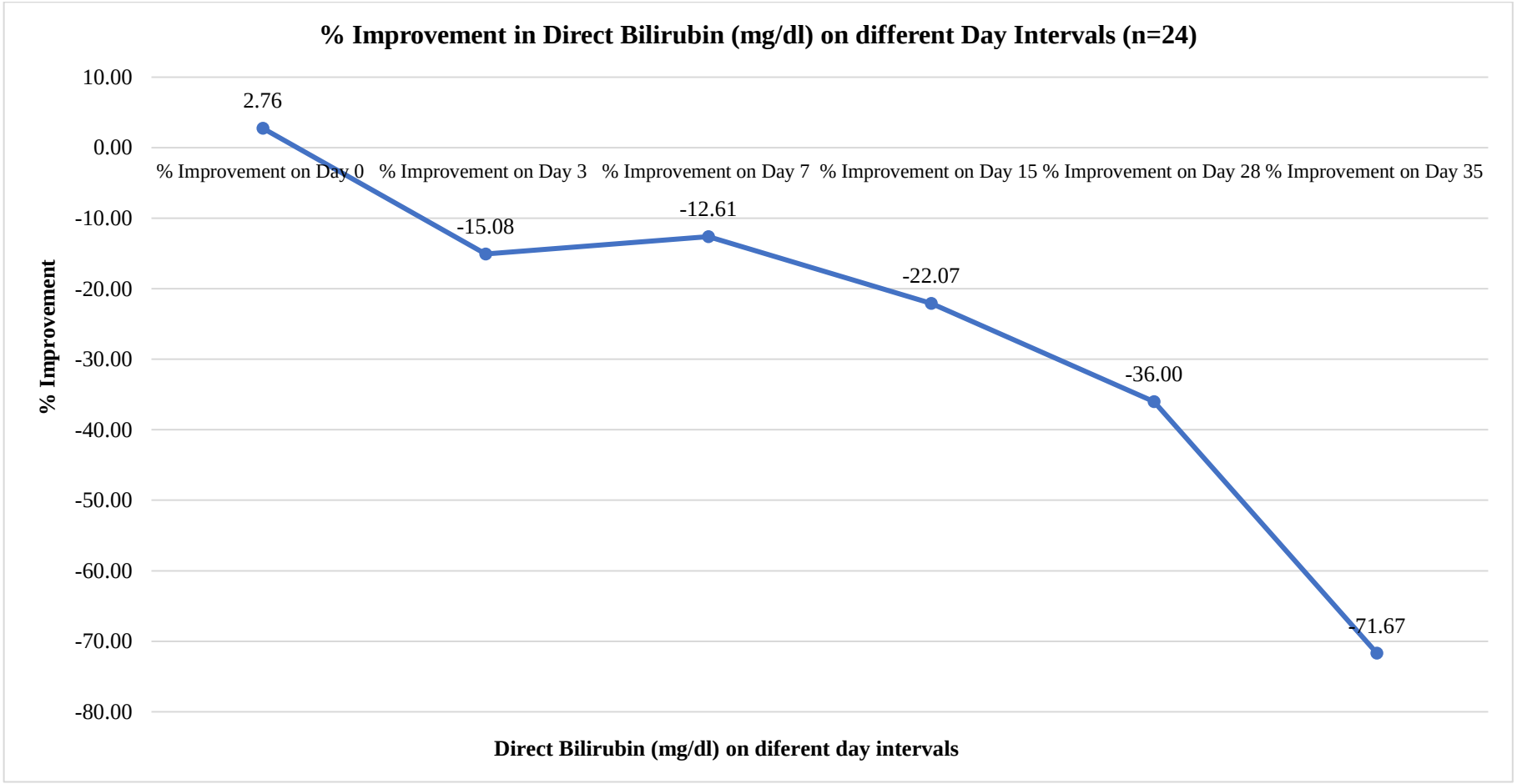


Figure 4.16 % Improvement in Direct Bilirubin (mg/dl) in Anaemic dogs on different Day Intervals (n=24)

Table 4.41: Mean ± SE Bilirubin Direct (mg/dl) in healthy control group and different anaemic groups at all the time intervals

Time Intervals	CKD (a) (n=6)	*E. canis (b)* (n=6)	*B. canis (c)* (n=6)	Pyometra(d) (n=6)	Healthy(e) (n=6)	Overall (a+b+c+d) (n=24)	F value	P value	S / NS
BT	0.12±0.01^{pq}	2.60±1.16^{p}	1.97±0.58^{pq}	0.52±0.37^{q}	0.31±0.05^{q}	1.31±0.38	3.33	0.025	*
Day 0	0.10±0.00^{q}	2.80±1.13^{p}	2.26±0.42^{p}	0.20±0.08^{q}	0.20±0.02^{q}	1.34±0.38	5.82	0.001	**
Day 3	0.13±0.03^{q}	2.15±0.70^{p}	1.91±0.47^{p}	0.23±0.06^{q}	0.25±0.05^{q}	1.11±0.28	6.96	0.000	**
Day 7	0.13±0.03	2.13±0.65	1.43±0.48	0.86±0.76	0.30±0.05	1.14±0.30	2.71	0.052	NS
Day 15	0.16±0.02^{q}	1.96±0.50^{p}	1.78±0.24^{p}	0.15±0.07^{q}	0.36±0.08^{q}	1.02±0.22	12.58	0.000	**
Day 28	0.13±0.02^{q}	1.55±0.53^{p}	1.46±0.24^{p}	0.18±0.03^{q}	0.33±0.10^{q}	0.84±0.20	7.05	0.000	**
Day 35	0.10±0.00^{r}	0.53±0.22^{pq}	0.65±0.13^{p}	0.20±0.05^{qr}	0.21±0.05^{qr}	0.37±0.08	3.73	0.016	*
Overall	0.13±0.01	1.96±0.29	1.64±0.16	0.34±0.12	0.28±0.02				
F value	1.07	0.95	1.71	0.65	0.93				
P value	0.399	0.471	0.145	0.689	0.485				
S / NS	NS	NS	NS	NS	NS				

Table F-value for Days is 2.37 (5 %) and 2.75 (1 %); Table F value for Groups is 3.36 (5 %) and 4.17 (1 %).
* $p \leq 0.05$; ** $p \leq 0.01$ and NS – Non Significant
Means bearing superscripts p,q,r,s in rows show statistical significance between the groups while means bearing superscripts a,b,c,d in columns show the statistical significance within the groups.

Table 4.42: Mean ± SE Bilirubin Indirect (mg/dl) in healthy control group and different anaemic groups at all the time intervals

Time Intervals	CKD (a) (n=6)	*E. canis (b)* (n=6)	*B. canis (c)* (n=6)	Pyometra(d) (n=6)	Healthy(e) (n=6)	Overall (a+b+c+d) (n=24)	F value	P value	S / NS
BT	0.20±0.06^{q}	2.29±1.02^{p}	1.87±0.50^{ap}	0.24±0.11^{q}	0.21±0.04^{q}	1.16±0.33	4.01	0.011	*
Day 0	0.11±0.01^{q}	2.50±0.85^{p}	2.30±0.29^{ap}	0.15±0.05^{q}	0.18±0.03^{q}	1.27±0.32	9.31	0.000	**
Day 3	0.15±0.03^{q}	2.13±0.83^{p}	1.96±0.37^{ap}	0.21±0.04^{q}	0.11±0.03^{q}	1.12±0.29	6.39	0.001	**
Day 7	0.11±0.01	1.78±0.67	1.38±0.38^{ab}	0.90±0.78	0.18±0.03	1.05±0.29	2.20	0.097	NS
Day 15	0.11±0.01^{q}	1.83±0.59^{p}	1.85±0.27^{ap}	0.12±0.03^{q}	0.20±0.04^{q}	0.98±0.24	10.00	0.000	**
Day 28	0.11±0.01^{q}	1.64±0.64^{p}	1.30±0.21^{abp}	0.14±0.03^{q}	0.20±0.04^{q}	0.80±0.21	6.24	0.001	**
Day 35	0.11±0.01	0.90±0.51	0.78±0.09^{b}	0.16±0.04	0.25±0.05	0.49±0.14	2.44	0.072	NS
Overall	0.13±0.01	1.87±0.27	1.64±0.14	0.28±0.11	0.19±0.02				
F value	1.13	0.49	2.37	0.85	0.94				
P value	0.364	0.811	0.049	0.537	0.477				
S / NS	NS	NS	*	NS	NS				

Table F-value for Days is 2.37 (5 %) and 2.75 (1 %); Table F value for Groups is 3.36 (5 %) and 4.17 (1 %).

* $p \leq 0.05$; ** $p \leq 0.01$ and NS – Non Significant

Means bearing superscripts p,q,r,s in rows show statistical significance between the groups while means bearing superscripts a,b,c,d in columns show the statistical significance within the groups.

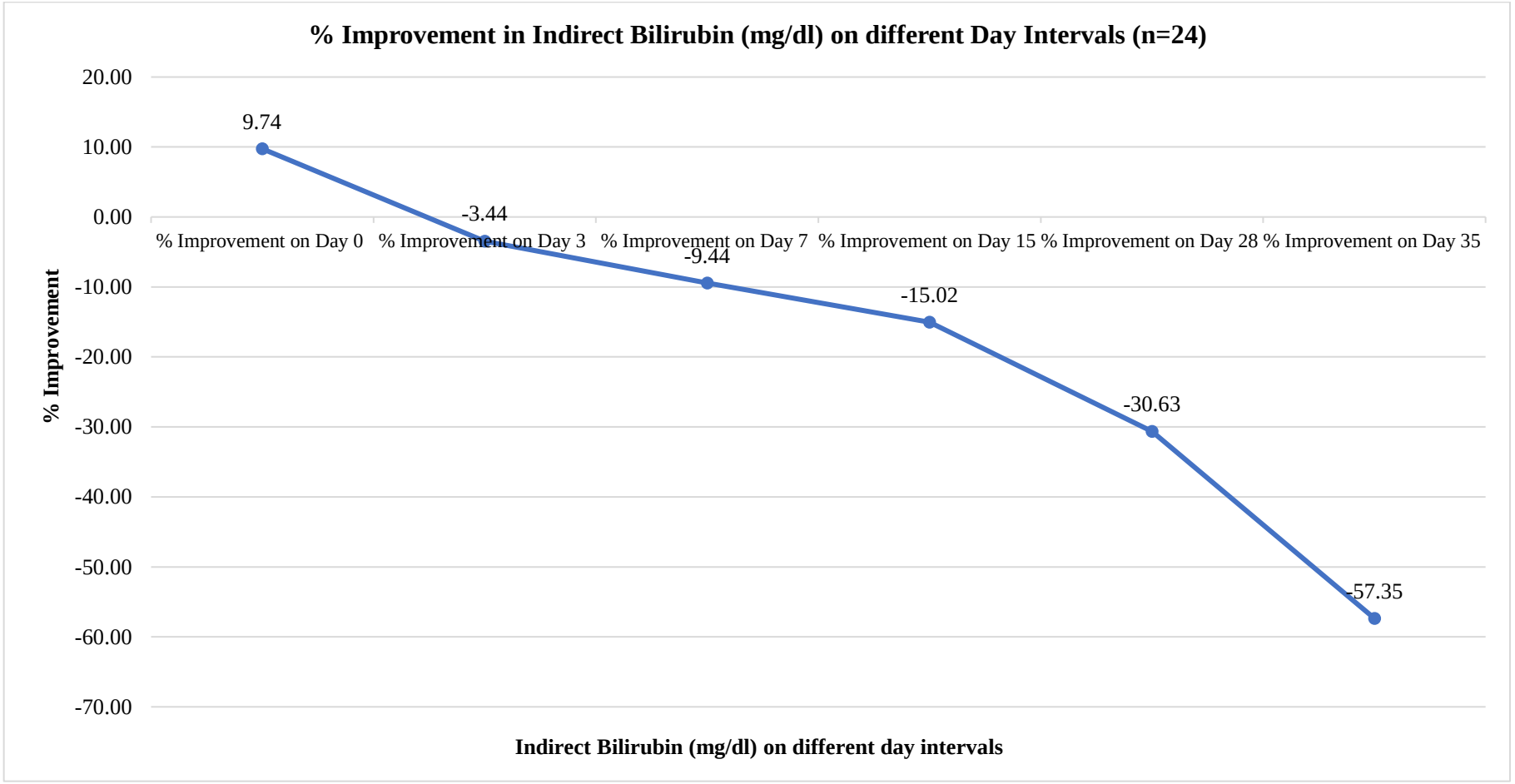


Figure 4.17 % Improvement in Indirect Bilirubin (mg/dl)in Anaemic dogs on different Day Intervals (n=24)

The overall Total Bilirubin (2.46 ± 0.70, 2.61 ± 0.68, 2.22 ± 0.56, 2.19 ± 0.58, 2.00 ± 0.45, 1.64 ± 0.40 and 0.86 ± 0.22) mg/dl, Direct Bilirubin (1.31 ± 0.38, 1.34 ± 0.38, 1.11 ± 0.28, 1.14 ± 0.30, 1.02 ± 0.22, 0.84 ± 0.20 and 0.37 ± 0.08) mg/dl and Indirect Bilirubin (1.16 ± 0.33, 1.27 ± 0.32, 1.12 ± 0.29, 1.05 ± 0.29, 0.98 ± 0.24, 0.80 ± 0.21 and 0.49 ± 0.14) mg/dl values were in before treatment, Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35.

A close look on Table 4.22, the overall increment of 6.02 % and decrement of 9.63 %, 11.10 %, 18.77%, 33.49% and 65.12% in Total Bilirubin was noted on Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35, respectively. Similarly, an overall increment of 2.76 % and decrement of 15.08 %, 12.61%, 22.07%, 36.00% and 71.67% in direct bilirubin was noted on Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35, respectively. The indirect bilirubin values revealed an increment of 9.74 % and a decrement of 3.44%, 9.44%, 15.02%, 30.63% and 57.35% on Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35, respectively.

Comparative analysis of the Total Bilirubin, Direct Bilirubin and Indirect Bilirubin values between CKD, *E. canis*, *B. canis* and pyometra groups revealed significant ($p \leq 0.05$) differences throughout all the Day intervals except on Day 7 when compared individually with the healthy control groups on respective days.

The Total Bilirubin, Direct Bilirubin and Indirect Bilirubin values in CKD, *E. canis*, *B. canis* and pyometra anaemic groups on before treatment were (0.33 ± 0.08, 4.90 ± 2.13, 3.84 ± 1.04 and 0.76 ± 0.48) (0.12 ± 0.01, 2.60 ± 1.16, 1.97 ± 0.58 and 0.52 ± 0.37) and (0.20 ± 0.06, 2.29 ± 1.02, 1.87 ± 0.50 and 0.24 ± 0.11) and on Day 35 (0.21± 0.01, 1.41±0.74, 1.43 ± 0.17 and 0.36 ± 0.09), (0.10±0.00, 0.53 ± 0.22, 0.65 ± 0.13 and 0.20 ± 0.05) (0.11 ± 0.01, 0.90 ± 0.51, 0.78 ± 0.09, 0.16 ± 0.04) respectively having overall per cent decrement in total bilirubin (34.83, 71.08, 62.76 and 51.90), in direct bilirubin (21.88, 79.63, 67.09, and 61.54) and in indirect bilirubin (42.93, 60.70, 58.20 and 31.28) anaemic groups (Table 4.23).

The highest percentage of decrement in the Total Bilirubin, Direct Bilirubin and indirect Bilirubin was noted in *E. canis* group followed by *B. canis*, pyometra and CKD group of dogs.

There was statistically non-significant decrease in the Total Bilirubin and Direct Bilirubin values on Day 35 in all the anaemic groups when respective diseases were compared with before-treatment values. There was also statistically non-significant decrease in the Indirect Bilirubin on Day 35 in all the anaemic groups except *B. canis* group when respective diseases were compared with before-treatment values.

Conclusively, post-treatment Total Bilirubin, Direct Bilirubin and Indirect Bilirubin study revealed that since there is a decrease in the aforementioned values on Day 35 than before treatment values in CKD, *E. canis*, *B. canis* and pyometra groups, showed that the whole blood transfusion and disease-specific treatment had a beneficial impact on reducing icterus and enhancing hepatic health resulting in substantial improvement in the clinical condition of the patient.

4.2.14 SGOT (IU/L), SGPT (IU/L) and Alkaline Phosphatase (IU/L) in healthy control group and different anaemic groups at all the time intervals:

Tables 4.43, Table 4.44 and Table 4.45 indicate SGOT (IU/L), SGPT (IU/L) and Alkaline phosphatase (IU/L) (Mean± SE) of dogs in different anaemic groups as well as healthy control group at all the time intervals. The overall SGOT, SGPT and Alkaline phosphatase values before treatment, Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35 were (138.74 ± 40.60, 144.19 ± 49.60, 95.18 ± 27.53, 73.38 ± 6.83, 70.49 ± 6.01, 68.33 ± 9.59 and 53.74 ± 2.91), (139.67 ± 42.78, 188.62 ± 67.13, 94.33 ± 16.30, 84.58 ± 14.73, 78.81 ± 13.71, 75.57 ± 7.59 and 59.88 ± 8.50) and (182.74 ± 37.05, 145.27 ± 30.19, 162.57 ± 28.62, 270.67 ± 74.16, 173.70 ± 63.44, 122.07 ± 14.44, and 110.67 ± 13.11), respectively.

On perusal of Table 4.22, an increment of 3.93 % and decrement of 31.40 %, 47.11 %, 49.19 %, 50.75 % and 61.26 % was observed on Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35 SGOT values respectively. A similar trend was observed in SGPT values wherein an increment of 35.05 % on Day 0 was followed by decrement of 32.46 %, 39.44 %, 43.57 %, 45.90 % and 57.13 % on Day 3, Day 7, Day 15, Day 28 and Day 35 respectively. The percent values of alkaline phosphatase were found to be decreased by 20.50%., 11.04 %, 4.95%, 33.20 %and 39.20,% on Day 0, Day 3, Day 15, Day 28 and Day 35 respectively

Table 4.43: Mean ± SE SGOT (IU/L) values in the healthy control group and different anaemic groups at all the time intervals

Time Intervals	CKD (a) (n=6)	*E. canis (b)* (n=6)	*B. canis (c)* (n=6)	Pyometra(d) (n=6)	Healthy(e) (n=6)	Overall (a+b+c+d) (n=24)	F value	P value	S / NS
BT	50.72±10.47	288.00±145.80	142.28±33.74	73.93±31.17	57.66±7.65	138.74±40.60	2.09	0.111	NS
Day 0	54.83±7.40^{q}	384.00±170.03^{p}	101.59±22.30^{q}	36.33±2.51^{q}	60.33±5.20^{q}	144.19±49.60	3.58	0.019	*
Day 3	57.66±7.62	207.00±100.00	76.50±20.27	39.53±2.54	57.50±2.52	95.18±27.53	2.20	0.097	NS
Day 7	70.50±17.79	94.50±12.22	72.33±12.06	56.16±9.82	64.16±6.45	73.38±6.83	1.36	0.274	NS
Day 15	58.13±6.45^{qr}	98.16±6.89^{p}	79.83±14.52^{pq}	45.83±6.51^{r}	58.66±4.75^{qr}	70.49±6.01	5.90	0.001	**
Day 28	48.33±9.35	72.00±11.85	91.33±35.36	61.66±3.82	58.16±3.72	68.33±9.59	0.88	0.486	NS
Day 35	47.16±6.81	46.33±6.42	59.79±5.11	61.66±1.64	57.00±4.48	53.74±2.91	1.88	0.144	NS
Overall	55.34±3.71	170.00±37.31	89.10±8.91	53.59±4.87	59.07±1.86				
F value	0.62	1.86	1.38	1.13	0.22				
P value	0.712	0.114	0.247	0.364	0.964				
S / NS	NS	NS	NS	NS	NS				

Table F-value for Days is 2.37 (5 %) and 2.75 (1 %); Table F value for Groups is 3.36 (5 %) and 4.17 (1 %).

* $p \leq 0.05$; ** $p \leq 0.01$ and NS – Non Significant

Means bearing superscripts p,q,r,s in rows show statistical significance between the groups while means bearing superscripts a,b,c,d in columns show the statistical significance within the groups.

Table 4.44 : Mean ± SE SGPT (IU/L) values in healthy control group and different anaemic groups at all the time intervals

Time Intervals	CKD (a) (n=6)	*E. canis (b)* (n=6)	*B. canis (c)* (n=6)	Pyometra(d) (n=6)	Healthy(e) (n=6)	Overall (a+b+c+d) (n=24)	F value	P value	S / NS
BT	50.10±8.01	209.66±159.90	112.22±30.86	186.68±62.47	55.33±4.67	139.67±42.78	0.88	0.487	NS
Day 0	43.33±4.91	444.00±250.30	124.98±31.86	142.16±22.08	63.50±1.83	188.62±67.13	2.03	0.119	NS
Day 3	43.33±4.28	112.00±59.06	105.33±76.86	116.64±20.85	61.00±2.88	94.33±16.30	1.31	0.290	NS
Day 7	54.83±10.61	100.16±52.67	94.33±162.21	89.00±26.67	57.50±5.43	84.58±14.73	0.60	0.662	NS
Day 15	44.57±9.16	98.00±46.50	90.00±17.20	82.66±19.11	63.83±4.37	78.81±13.71	0.74	0.573	NS
Day 28	49.50±9.90	66.93±14.84	88.33±12.53	97.50±3.16	62.33±4.39	75.57±7.59	2.40	0.076	NS
Day 35	30.50±1.97	49.06±9.92	91.12±11.87	68.83±6.51	62.50±5.71	59.88±8.50	2.67	0.055	NS
Overall	45.17±2.88	154.26±45.66	100.90±8.92	111.93±11.77	60.86±1.60				
F value	1.03	1.36	0.30	1.95	0.54				
P value	0.421	0.255	0.928	0.099	0.771				
S / NS	NS	NS	NS	NS	NS				

Table F-value for Days is 2.37 (5 %) and 2.75 (1 %); Table F value for Groups is 3.36 (5 %) and 4.17 (1 %).

* $p \leq 0.05$; ** $p \leq 0.01$ and NS – Non Significant

Means bearing superscripts p,q,r,s in rows show statistical significance between the groups while means bearing superscripts a,b,c,d in columns show the statistical significance within the groups.

Table 4.45: Mean ± SE Alkaline Phosphatase (IU/L) in the healthy control group and different anaemic groups at all the time intervals

Time Intervals	CKD (a) (n=6)	*E. canis (b)* (n=6)	*B. canis (c)* (n=6)	Pyometra(d) (n=6)	Healthy(e) (n=6)	Overall (a+b+c+d) (n=24)	F value	P value	S / NS
BT	65.73±15.77	226.00±82.09	186.55±30.86	252.66±114.96	111.66±18.74	182.74±37.05	1.42	0.255	NS
Day 0	65.00 ± 14.83^{q}	275.66 ± 100.82^{p}	149.75 ± 31.86^{pq}	90.667 ± 8.98^{q}	121.00 ± 16.92^{q}	145.27±30.19	2.85	0.044	*
Day 3	61.00 ± 16.94^{r}	269.50 ± 55.72^{p}	218.00 ± 76.86^{pq}	101.76 ± 8.14^{qr}	119.50 ± 18.13^{qr}	162.57±28.62	3.86	0.014	*
Day 7	72.50±10.81	330.33±98.04	309.83±162.21	370.00±233.05	121.00±14.70	270.67±74.16	0.99	0.428	NS
Day 15	85.46±12.89	399.50±244.47	85.16±17.20	124.66±4.77	125.00±15.27	173.70±63.44	1.46	0.242	NS
Day 28	89.00 ± 8.11^{q}	186.51 ± 46.11^{p}	83.91 ± 12.53^{q}	128.83 ± 11.62^{pq}	128.33 ± 18.43^{pq}	122.07±14.44	3.00	0.037	*
Day 35	73.83±6.55	138.00±44.65	90.83±11.87	140.00±17.71	106.16±16.02	110.67±13.11	1.53	0.222	NS
Overall	73.22±4.73	260.79±42.31	160.58±27.48	172.66±37.48	118.95±6.02				
F value	0.68	0.57	1.43	1.06	0.20				
P value	0.663	0.746	0.229	0.400	0.973				
S / NS	NS	NS	NS	NS	NS				

Table F-value for Days is 2.37 (5 %) and 2.75 (1 %); Table F value for Groups is 3.36 (5 %) and 4.17 (1 %).
* $p \leq 0.05$; ** $p \leq 0.01$ and NS – Non Significant
Means bearing superscripts p,q,r,s in rows show statistical significance between the groups while means bearing superscripts a,b,c,d in columns show the statistical significance within the groups.

except an increment of 48.12 % noted on Day 7.

Comparative analysis of the SGOT, SGPT and Alkaline phosphatase values between CKD, *E. canis*, *B. canis* and pyometra groups revealed non-significant differences throughout all the study intervals except Day 0 and Day15 in SGOT, while Day 0, Day 3 and Day 28 in Alkaline phosphatase where significant ($p \leq 0.05$) difference was noted when compared individually with the healthy control groups on respective days. The SGOT, SGPT and Alkaline phosphatase values in CKD, *E. canis*, *B. canis* and pyometra anaemic groups on before treatment were (50.72 ± 10.47, 288.00 ± 145.80, 142.28 ± 33.74 and 73.93 ± 31.17), (50.10 ± 8.01, 209.66 ± 159.90, 122.22 ± 30.86 and 186.68 ± 62.47) and (65.73 ± 15.77, 226.00 ± 82.09, 186.55 ± 30.86 and 252.66 ± 114.96), respectively and on Day 35 (47.16 ± 6.81, 46.33 ± 6.42, 59.79 ± 5.11 and 61.66 ± 1.64) (30.50 ± 1.97, 49.06 ± 9.92, 91.12 ± 11.87, 68.83 ± 6.51) and (73.83 ± 138.00 ± 44.65, 90.83 ± 11.87 and 140.00 ± 17.71)respectively having overall percent decrement as (7.02, 83.91, 57.97 and 16.60) in SGOT, (39.13, 76.60, 18.81 and 63.13) in SGPT and (12.32, 38.94, 51.31 and 44.59) in Alkaline phosphatase values (Table 4.23)

The highest percentage of improvement (Decrease) in SGOT was documented in *E. canis,* followed by *B. canis,* pyometra, and CKD while in SGPT highest percentage (Decrease) was in *E. canis,* pyometra, CKD and *B. canis* anaemic groups. The order of percent improvement in ALP values was *B. canis*, pyometra, *E. canis* and CKD anaemic groups.

There was statistically non-significant decrease in the SGOT, SGPT and Alkaline phosphatase values of CKD, *E. canis. B. canis* and pyometra anaemic groups on Day 35 when respective disease values were compared with before-treatment values.

Conclusively, post-treatment SGOT, SGPT and Alkaline phosphatase study revealed that there is decrease in aforementioned values on Day 35 than before treatment values in CKD, *E. canis*, *B. canis* and pyometra groups. This showed that the whole blood transfusion and disease-specific treatment had beneficial impact on decreasing the liver enzymes and improving the clinical

condition of the patient.

4.2.15 Serum Total Proteins (g/dl) in healthy control group and different anaemic groups at all the time intervals:

Table 4.46 (Figure 4.18) depicts serum total protein (g/dl) values (Mean± SE) of dogs in different anaemic groups as well as healthy control group at all the time intervals. The overall total protein values in before treatment, Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35 were 6.85 ± 0.28, 7.04 ± 0.26, 6.57 ± 0.19, 6.55 ± 0.24, 6.35 ± 0.18, 6.14 ± 0.17 and 6.15 ± 0.17, respectively with percent increment of 2.69 on Day 0 followed by decrement of 4.08 %, 4.35 %, 7.41 %, 10.40% and 10.27 % on Day 3, Day 7, Day 15, Day 28 and Day 35 respectively (Table 4.22)

Comparative analysis of the total protein values between CKD, *E. canis*, *B. canis* and pyometra groups revealed statistically significant ($p \leq 0.01$) differences throughout all the day intervals except Day 7 and Day 15 where non-significant difference was noted when compared individually with the healthy control groups on respective days.

The total protein values in CKD, *E. canis*, *B. canis* and pyometra anaemic groups on before treatment were 7.56 ± 0.76, 7.57 ± 0.51, 5.89 ± 0.08 and 6.36 ± 0.39 and on Day 35 (6.31 ± 0.09, 6.55 ± 0.36, 6.48 ± 0.26 and 5.23 ± 0.25), respectively. There was an overall percent decrement of 16.53, 13.47 and 17.81 in CKD, E. canis and pyometra groups respectively except for the *B. canis* group which showed an increment of 10.02 % on Day 35.

The highest percentage of difference in total protein was noted in the following order: pyometra (decrement of 17.81 %), CKD (decrement of 16.53%), *E. canis* (decrement of 13.47 %) and *B. canis* (increment of 10.02 %) (Table 4.23)

Total protein values were found to be significantly decreased in pyometra while non-significantly increased in *B. canis* and non-significantly decreased in CKD and *E. canis* anaemic groups when Day 35 values of respective disease were compared with before treatment values.

Table 4.46: Mean ± SE Total Protein (g/dl) in healthy control group and different anaemic groups at all the time intervals

Time Intervals	CKD (a) (n=6)	*E. canis (b)* (n=6)	*B. canis (c)* (n=6)	Pyometra(d) (n=6)	Healthy(e) (n=6)	Overall (a+b+c+d) (n=24)	F value	P value	S / NS
BT	7.56±0.76^{p}	7.57±0.51^{p}	5.89±0.08^{q}	6.36±0.39^{apq}	6.41±0.04^{pq}	6.85±0.28	2.89	0.042	*
Day 0	7.23±0.54^{pq}	8.22±0.65^{p}	6.36±0.15^{q}	6.32±0.23^{aq}	6.43±0.04^{q}	7.04±0.26	4.16	0.010	*
Day 3	6.40±0.22^{q}	7.46±0.54^{p}	6.45±0.19^{q}	5.97±0.22^{abcq}	6.45±0.04^{q}	6.57±0.19	3.40	0.023	*
Day 7	6.43±0.39	7.51±0.56	6.10±0.54	6.16±0.25^{ab}	6.35±0.04	6.55±0.24	1.97	0.129	NS
Day 15	6.02±0.34	7.11±0.42	6.30±0.24	5.93±0.21^{abc}	6.35±0.07	6.35±0.18	2.69	0.053	NS
Day 28	5.98±0.33^{qr}	6.78±0.16^{p}	6.46±0.22^{pq}	5.32±0.29^{bcr}	6.37±0.03^{pq}	6.14±0.17	5.57	0.002	**
Day 35	6.31±0.09^{p}	6.55±0.36^{p}	6.48±0.26^{p}	5.23±0.25^{cq}	6.35±0.03^{p}	6.15±0.17	5.36	0.002	**
Overall	6.57±0.18	7.32±0.19	6.30±0.10	5.90±0.12	6.39±0.02				
F value	1.92	1.31	0.61	2.78	0.73				
P value	0.103	0.275	0.717	0.025	0.626				
S / NS	NS	NS	NS	**	NS				

Table F-value for Days is 2.37 (5 %) and 2.75 (1 %); Table F value for Groups is 3.36 (5 %) and 4.17 (1 %).
* $p \leq 0.05$; ** $p \leq 0.01$ and NS – Non Significant
Means bearing superscripts p,q,r,s in rows show statistical significance between the groups while means bearing superscripts a,b,c,d in columns show the statistical significance within the groups.

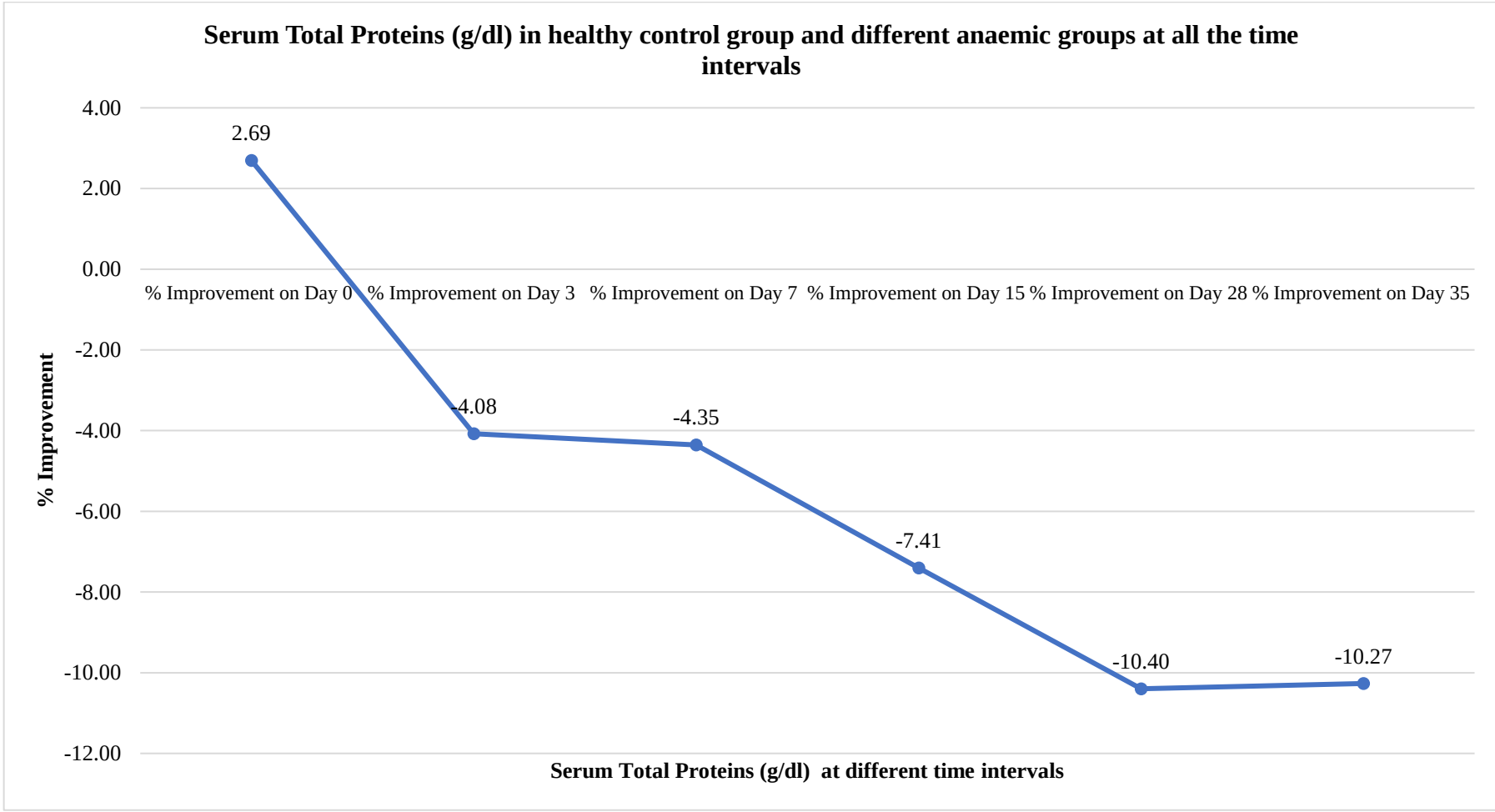


Figure 4.18 Serum Total Proteins (g/dl) in healthy control group and different anaemic groups at all the time intervals (n=24).

Table 4.47: Mean ± SE Albumin (g/dl) in healthy control group and different anaemic groups at all the time intervals

Time Intervals	CKD (a) (n=6)	*E. canis (b)* (n=6)	*B. canis (c)* (n=6)	Pyometra(d) (n=6)	Healthy(e) (n=6)	Overall (a+b+c+d) (n=24)	F value	P value	S / NS
BT	1.95±0.14^{q}	2.18±0.15^{q}	1.73±0.07^{dq}	2.00±0.30^{q}	3.19±0.02^{bcp}	1.97±0.10	10.98	0.000	**
Day 0	2.10±0.11^{q}	2.23±0.11^{q}	2.25±0.14^{cdq}	2.01±0.21^{q}	3.27±0.03^{abp}	2.15±0.07	14.10	0.000	**
Day 3	2.06±0.08^{q}	2.40±0.14^{q}	2.25±0.08^{cdq}	2.12±0.23^{q}	3.29±0.03^{ap}	2.21±0.08	13.49	0.000	**
Day 7	2.06±0.06^{q}	2.42±0.05^{q}	2.33±0.20^{bcq}	2.15±0.22^{q}	3.10±0.03^{cp}	2.24±0.08	8.54	0.000	**
Day 15	1.94±0.15^{s}	2.44±0.07^{qr}	2.64±0.22^{abcq}	2.04±0.20^{rs}	3.15±0.02^{cp}	2.27±0.10	9.67	0.000	**
Day 28	2.13±0.29^{q}	2.72±0.12^{pq}	2.88±0.25^{abp}	2.16±0.26^{q}	3.16±0.03^{cp}	2.48±0.13	4.39	0.007	**
Day 35	2.58±0.17^{pq}	2.31±0.34^{q}	3.08±0.25^{ap}	2.10±0.25^{q}	3.16±0.03^{cp}	2.52±0.15	3.91	0.013	*
Overall	2.12±0.07	2.39±0.06	2.45±0.09	2.09±0.09	3.19±0.01				
F value	1.76	1.05	5.67	0.06	4.62				
P value	0.136	0.406	0.000	0.998	0.001				
S / NS	NS	NS	**	NS	**				

Table F-value for Days is 2.37 (5 %) and 2.75 (1 %); Table F value for Groups is 3.36 (5 %) and 4.17 (1 %).
* $p \leq 0.05$; ** $p \leq 0.01$ and NS – Non Significant
Means bearing superscripts p,q,r,s in rows show statistical significance between the groups while means bearing superscripts a,b,c,d in columns show the statistical significance within the groups.

4.2.16 Serum Albumin (g/dl) in healthy control group and different anaemic groups at all the time intervals:

Table 4.47 (Figure 4.19) depicts albumin (g/dl) (Mean± SE) of dogs in different anaemic groups as well as healthy control group at all the time intervals. The overall albumin values in before treatment, Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35 were 1.97 ± 0.10, 2.15 ± 0.07, 2.21 ± 0.08, 2.24 ± 0.08, 2.27 ± 0.10, 2.48 ± 0.13 and 2.52 ± 0.15, respectively with percent increment of 9.29, 12.34, 13.99, 15.27, 25.83 and 28.12 on Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35 respectively (Table 4.22).

Comparative analysis of the albumin values between CKD, *E. canis*, *B. canis* and pyometra groups revealed significant ($p \leq 0.01$) differences throughout all the study intervals when compared individually with the healthy control groups on respective days. This showed that there was a significant ($p \leq 0.01$) decrease in the albumin value in all the anaemic groups than the Healthy control group.

The albumin values in CKD, *E. canis*, *B. canis* and pyometra anaemic groups on before treatment were 1.95 ± 0.14, 2.18 ± 0.15, 1.73 ± 0.07 and 2.00 ± 0.30 and on Day 35 were 2.58 ± 0.17, 2.31 ± 0.34, 3.08 ± 0.25 and 2.10 ± 0.25 respectively having overall increment of 32.30 %, 5.96 %, 78.03 % and 5.00 %. The highest percentage of improvement in albumin was noted in *B. canis* (78.03) followed by CKD (32.30), *E. canis* (5.96) and pyometra (5.00) anaemic dogs. There is continuous albumin synthesis and degredation in dogs. This degradation is caused by number of factors including malnutrition, kidney disease, protein losing enteropathy, inflammation, infection and liver disease. Synthesis of albumin takes a longer time as compared to its degradation. There was statistically significant ($p \leq 0.05$) increase in the albumin values of *B. canis* while non-significant increase in CKD, *E. canis* and pyometra anaemic groups when Day 35 values of respective disease were compared with before treatment values.Intravenous supplementation of amino acids, gradual increase in the food intake and decreased proteinuria might have helped in restoration of the albumin level in the anaemic dogs.

Conclusively, a post-treatment albumin study revealed that since there is a significant increase in the albumin values on Day 35 in CKD, *E. canis*, *B. canis*

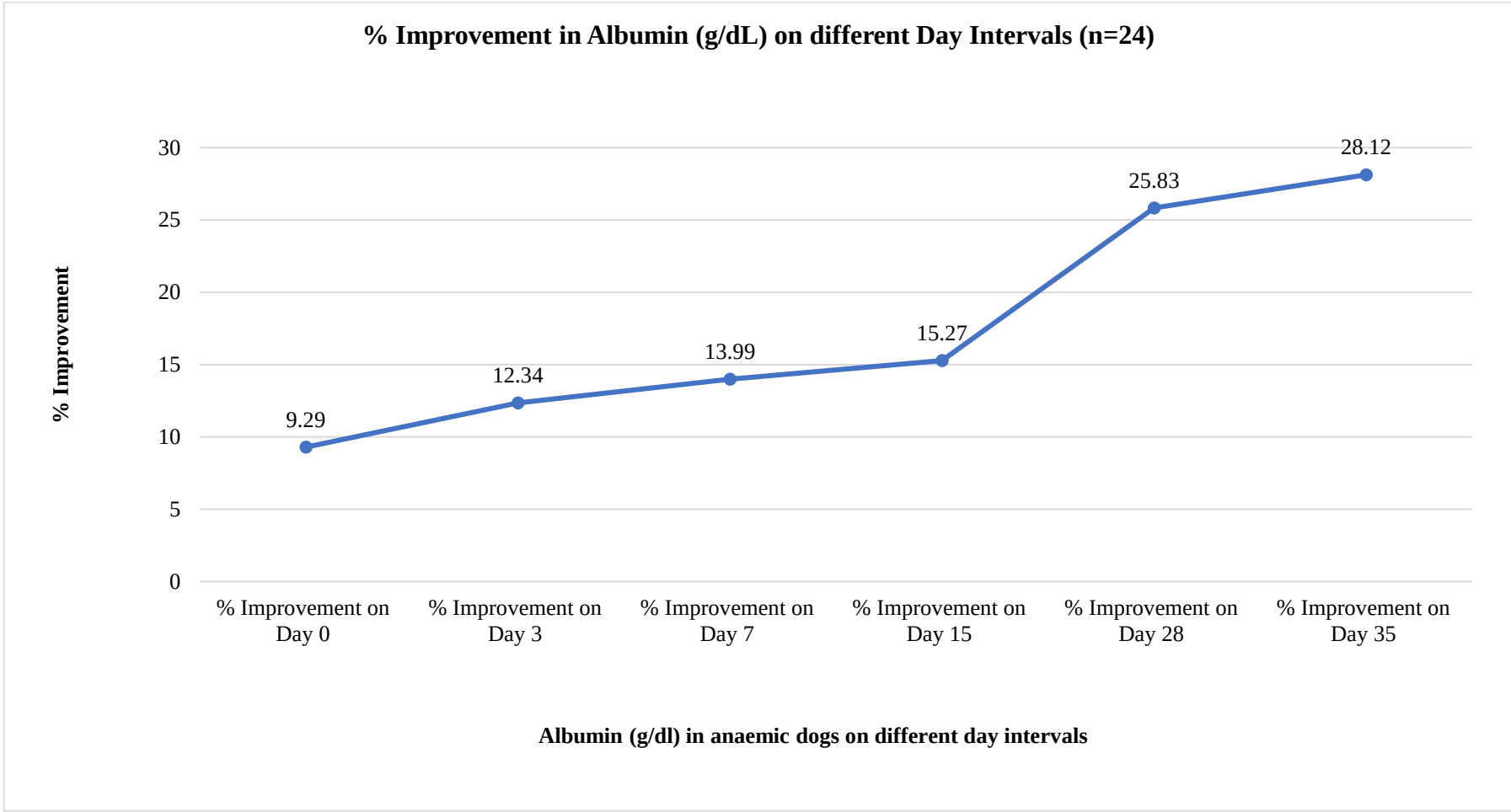


Figure 4.19 Percent Improvement in Albumin in Anaemic dogs on different Day Intervals (n=24)

and pyometra groups, it showed that the whole blood transfusion in which albumin was readily available combined with disease-specific treatment had a beneficial impact on increasing albumin level. The relative rise in the albumin can also be attributed to the lowering of globulin levels in the blood.

4.2.17 Serum Globulin (g/dl) in healthy control group and different anaemic groups at all the time intervals:

Table 4.48 (Figure 4.20) shows serum globulin (g/dl) (Mean± SE) of dogs in different anaemic groups as well as healthy control group at all the time intervals. The overall globulin values in before treatment, Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35 were 4.86 ± 0.25, 4.89 ± 0.25, 4.36 ± 0.16, 4.31 ± 0.22, 4.08 ± 0.16, 3.67 ± 0.12 and 3.63 ± 0.14, respectively with percent increment of 0.67 on Day 0 followed by decrement of 10.16%, 11.21 %, 16.06 %, 24.34 % and 25.26 % on Day 3, Day 7, Day 15, Day 28 and Day 35 respectively (Table 4.22).

Comparative analysis of the globulin values between CKD, *E. canis*, *B. canis* and pyometra groups revealed significant ($p \leq 0.01$) differences throughout all the study intervals when compared individually with the healthy control groups on respective days. This showed that there was a significant ($p \leq 0.01$) increase in the globulin value in all the anaemic groups than the Healthy control group.

The globulin values in CKD, *E. canis*, *B. canis* and pyometra anaemic groups on before treatment were 5.51 ± 0.70, 5.39 ± 0.57, 4.16 ± 0.04 and 4.35 ± 0.20 and on Day 35 were 3.73 ± 0.22, 4.24 ± 0.19, 3.40 ± 0.20 and 3.13 ± 0.31 respectively having overall decrement as 32.29, 21.24, 18.15, 28.11 and 1.09 in respective anaemic groups. The highest percentage of decrement in globulin was noted in CKD (32.29,) followed by pyometra (28.11), *E. canis* (21.24), *B. canis* (18.15) anaemic dogs.

There was statistically significant ($p \leq 0.05$) decrease in the globulin values of CKD and pyometra groups while non-significant decrease in *E. canis* and *B. canis* anaemic groups when Day 35 values of respective diseases were compared with before treatment values.

Table 4.48: Mean ± SE Globulin (g/dl) in the healthy control group and different anaemic groups at all the time intervals

Time Intervals	CKD (a) (n=6)	*E. canis (b)* (n=6)	*B. canis (c)* (n=6)	Pyometra(d) (n=6)	Healthy(e) (n=6)	Overall (a+b+c+d) (n=24)	F value	P value	S / NS
BT	5.51 ± 0.70^{ap}	5.39 ± 0.57^{pq}	4.16 ± 0.04^{qr}	4.35 ± 0.20^{apqr}	3.21 ± 0.04^{r}	4.86 ± 0.25	5.22	0.003	**
Day 0	5.13 ± 0.51^{abpq}	5.98 ± 0.64^{p}	4.11 ± 0.16^{qr}	4.31 ± 0.13^{aqr}	3.15 ± 0.04^{r}	4.89 ± 0.25	7.82	0.000	**
Day 3	4.33 ± 0.22^{abcpq}	5.06 ± 0.48^{p}	4.20 ± 0.12^{q}	3.85 ± 0.13^{aqr}	3.15 ± 0.03^{r}	4.36 ± 0.16	7.57	0.000	**
Day 7	4.36 ± 0.37^{abcpq}	5.09 ± 0.53^{p}	3.76 ± 0.41^{q}	4.01 ± 0.26^{apq}	3.25 ± 0.05^{q}	4.31 ± 0.22	3.54	0.020	*
Day 15	4.08 ± 0.30^{bcpq}	4.67 ± 0.41^{p}	3.65 ± 0.19^{qr}	3.88 ± 0.21^{apqr}	3.19 ± 0.07^{r}	4.08 ± 0.16	4.23	0.009	**
Day 28	3.84 ± 0.20^{cp}	4.06 ± 0.19^{p}	3.61 ± 0.18^{pq}	3.16 ± 0.28^{bq}	3.21 ± 0.03^{q}	3.67 ± 0.12	4.03	0.011	*
Day 35	3.73 ± 0.22^{cpq}	4.24 ± 0.19^{p}	3.40 ± 0.20^{q}	3.13 ± 0.31^{bq}	3.18 ± 0.03^{q}	3.63 ± 0.14	4.59	0.006	**
Overall	4.43 ± 0.17	4.93 ± 0.19	3.85 ± 0.09	3.82 ± 0.11	3.20 ± 0.02				
F value	2.71	2.06	2.05	4.63	0.54				
P value	0.028	0.083	0.083	0.001	0.768				
S / NS	*	NS	NS	**	NS				

Table F-value for Days is 2.37 (5 %) and 2.75 (1 %); Table F value for Groups is 3.36 (5 %) and 4.17 (1 %).
* $p\leq0.05$; ** $p\leq0.01$ and NS – Non Significant
Means bearing superscripts p,q,r,s in rows show statistical significance between the groups while means bearing superscripts a,b,c,d in columns show the statistical significance within the groups.

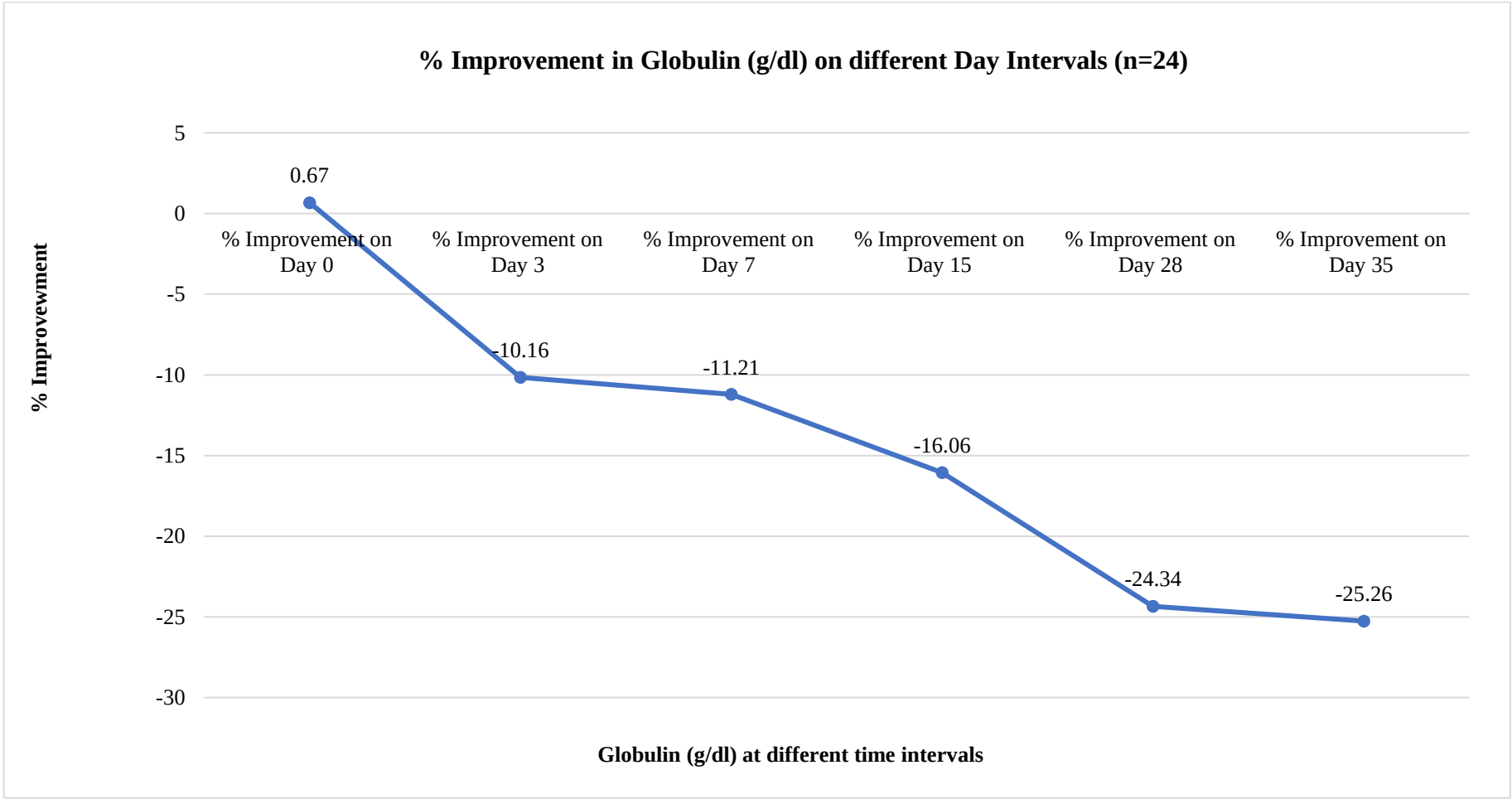


Figure 4.20 Percent Improvement in Globulin (g/dl) in different Anaemic dogs on different Day Intervals (n=24)

The use of immunosuppressive medications might have caused the reduced activity of inflammatory mediators such as peptides, glycoproteins, cytokines, arachidonic acid metabolites (prostaglandins and leukotrienes), nitric oxide, and oxygen free radicals.

In the present study the significnat hypoalbuminia was noted in all the anaemic dogs, hence this may act as compensatory mechanism for the hyprgloblulinemic state in order to maintain the oncotic pressure and prevent increae in the blood viscocity.

Conclusively, post-treatment globulin study revealed that since there is a significant decrease in the globulin value on Day 35 in CKD, *E. canis*, *B. canis* and pyometra groups, it showed that the whole blood transfusion and disease-specific treatment has a beneficial impact on globulin. Administration of glucocorticoids at immune-suppressive doses might have resulted in terminating the immune complex cascade which would otherwise have led to increased globulin levels. Also, the relative rise in the albumin might have contributed to the lowering of globulin levels in the blood.

4.2.18 A: G ratio in healthy control group and different anaemic groups at all the time intervals:

Table 4.49 (Figure 4.21) indicates the A: G ratio (Mean± SE) of dogs in different anaemic groups as well as healthy control group at all the time intervals. The overall A: G ratio values in before treatment, Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35 were 0.42 ± 0.02, 0.46 ± 0.03, 0.52 ± 0.02, 0.55 ± 0.03, 0.58 ± 0.04, 0.70 ± 0.05 and 0.74 ± 0.06, respectively with percent increment in A: G ratio as 10.78, 24.31, 31.86, 38.20, 67.78 and 75.99 on Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35, respectively (Table 4.22).

Comparative analysis of the A: G ratio values between CKD, *E. canis*, *B. canis* and pyometra groups on BT, Day 0, 3, 7,15, 28 and 35 revealed significant ($p \leq 0.01$) skewed difference when compared individually with the healthy control groups on respective days. This showed that there was a significant ($p \leq 0.01$) decrease in the A: G ratio value in all the anaemic groups than the Healthy control group.

Table 4.49: Mean ± SE A: G Ratio in the healthy control group and different anaemic groups at all the time intervals

Time Intervals	CKD (a) (n=6)	*E. canis (b)* (n=6)	*B. canis (c)* (n=6)	Pyometra(d) (n=6)	Healthy(e) (n=6)	Overall (a+b+c+d) (n=24)	F value	P value	S / NS
BT	0.36 ± 0.03^{cq}	0.43 ± 0.05^{bq}	0.41 ± 0.01^{dq}	0.46 ± 0.07^{q}	0.99 ± 0.01^{p}	0.42 ± 0.02	33.29	0.000	**
Day 0	0.42 ± 0.04^{bcq}	0.39 ± 0.04^{bq}	0.55 ± 0.05^{cdq}	0.47 ± 0.05^{q}	1.04 ± 0.01^{p}	0.46 ± 0.03	33.49	0.000	**
Day 3	0.48 ± 0.04^{bcq}	0.49 ± 0.04^{bq}	0.53 ± 0.01^{cdq}	0.55 ± 0.07^{q}	1.04 ± 0.02^{p}	0.52 ± 0.02	30.15	0.000	**
Day 7	0.48 ± 0.03^{bcq}	0.50 ± 0.05^{abq}	0.65 ± 0.07^{bcq}	0.55 ± 0.08^{q}	0.95 ± 0.02^{p}	0.55 ± 0.03	11.02	0.000	**
Day 15	0.48 ± 0.05^{bcr}	0.54 ± 0.05^{abr}	0.74 ± 0.08^{abcq}	0.53 ± 0.06^{r}	0.99 ± 0.02^{p}	0.58 ± 0.04	12.21	0.000	**
Day 28	0.56 ± 0.09^{abq}	0.68 ± 0.05^{aq}	0.81 ± 0.09^{abpq}	0.73 ± 0.13^{pq}	0.98 ± 0.01^{p}	0.70 ± 0.05	3.25	0.027	*
Day 35	0.71 ± 0.08^{apq}	0.55 ± 0.09^{abq}	0.93 ± 0.11^{ap}	0.73 ± 0.14^{pq}	0.99 ± 0.01^{p}	0.74 ± 0.06	3.19	0.030	*
Overall	0.50 ± 0.03	0.51 ± 0.02	0.66 ± 0.04	0.58 ± 0.04	1.00 ± 0.01				
F value	3.57	2.47	5.94	1.44	2.31				
P value	0.007	0.042	0.000	0.225	0.054				
S / NS	**	*	**	NS	NS				

Table F-value for Days is 2.37 (5 %) and 2.75 (1 %); Table F value for Groups is 3.36 (5 %) and 4.17 (1 %).
* $p \leq 0.05$; ** $p \leq 0.01$ and NS – Non Significant
Means bearing superscripts p,q,r,s in rows show statistical significance between the groups while means bearing superscripts a,b,c,d in columns show the statistical significance within the groups.

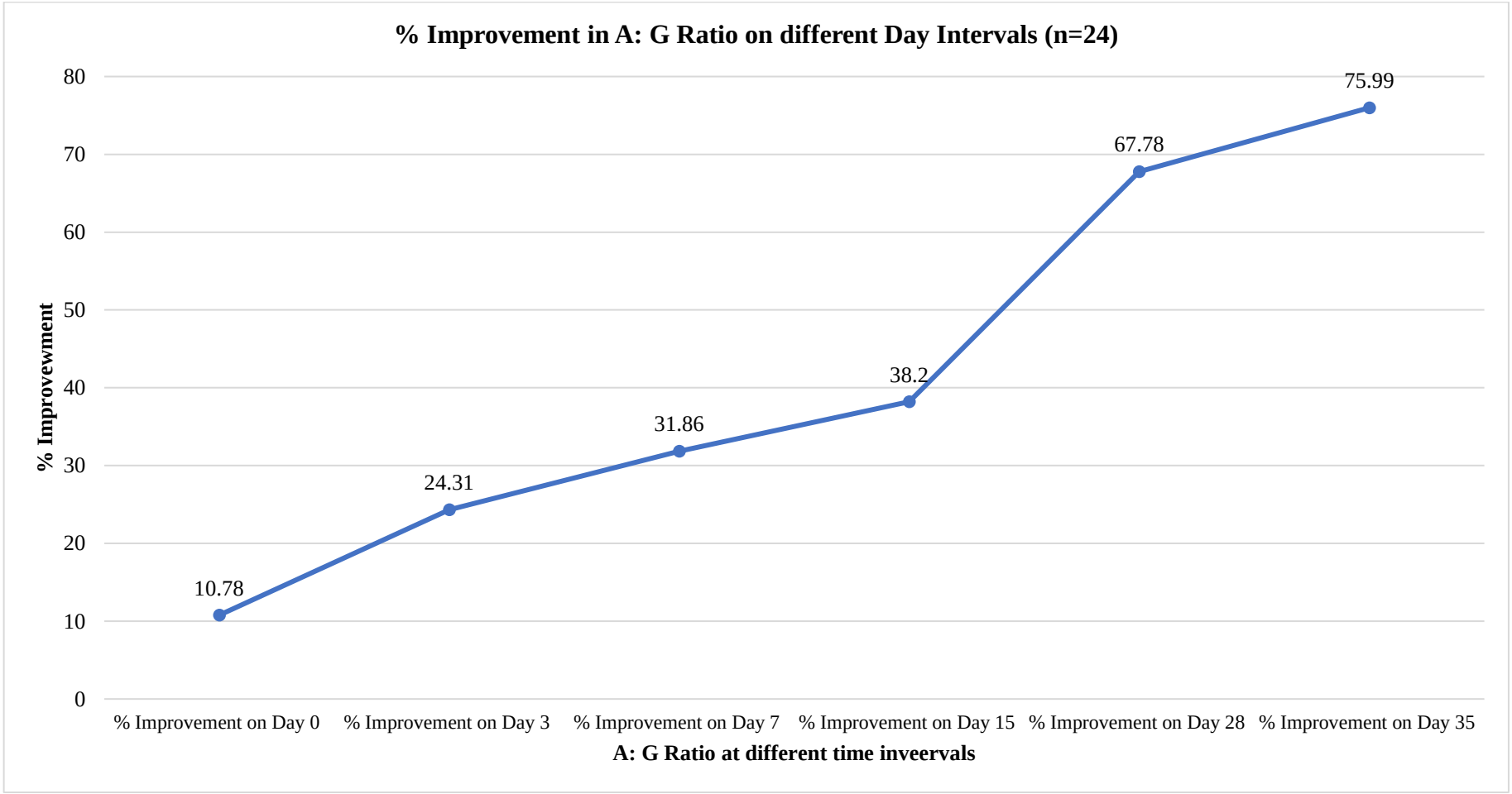


Figure 4.21 Percent Improvement in A: G Ratio in Anaemic dogs on different Day Intervals (n=24)

The A: G ratio values in CKD, *E. canis*, *B. canis* and pyometra anaemic groups on before treatment were 0.36 ± 0.03, 0.43 ± 0.05, 0.41 ± 0.01 and 0.46 ± 0.07 and on Day 35 were 0.71 ± 0.08, 0.55 ± 0.09, 0.93 ± 0.11 and 0.73 ± 0.14 respectively having overall percent improvement as 99.44, 27.91, 123.74 and 59.40 in respective anaemic groups.

The highest percentage of increment in the A: G ratio was noted in *B. canis* (123.74) followed by CKD (99.44), pyometra (59.40) and *E. canis* (27.91) anaemic dogs. There was statistically significant increase in the A: G ratio values of CKD, *E. canis* and *B. canis* anaemic groups while non-significant increase in the pyometra group when Day 35 values of respective disease were compared with before treatment values.

Conclusively, post treatment A: G ratio study revealed that there is a significant increase in the A: G ratio between before treatment and Day 35 in CKD, *E. canis*, *B. canis* and pyometra groups. The increasing trend ratio of A: G from Day 0 to Day 35 in every anaemic group indicated that the hyperglobulinemia was decreasing consistently and consecutively. The rise in albumin and the relative decrease in globulin levels contributed in increasing the A: G ratio in the present study.

4.2.19 Blood lactate (mmol/L) values in healthy control group and different anaemic groups at all the time intervals:

Table 4.50 (Figure 4.22) describes blood lactate (mmol/L) (Mean± SE) of dogs in different anaemic groups as well as healthy control group at all the time intervals. The overall lactate values in before treatment, Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35 were 4.80 ± 0.31, 4.41 ± 0.31, 4.10 ± 0.25, 3.83 ± 0.25, 3.42 ±0.26, 2.98 ± 0.22 and 2.33 ± 0.26, respectively with percent decrement in lactate as 8.19, 14.50,20.20, 28.74, 37.96 and 51.44 on Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35, respectively (Table 4.22).

Comparative analysis of the lactate values between CKD, *E. canis*, *B. canis* and pyometra groups on BT, Day 0, 3, 7,15, 28 and 35 revealed significant ($p \leq 0.05$) differences when compared individually with the healthy control groups on respective days. This showed that the anaemic group had a significantly

Table 4.50: Mean ± SE Lactate (mmol/L) level in healthy control group and different anaemic groups at all the time intervals

Time Intervals	CKD (a) (n=6)	*E. canis (b)* (n=6)	*B. canis (c)* (n=6)	Pyometra(d) (n=6)	Healthy(e) (n=6)	Overall (a+b+c+d) (n=24)	F value	P value	S / NS
BT	6.11±1.05^{p}	4.85±0.20^{apq}	4.56±0.14aq	3.67±0.08^{aq}	0.86±0.14^{br}	4.80±0.31	16.14	0.000	**
Day 0	5.59±0.92^{p}	4.87±0.10^{apq}	4.15±0.09^{abqr}	3.00±0.45^{abr}	1.18±0.18^{abs}	4.41±0.31	13.45	0.000	**
Day 3	5.48±0.66^{p}	4.20±0.23^{abq}	3.71±0.10^{bcqr}	3.01±0.09^{abr}	1.58±0.15^{as}	4.10±0.25	19.41	0.000	**
Day 7	5.60±0.14^{p}	3.65±0.32^{bq}	3.41±0.18^{cdq}	2.65±0.06^{bcr}	1.55±0.09^{as}	3.83±0.25	62.44	0.000	**
Day 15	5.11±0.08^{p}	2.66±0.46^{cq}	3.11±0.11^{deq}	2.78±0.45^{bq}	1.53±0.13^{ar}	3.42±0.26	18.56	0.000	**
Day 28	4.60±0.14^{p}	2.55±0.33^{cqr}	2.73±0.15^{eq}	2.01±0.09^{cdrs}	1.48±0.13^{as}	2.98±0.22	38.46	0.000	**
Day 35	4.22±0.13^{p}	2.10±0.25^{cq}	1.43±0.30^{fr}	1.56±0.11^{dqr}	1.26±0.07^{abr}	2.33±0.26	37.97	0.000	**
Overall	5.25±0.23	3.56±0.19	3.30±0.16	2.67±0.13	1.35±0.06				
F value	1.19	15.02	35.14	7.49	3.67				
P value	0.329	0.000	0.000	0.000	0.006				
S / NS	NS	**	**	**	**				

Table F-value for Days is 2.37 (5 %) and 2.75 (1 %); Table F value for Groups is 3.36 (5 %) and 4.17 (1 %).
* $p \leq 0.05$; ** $p \leq 0.01$ and NS – Non Significant
Means bearing superscripts p,q,r,s in rows show statistical significance between the groups while means bearing superscripts a,b,c,d in columns show the statistical significance within the groups.

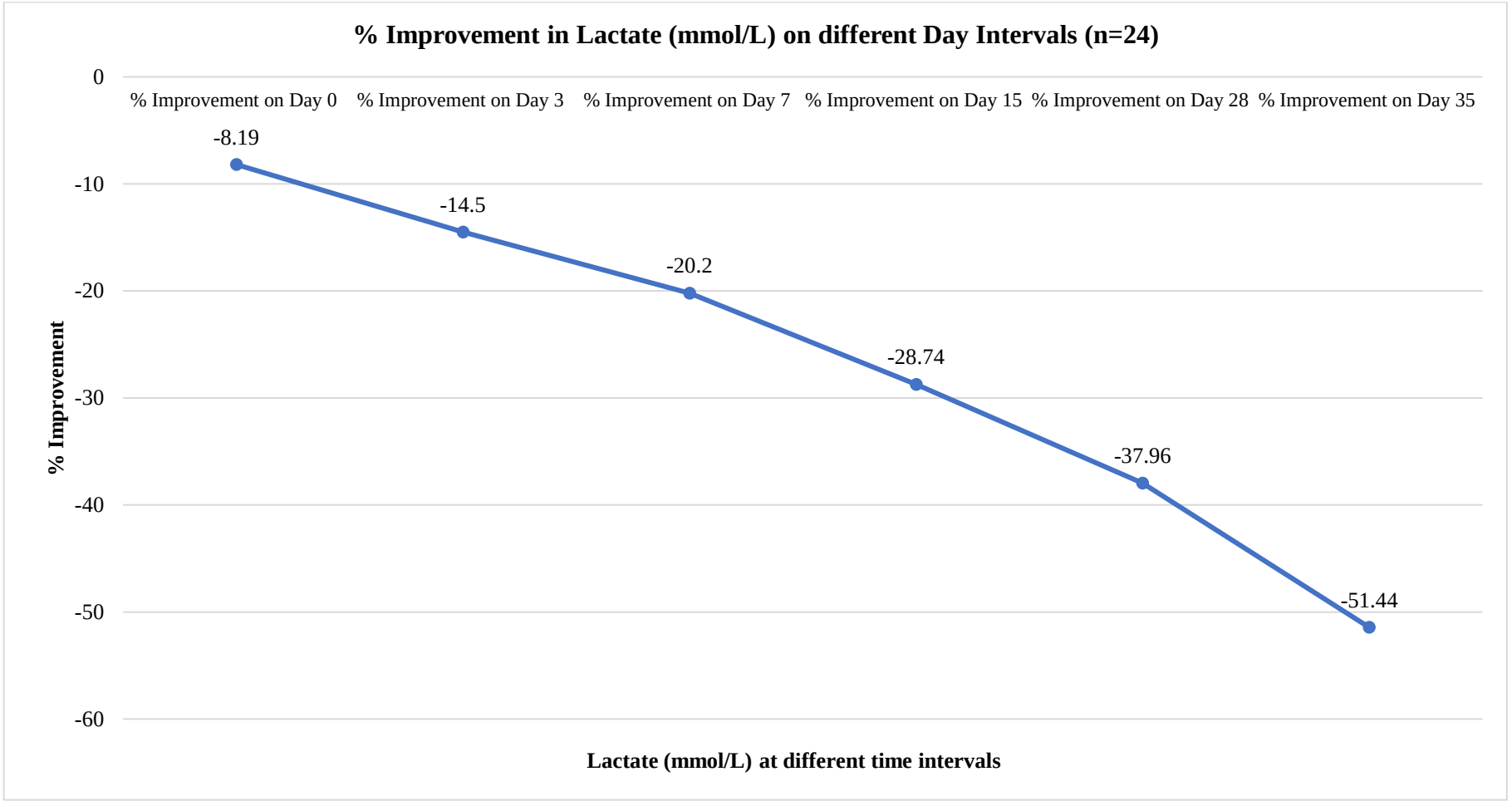


Figure 4.22 Percent Improvement in Lactate (mmol/L) in anaemic dogs on different Day Intervals (n=24)

higher lactate value than the healthy control group.

The lactate values in CKD, *E. canis*, *B. canis* and pyometra anaemic groups on before treatment was 6.11 ± 1.05, 4.85 ± 0.20, 4.56 ± 0.14 and 3.67 ± 0.08 and on Day 35 were 4.22 ± 0.13, 2.10 ± 0.25, 1.43 ± 0.30 and 1.56 ± 0.11, respectively having overall percent decrement as 30.90, 56.72, 68.60, and 7.39 in respective anaemic groups. The highest percentage of decrement in lactate was noted in *B. canis* (68.60) followed by pyometra (57.39), *E. canis* (56.72), and CKD (30.90) anaemic dogs.

There was statistically significant decrease in the lactate values of *E. canis*, *B. canis* and pyometra anaemic groups while a non-significant decrease in CKD when Day 35 values of respective disease were compared with before treatment values.

When the amount of lactate produced in oxygen-deprived tissue is higher than the amount of lactate broken down in the body, the concentration of lactate in the blood rises. Hyperlactatemia can occur in dogs with immunological hemolytic anaemia (IMHA) as a consequence of reduced oxygen supply to tissues caused by severe anaemia or systemic hypoperfusion. Post-treatment lactate level was decreasing gradually owing to increased Iron stores, increased haemoglobin and in turn minimizing the hypoxia in anaemic dogs.

Conclusively, a post-treatment lactate study revealed that there is a decrease in the lactate values between before treatment and Day 35 in CKD, *E. canis*, *B. canis* and pyometra groups, it showed that disease-specific treatment was efficacious in decreasing the hyperlactatemia in anaemic dogs.

4.2.20 Reticulocyte (%) and Reticulocyte Production Index (RPI) in healthy control group and different anaemic groups at all the time intervals:

Tables 4.51 and Table 4.53 (Figure 4.23) exhibit reticulocyte (%) and reticulocyte production index (Mean± SE) of dogs in different anaemic groups as well as healthy control group at all the time intervals. The overall reticulocyte %) in before treatment, Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35 were 4.66 ± 1.33, 4.37 ± 1.05, 6.11 ± 1.57, 4.32 ± 1.03, 3.67 ± 0.96, 4.19 ± 0.98 and 4.32 ± 1.02 respectively with respective percent decrement of 6.34, 7.30, 21.24, 10.14

Table 4.51: Mean ± SE Reticulocyte (%) count in healthy control group and different anaemic groups at all the time intervals

Time Intervals	CKD (a) (n=6)	*E. canis (b)* (n=6)	*B. canis (c)* (n=6)	Pyometra(d) (n=6)	Healthy(e) (n=6)	Overall (a+b+c+d) (n=24)	F value	P value	S / NS
BT	0.83±0.06^{dq}	2.33±1.49^{q}	14.49±2.07^{p}	0.98±0.13^{q}	0.75±0.10^{q}	4.66±1.33	26.96	0.000	**
Day 0	1.09±0.18b^{cdq}	3.12±1.80^{q}	12.16±0.24^{p}	1.08±0.17^{q}	0.86±0.10^{q}	4.37±1.05	34.39	0.000	**
Day 3	1.22±0.01^{abq}	8.98±5.04^{p}	12.87±0.16^{p}	1.35±0.22^{q}	0.76±0.11^{q}	6.11±1.57	6.04	0.001	**
Day 7	0.94±0.11^{bcdq}	2.65±1.51^{q}	12.25±0.46^{p}	1.43±0.31^{q}	0.90±0.14^{q}	4.32±1.03	44.90	0.000	**
Day 15	0.90±0.06^{cdq}	0.91±0.14^{q}	11.42±0.75^{p}	1.44±0.41^{q}	0.93±0.07^{q}	3.67±0.96	139.14	0.000	**
Day 28	1.17±0.11^{abcq}	2.17±0.68^{q}	11.61±1.40^{p}	1.79±0.66^{q}	0.93±0.19^{q}	4.19±0.98	35.28	0.000	**
Day 35	1.40±0.05^{aq}	2.27±0.41^{q}	11.58±2.04^{p}	2.00±0.71^{q}	1.03±0.10^{q}	4.32±1.02	20.33	0.000	**
Overall	1.08±0.05	3.21±0.86	12.34±0.47^{p}	1.44±0.16	0.88±0.05				
F value	4.07	1.43	0.70	0.69	0.63				
P value	0.003	0.229	0.645	0.658	0.702				
S / NS	**	NS	NS	NS	NS				

Table F-value for Days is 2.37 (5 %) and 2.75 (1 %); Table F value for Groups is 3.36 (5 %) and 4.17 (1 %).
* $p \leq 0.05$; ** $p \leq 0.01$ and NS – Non Significant
Means bearing superscripts p,q,r,s in rows show statistical significance between the groups while means bearing superscripts a,b,c,d in columns show the statistical significance within the groups.

Table 4.52: Mean ± SE Absolute Reticulocyte (thousand cells/µl) count in healthy control group and different anaemic groups at all the time intervals

Time Intervals	CKD (a) (n=6)	*E. canis (b)* (n=6)	*B. canis (c)* (n=6)	Pyometra(d) (n=6)	Healthy(e) (n=6)	Overall (a+b+c+d) (n=24)	F value	P value	S / NS
BT	15.77±2.02^{cq}	38.28±24.57^{q}	224.33±7.75^{p}	19.92±2.92^{q}	44.86±9.35^{q}	74.58±19.10	50.53	0.000	**
Day 0	19.10±3.57^{cq}	56.65±34.98^{q}	259.53±22.93^{p}	24.78±5.63^{q}	53.62±10.48^{q}	90.02±22.87	26.38	0.000	**
Day 3	22.66±2.47^{cr}	120.26±59.87^{q}	329.29±31.61^{p}	33.86±7.00^{qr}	48.75±6.05^{qr}	126.52±30.17	17.47	0.000	**
Day 7	19.41±3.62^{cq}	53.62±30.87^{q}	345.23±40.26^{p}	34.23±11.04^{q}	55.10±6.24^{q}	113.13±30.57	34.15	0.000	**
Day 15	23.02±1.81^{cq}	23.32±3.38^{q}	339.17±30.48^{p}	47.86±18.26^{q}	63.17±8.28^{q}	108.35±29.09	67.84	0.000	**
Day 28	33.50±4.37^{bq}	55.29±11.43^{q}	352.03±34.29^{p}	62.29±23.57^{q}	63.66±15.15^{q}	125.78±29.14	42.49	0.000	**
Day 35	45.24±3.60^{aq}	64.71±6.64^{q}	355.10±54.96^{p}	70.61±26.20^{q}	67.66±7.93^{q}	133.92±30.29	22.56	0.000	**
Overall	25.53±1.87	58.88±11.55	314.96±14.17	41.94±6.22	56.69±3.54				
F value	10.43	0.98	2.23	1.42	0.78				
P value	0.000	0.449	0.062	0.234	0.588				
S / NS	**	NS	NS	NS	NS				

Table F-value for Days is 2.37 (5 %) and 2.75 (1 %); Table F value for Groups is 3.36 (5 %) and 4.17 (1 %).
* $p \leq 0.05$; ** $p \leq 0.01$ and NS – Non Significant
Means bearing superscripts p,q,r,s in rows show statistical significance between the groups while means bearing superscripts a,b,c,d in columns show the statistical significance within the groups.

Table 4.53: Mean ± SE RPI values in healthy control group and different anaemic groups at all the time intervals

Time Intervals	CKD (a) (n=6)	*E. canis (b)* (n=6)	*B. canis (c)* (n=6)	Pyometra(d) (n=6)	Healthy(e) (n=6)	Overall (a+b+c+d) (n=24)	F value	P value	S / NS
BT	0.13±0.01^{cq}	0.32±0.20^{q}	2.01±0.10^{p}	0.15±0.02^{q}	0.34±0.06^{q}	0.66±0.17	55.16	0.000	**
Day 0	0.15±0.02^{cq}	0.45±0.27^{q}	2.21±0.32^{p}	0.17±0.03^{q}	0.41±0.07^{q}	0.75±0.20	19.70	0.000	**
Day 3	0.18±0.02^{cq}	1.24±0.64^{q}	2.75±0.39^{p}	0.24±0.05^{q}	0.38±0.05^{q}	1.11±0.28	10.43	0.000	**
Day 7	0.16±0.03^{cq}	0.45±0.24^{q}	3.05±0.36^{p}	0.26±0.07^{q}	0.41±0.05^{q}	0.98±0.27	37.00	0.000	**
Day 15	0.18±0.01^{cq}	0.19±0.02^{q}	3.19±0.27^{p}	0.33±0.12^{q}	0.41±0.02^{q}	0.98±0.28	91.16	0.000	**
Day 28	0.26±0.03^{bq}	0.45±0.09^{q}	2.99±0.32^{p}	0.46±0.18^{q}	0.44±0.08^{q}	1.04±0.25	43.00	0.000	**
Day 35	0.36±0.02^{aq}	0.52±0.06^{q}	2.87±0.44^{p}	0.54±0.20^{q}	0.50±0.04^{q}	1.08±0.25	23.72	0.000	**
Overall	0.21±0.01	0.52±0.11	2.73±0.13	0.31±0.05	0.41±0.02				
F value	9.28	1.32	1.77	1.49	0.68				
P value	0.000	0.274	0.132	0.208	0.662				
S / NS	**	NS	NS	NS	NS				

Table F-value for Days is 2.37 (5 %) and 2.75 (1 %); Table F value for Groups is 3.36 (5 %) and 4.17 (1 %).
* $p \leq 0.05$; ** $p \leq 0.01$ and NS – Non Significant
Means bearing superscripts p,q,r,s in rows show statistical significance between the groups while means bearing superscripts a,b,c,d in columns show the statistical significance within the groups.

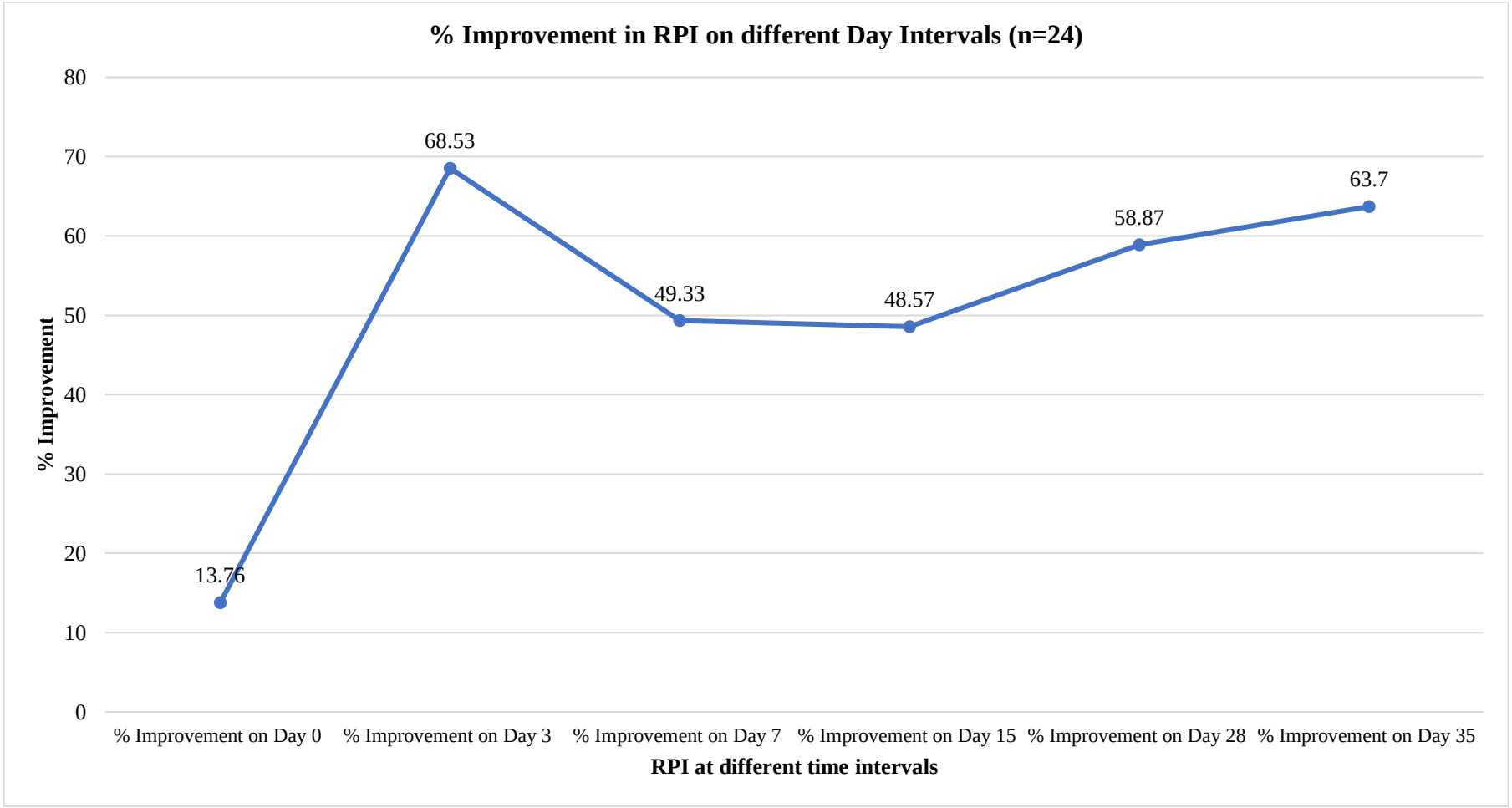


Figure 4.23 Percent Improvement in RPI in Anaemic dogs on different Day Intervals (n=24)

and 7.44 on Day 0, Day 7, Day 15, Day 28 and Day 35 except for Day 3 which witnessed percent increment of 31.08.

The reticulocyte production index values in before treatment, Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35 were 0.66 ± 0.17, 0.75 ± 0.20, 1.11 ± 0.28, 0.98 ± 0.27, 0.98 ± 0.28, 1.04 ± 0.25 and 1.08 ± 0.25 respectively with respective percent increment of 13.76, 68.53, 49.33, 48.57, 58.87 and 63.70 on Day 0, Day 7, Day 15, Day 28 and Day 35.

Comparative analysis of the reticulocyte and reticulocyte production index values between CKD, *E. canis*, *B. canis* and pyometra groups revealed significant ($p \leq 0.05$) differences throughout all the study intervals when compared individually with the healthy control groups on respective days.

Overall reticulocyte and reticulocyte production index revealed higher values in *B. canis* anaemic group followed by *E. canis*, pyometra and CKD groups.

The reticulocyte count in CKD, *E. canis*, *B. canis* and pyometra anaemic groups on before treatment were 0.83 ± 0.06, 2.33 ± 1.49, 14.49 ± 2.07 and 0.98 ± 0.13 having increment of 67.86%, decrement of 2.61%, decrement of 20.06% and increment of 102.43% respectively.

The reticulocyte production index values in CKD, *E. canis*, *B. canis* and pyometra anaemic groups on before treatment were 0.13 ± 0.01, 0.32 ± 0.20, 2.01 ± 0.10 and 0.15 ± 0.02 having increment of 174.44 %, 62.54%, 42.32% and 247.13% respectively in respective anaemic groups.

The highest percentage of improvement in reticulocyte count was in pyometra, followed by CKD, *E. canis* and *B. canis* while the percentage of improvement in reticulocyte production index was in pyometra (247.13) followed by CKD (174.44), *E. canis* (62.54) and *B. canis* (42.32) anaemic dogs.

There was statistically non-significant increase in the reticulocyte and reticulocyte production index values of *E. canis*, *B. canis* and pyometra anaemic groups while significant increase in CKD when Day 35 values of respective diseases were compared with before treatment values.

Upon analyzing Tables 4.51 and Table 4.52, it is evident that the reticulocyte count (%) and RPI have at par values after the transfusion.

Blood transfusion may be able to stimulate hematopoiesis, according to Robertson (1941). Jain *et al.* (1986) reported that immature erythrocytes appeared in the bloodstream as a result of hemopoiesis. In normal dogs, Sastry (1989) found that reticulocyte levels ranged from 0.0 to 1.5%. According to Bellamy *et al.* (1978), reticulocyte counts increased on the fifth- and sixth day following a blood transfusion in a dog suffering from hemolytic anaemia.

The administration of blood from a normal donor animal, which has fewer reticulocytes according to Sastry (1989), may be the cause of the non-significant difference in reticulocyte levels seen following transfusion in our study.

4.2.21 Serum electrolytes [Sodium (mEq/L), Potassium (mEq/L) and Chloride (mEq/L) in healthy control group and different anaemic groups at all the time intervals

Tables 4.54, Table 4.55 and Table 4.56 show Sodium (mEq/L), Potassium (mEq/L), and Chloride (mEq/L) (Mean± SE) of dogs in different anaemic groups as well as healthy control group at all the time intervals.

The overall Sodium, Potassium and Chloride values in before treatment, Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35 were (Sodium: 139.91 ± 5.05, 137.66 ± 4.26, 139.29 ± 3.67, 138.21 ± 2.85, 143.50 ± 2.54, 144.98 ± 1.56 and 149.55 ± 2.37); (Potassium: 5.03 ± 0.23, 4.96 ± 0.22, 6.22 ± 1.44, 5.01 ± 0.16, 4.80 ± 0.12, 4.90 ± 0.10 and 4.93 ± 0.11) and (Chloride: 107.54 ± 0.95, 107.40 ± 0.83, 107.74 ± 0.84, 108.44 ± 0.89, 108.80 ± 0.84, 109.42 ± 0.83 and 111.20 ± 1.22) respectively.

The percent change in Sodium revealed a decrement of 1.61, 0.44 and 1.21 on Day 0, Day 3 and Day 7 respectively followed by respective increments of 2.56, 3.62 and 6.89 on Day 15, Day 28 and Day 35. The Potassium values witnessed a decrement of 1.33, 0.42, 4.64, 2.57 and 1.91 on Day 0, Day 7, Day 15, Day 28 and Day 35 respectively except for an increment of 0.18 on Day 3.

Table 4.54: Mean ± SE Sodium (mEq/L) in healthy control group and different anaemic groups at all the time intervals

Time Intervals	CKD (a) (n=6)	*E. canis (b)* (n=6)	*B. canis (c)* (n=6)	Pyometra(d) (n=6)	Healthy(e) (n=6)	Overall (a+b+c+d) (n=24)	F value	P value	S / NS
BT	142.75±3.01^{qr}	126.66±11.03^{bcrs}	119.88±5.88^{ds}	170.33±1.43^{ap}	147.66±2.15^{q}	139.91±5.05	11.31	0.000	**
Day 0	143.08±1.73^{r}	115.91±4.71^{ct}	126.18±3.89^{cds}	165.46±3.53^{abp}	155.33±2.36^{q}	137.66±4.26	35.43	0.000	**
Day 3	144.13±2.59^{q}	124.93±5.08^{bcr}	125.70±4.39^{cdr}	162.38±2.54^{abcp}	151.00±4.34^{q}	139.29±3.67	17.11	0.000	**
Day 7	141.58±1.15^{qr}	127.00±2.68^{bcs}	130.66±5.76^{bcdrs}	153.60±4.66^{cdp}	148.16±3.14^{pq}	138.21±2.85	8.68	0.000	**
Day 15	144.80±1.93^{qr}	132.63±3.47^{bcs}	137.01±3.67^{abcrs}	159.53±2.34^{bcdp}	151.66±5.40^{pq}	143.50±2.54	9.21	0.000	**
Day 28	141.88±1.16	142.66±3.44^{ab}	141.66±2.82^{ab}	153.68±1.74^{cd}	146.33±4.79	144.98±1.56	2.73	0.051	NS
Day 35	142.96±1.30	154.21±8.46^{a}	148.40±2.53^{a}	152.63±2.92^{d}	157.66±2.18	149.55±2.37	1.72	0.175	NS
Overall	143.03±0.71	132.00±2.85	132.79±2.09	159.66±1.42	151.12±1.43				
F value	0.33	4.14	5.42	5.40	1.27				
P value	0.911	0.003	0.000	0.000	0.296				
S / NS	NS	**	**	**	NS				

Table F-value for Days is 2.37 (5 %) and 2.75 (1 %); Table F value for Groups is 3.36 (5 %) and 4.17 (1 %).

* $p \leq 0.05$; ** $p \leq 0.01$ and NS – Non Significant

Means bearing superscripts p,q,r,s in rows show statistical significance between the groups while means bearing superscripts a,b,c,d in columns show the statistical significance within the groups.

Table 4.55: Mean ± SE Potassium (mEq/L) in healthy control group and different anaemic groups at all the time intervals

Time Intervals	CKD (a) (n=6)	*E. canis (b)* (n=6)	*B. canis (c)* (n=6)	Pyometra(d) (n=6)	Healthy(e) (n=6)	Overall (a+b+c+d) (n=24)	F value	P value	S / NS
BT	3.56±0.16^{r}	5.88±0.34^{abp}	5.96±0.09^{ap}	4.70±0.12^{q}	4.23±0.09^{q}	5.03±0.23	30.58	0.000	**
Day 0	3.53±0.20^{r}	6.00±0.17^{ap}	5.68±0.12^{abp}	4.63±0.25^{q}	4.66±0.20^{q}	4.96±0.22	23.82	0.000	**
Day 3	3.62 ±5.90	5.61±0.19^{ab}	5.50±0.13^{bc}	4.28±0.39	4.91±0.21	6.22±1.44	0.58	0.674	NS
Day 7	4.00±0.14^{s}	5.86±0.08^{abp}	5.38±0.15^{bcpq}	4.78±0.17^{r}	4.90±0.25^{qr}	5.01±0.16	16.54	0.000	**
Day 15	4.16±0.15^{r}	5.30±0.20^{bcp}	5.20±0.12^{cp}	4.51±0.12^{qr}	4.93±0.16^{pq}	4.80±0.12	9.40	0.000	**
Day 28	4.56±0.08^{q}	5.31±0.14^{bcp}	5.13±0.15^{cp}	4.58±0.22^{q}	4.83±0.19^{pq}	4.90±0.10	4.03	0.011	*
Day 35	4.63±0.16	4.90±0.21^{c}	5.11±0.13^{c}	5.08±0.34	4.63±0.24	4.93±0.11	1.01	0.416	NS
Overall	4.85±0.84	5.55±0.09	5.43±0.07	4.65±0.10	4.73±0.08				
F value	0.86	3.66	5.52	0.95	1.53				
P value	0.529	0.006	0.000	0.472	0.197				
S / NS	NS	**	**	NS	NS				

Table F-value for Days is 2.37 (5 %) and 2.75 (1 %); Table F value for Groups is 3.36 (5 %) and 4.17 (1 %).
* $p \leq 0.05$; ** $p \leq 0.01$ and NS – Non Significant
Means bearing superscripts p,q,r,s in rows show statistical significance between the groups while means bearing superscripts a,b,c,d in columns show the statistical significance within the groups.

Table 4.56: Mean ± SE Chloride (mEq/L) in healthy control group and different anaemic groups at all the time intervals

Time Intervals	CKD (a) (n=6)	*E. canis (b)* (n=6)	*B. canis (c)* (n=6)	Pyometra(d) (n=6)	Healthy(e) (n=6)	Overall (a+b+c+d) (n=24)	F value	P value	S / NS
BT	105.15±3.00	108.68±1.52	109.86±1.39	106.46±0.81	106.50±0.56	107.54±0.95	1.26	0.310	NS
Day 0	104.15±1.33^{q}	109.81±1.52^{p}	109.46±1.45^{p}	106.18±1.43^{pq}	110.33±1.05^{p}	107.40±0.83	3.88	0.013	*
Day 3	103.88±1.47	108.56±1.34	110.06±1.84	108.43±1.09	110.50±3.81	107.74±0.84	1.49	0.235	NS
Day 7	104.86±1.29^{q}	110.16±1.60^{q}	110.65±1.84^{q}	108.06±1.68^{q}	121.50±4.68^{p}	108.44±0.89	6.05	0.001	**
Day 15	107.00±1.54	110.50±1.30	110.08±1.76	107.61±1.97	116.66±4.73	108.80±0.84	2.18	0.099	NS
Day 28	106.18±1.68	110.90±1.26	110.08±1.31	110.51±1.88	110.66±3.21	109.42±0.83	0.97	0.440	NS
Day 35	107.73±2.04	112.71±1.54	110.41±1.41	113.91±3.75	117.50±6.95	111.20±1.22	0.95	0.451	NS
Overall	105.57±0.68	110.19±0.55	110.09±0.56	108.74±0.80	113.38±1.63				
F value	0.61	0.95	0.05	1.78	1.62				
P value	0.719	0.470	0.999	0.130	0.168				
S / NS	NS	NS	NS	NS	NS				

Table F-value for Days is 2.37 (5 %) and 2.75 (1 %); Table F value for Groups is 3.36 (5 %) and 4.17 (1 %).

* $p \leq 0.05$; ** $p \leq 0.01$ and NS – Non Significant

Means bearing superscripts p,q,r,s in rows show statistical significance between the groups while means bearing superscripts a,b,c,d in columns show the statistical significance within the groups.

The percent change in Chloride revealed a decrement of 0.13 on Day 0 followed by an increment of 0.18, 0.83, 1.17, 1.75 and 3.40 on Day 3, Day 7, Day 15, Day 28 and Day 35 respectively.

Comparative analysis of the Sodium Potassium and Chloride values between CKD, *E. canis*, *B. canis* and pyometra groups on BT, Day 0, 3, 7, 15, 28 and 35 revealed statistically significant sodium values throughout all the days except non-significant on Day 28 and Day 35, statistically significant potassium values throughout all the days except non-significant on Day 3 and Day 35, statistically non-significant chloride values throughout all the days except significant on Day 0 and Day 7 when compared individually with the healthy control groups on respective days.

The Sodium, Potassium, Chloride values in CKD, *E. canis, B. canis* and pyometra anaemic groups on before treatment were (142.75 ± 3.01, 126.66 ± 11.03, 119.88 ± 5.88 and 170.33 ± 1.43), (3.56 ± 0.16, 5.88 ± 0.34, 5.96 ± 0.09 and 4.70 ± 0.12) and (105.15 ± 3.00, 108.68 ± 1.52, 109.86 ± 1.39 and 106.46 ± 0.81) and on Day 35 (142.96 ± 1.30, 154.21 ± 8.46, 148.40 ± 2.53 and152.63 ± 2.92) (4.63 ± 0.16, 4.90 ± 0.21, 5.11 ± 0.13 and 5.08 ± 0.34) and (107.73 ± 0.68, 112.71 ± 1.54, 110.41 ± 1.41 and 113.91 ± 3.75) respectively.

The overall per cent difference in Sodium values were 1.61 (decrement), 0.44 (decrement), 1.21 (decrement), 2.56 (increment), 3.62 (increment) and 6.89 (increment), Potassium were 1.33 (decrement), 23.61 (increment), 0.42 (decrement), 4.64 (decrement), 2.57 (decrement) and 1.91 (decrement) while Chloride were 0.13 (decrement), 0.18 (increment), 0.83 (increment), 1.17 (increment), 1.75 (increment) and 3.40 (increment) on Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35 respectively.

Sodium level was found to be significantly increased in *E. canis* and *B. canis* and significantly decreased in pyometra while at par values in the CKD anaemic group when Day 35 values of respective disease were compared with before treatment values.

Table 4.57 : Mean ± SE Urinary Protein (mg/dl) in healthy control group and different anaemic groups at all the time intervals

Time Intervals	CKD (a) (n=6)	*E. canis (b)* (n=6)	*B. canis (c)* (n=6)	Pyometra(d) (n=6)	Healthy(e) (n=6)	Overall (a+b+c+d) (n=24)	F value	P value	S / NS
BT	116.93±30.33^{p}	11.18±2.69^{q}	14.11±4.33^{q}	22.66±7.38^{q}	15.08±1.00^{q}	41.23±11.76	10.31	0.000	**
Day 0	176.68±60.96^{p}	9.71±2.76^{q}	25.53±8.62^{q}	25.50±8.62^{q}	15.58±1.18^{q}	59.36±20.29	6.47	0.001	**
Day 3	176.92±48.29^{p}	21.33±9.91^{q}	29.36±11.09^{q}	60.18±33.42^{q}	15.35±1.13^{q}	71.95±19.19	6.15	0.001	**
Day 7	146.60±44.27^{p}	9.20±2.13^{q}	22.65±6.78^{q}	35.33±10.13^{q}	15.03±0.91^{q}	53.45±15.63	7.74	0.000	**
Day 15	145.81±45.51^{p}	42.26±32.41^{q}	23.21±7.50^{q}	51.18±31.09^{q}	14.36±0.63^{q}	65.62±17.97	3.34	0.025	*
Day 28	108.35±14.33^{p}	11.01±2.51^{q}	18.35±4.80^{q}	19.33±4.40^{q}	14.65±0.93^{q}	39.26±9.13	33.74	0.000	**
Day 35	116.38±20.58^{p}	9.66±2.74^{q}	21.81±4.17^{q}	16.50±4.32^{q}	14.45±0.73^{q}	41.09±10.41	21.90	0.000	**
Overall	141.10±14.82	16.34±4.87	22.15±2.60	32.96±6.85	14.93±0.34				
F value	0.48	0.88	0.46	0.84	0.23				
P value	0.812	0.519	0.827	0.546	0.963				
S / NS	NS	NS	NS	NS	NS				

Table F-value for Days is 2.37 (5 %) and 2.75 (1 %); Table F value for Groups is 3.36 (5 %) and 4.17 (1 %).
* $p \leq 0.05$; ** $p \leq 0.01$ and NS – Non Significant
Means bearing superscripts p,q,r,s in rows show statistical significance between the groups while means bearing superscripts a,b,c,d in columns show the statistical significance within the groups.

Table 4.58: Mean ± SE Urinary Creatinine (mg/dl) in healthy control group and different anaemic groups at all the time intervals

Time Intervals	CKD (a) (n=6)	*E. canis (b)* (n=6)	*B. canis (c)* (n=6)	Pyometra(d) (n=6)	Healthy(e) (n=6)	Overall (a+b+c+d) (n=24)	F value	P value	S / NS
BT	77.56±26.98	67.71±10.68	85.70±26.58	74.75±10.77	65.50±7.10	76.43±9.61	0.19	0.941	NS
Day 0	98.78±29.37	62.86±10.88	161.68±68.49	90.16±19.87	64.30±5.44	103.38±19.66	1.32	0.288	NS
Day 3	102.77±31.96	68.33±10.92	142.38±63.06	64.56±10.09	68.18±5.16	94.51±18.07	1.07	0.391	NS
Day 7	107.87±35.59	63.61±8.31	128.71±46.72	80.75±13.58	75.31±5.26	95.24±15.11	0.94	0.456	NS
Day 15	111.96±40.20	67.30±10.44	137.31±55.19	72.18±11.85	69.23±6.85	97.19±17.42	1.00	0.425	NS
Day 28	151.23±51.13	63.05±7.72	101.41±33.94	84.46±12.59	72.08±6.59	100.04±16.21	1.50	0.230	NS
Day 35	170.18±55.07	53.27±1.63	100.46±30.42	119.46±29.30	72.15±7.78	110.85±18.39	2.10	0.110	NS
Overall	117.20±14.69	63.74±3.30	122.53±17.50	83.76±6.38	69.54±2.30				
F value	0.64	0.31	0.31	1.13	0.37				
P value	0.690	0.924	0.925	0.360	0.888				
S / NS	NS	NS	NS	NS	NS				

Table F-value for Days is 2.37 (5 %) and 2.75 (1 %); Table F value for Groups is 3.36 (5 %) and 4.17 (1 %).
* $p \leq 0.05$; ** $p \leq 0.01$ and NS – Non Significant
Means bearing superscripts p,q,r,s in rows show statistical significance between the groups while means bearing superscripts a,b,c,d in columns show the statistical significance within the groups.

Potassium level was found to be significantly decreased in *E. canis* and *B. canis*, while non-significantly increased in CKD and pyometra anaemic dogs when Day 35 values of respective disease were compared with before treatment values.

Chloride level was found to be non-significantly increased throughout all the anaemic groups when Day 35 values of respective disease were compared with before treatment values.

4.2.22 UPC ratio in healthy control group and different anaemic groups at all the time intervals:

Table 4.59 describes the UPC ratios (Mean± SE) of dogs in different anaemic groups as well as healthy control group at all the time intervals. The overall UPC ratio in before treatment, Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35 were 0.64 ± 0.20, 0.69 ± 0.20, 1.06 ± 0.31, 0.68 ± 0.18, 0.98 ± 0.28, 0.50 ± 0.14 and 0.45 ± 0.13, respectively with percent difference in UPC ratio as 7.32, 65.10, 6.81, 53.33, 21.83 (decrement), 29.15 (decrement). (Table 4.22).

Comparative analysis of the UPC ratio values between CKD, *E. canis, B. canis* and pyometra groups revealed significant ($p \leq 0.01$) differences throughout all the day intervals except Day 15 where the non-significant difference was noted when compared individually with the healthy control groups on respective days. The UPC ratio values in CKD, *E. canis*, *B. canis* and pyometra anaemic groups on before treatment were 1.84 ± 0.55, 0.17 ± 0.04, 0.24 ± 0.06 and 0.29 ± 0.06 and on Day 35 were 1.23 ± 0.34, 0.17 ± 0.04, 0.25 ± 0.05 and 0.14 ± 0.01 respectively having overall percent decrement of 51.54 and 33.17 for pyometra and CKD respectively. However, *E. canis* and *B. canis* group showed percent increase of 1.14 and 7.08 respectively.

The highest percentage of improvement in UPC ratio was noted in pyometra, followed by CKD, *E. canis* and *B. canis* anaemic dogs.

There was a non-significant decrease in the UPC ratio values of CKD, *E. canis B. canis* and pyometra anaemic groups when Day 35 values of respective diseases were compared with before-treatment values.

Table 4.59: Mean ± SE UPC Ratio in healthy control group and different anaemic groups at all the time intervals

Time Intervals	CKD (a) (n=6)	*E. canis (b)* (n=6)	*B. canis (c)* (n=6)	Pyometra(d) (n=6)	Healthy(e) (n=6)	Overall (a+b+c+d) (n=24)	F value	P value	S / NS
BT	1.84±0.55^{p}	0.17±0.04^{q}	0.24±0.06^{q}	0.29±0.06^{q}	0.24±0.02^{q}	0.64±0.20	8.21	0.000	**
Day 0	2.08±0.46^{p}	0.16±0.05^{q}	0.21±0.06^{q}	0.27±0.06^{q}	0.24±0.01^{q}	0.69±0.20	14.87	0.000	**
Day 3	2.44±0.61^{p}	0.35±0.18^{q}	0.21±0.06^{q}	1.20±0.85^{pq}	0.22±0.01^{q}	1.06±0.31	4.01	0.011	*
Day 7	1.76±0.40^{p}	0.15±0.04^{q}	0.21±0.05^{q}	0.59±0.29^{q}	0.20±0.01^{q}	0.68±0.18	8.88	0.000	**
Day 15	1.87±0.38	0.88±0.72	0.21±0.05	0.95±0.71	0.21±0.01	0.98±0.28	1.94	0.134	NS
Day 28	1.38±0.40^{p}	0.18±0.04^{q}	0.20±0.05^{q}	0.22±0.04^{q}	0.21±0.01^{q}	0.50±0.14	8.30	0.000	**
Day 35	1.23±0.34^{p}	0.17±0.04^{q}	0.25±0.05^{q}	0.14±0.01^{q}	0.21±0.01^{q}	0.45±0.13	8.75	0.000	**
Overall	1.80±0.17	0.30±0.11	0.22±0.02	0.53±0.16	0.22±0.01				
F value	0.76	0.85	0.10	0.87	0.82				
P value	0.601	0.534	0.995	0.522	0.556				
S / NS	NS	NS	NS	NS	NS				

Table F-value for Days is 2.37 (5 %) and 2.75 (1 %); Table F value for Groups is 3.36 (5 %) and 4.17 (1 %).
* $p \leq 0.05$; ** $p \leq 0.01$ and NS – Non Significant
Means bearing superscripts p,q,r,s in rows show statistical significance between the groups while means bearing superscripts a,b,c,d in columns show the statistical significance within the groups.

4.2.23 Free Hb (g/dl) in healthy control group and different anaemic groups at all the time intervals:

Table 4.60 (Figure 4.24) indicates free Hb (g/dl) values (Mean± SE) of dogs in different anaemic groups as well as healthy control group at all the time intervals. The overall free Hb values in before treatment, Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35 were 0.20 ± 0.03, 0.17 ± 0.03, 0.14 ± 0.03, 0.13 ± 0.03, 0.10 ± 0.02, 0.05 ± 0.02 and 0.03 ± 0.01, respectively.

Comparative analysis of the free Hb values between CKD, *E. canis*, *B. canis* and pyometra groups on BT, Day 0, 3, 7,15, 28 and 35 revealed significant ($p \leq 0.05$) difference when compared individually with the healthy control groups on respective days. This showed that there was a significant ($p \leq 0.01$) increase in the free Hb value in all the anaemic groups than the healthy control group. The free Hb values in CKD, *E. canis*, *B. canis* and pyometra anaemic groups on before treatment were 0.06 ±0.02, 0.38 ± 0.03, 0.31 ± 0.03 and 0.03 ± 0.02 and on Day 35 were 0.00 ± 0.00, 0.06 ± 0.02, 0.05 ± 0.03 and 0.00 ± 0.00, respectively.

There was statistically significant decrease in the free Hb values of CKD, *E. canis* and *B. canis* anaemic groups while a non-significant decrease in the pyometra group when Day 35 values of respective disease were compared with before treatment values.

The percent decrement in free Hb values on Day 35 compared to before treatment was 100, 82.51, 84.23 and 100 for CKD, *E. canis*, *B. canis* and pyometra anaemic groups respectively.

Plasma-free haemoglobin may exert several deleterious effects and elevated PFH has been correlated to pulmonary hypertension. Lowering PFH may reduce the toxic effects of free heme and heme-derived iron which increases cellular susceptibility to oxidants and may cause endothelial dysfunction from depletion of Nitric oxide.

Conclusively, a post-treatment free Hb study revealed that since there is a decrease in the free Hb values between before treatment and Day 35 in CKD, *E. canis*, *B. canis* and pyometra groups, it showed that the whole blood transfusion and disease-specific treatment had a beneficial impact on controlling haemolysis

Table 4.60: Mean ± SE Free Hb (g/dl) values in the healthy control group and different anaemic groups at all the time intervals

Time Intervals	CKD (a) (n=6)	*E. canis (b)* (n=6)	*B. canis (c)* (n=6)	Pyometra(d) (n=6)	Healthy(e) (n=6)	Overall (a+b+c+d) (n=24)	F value	P value	S / NS
BT	0.06±0.02^{aq}	0.38±0.03^{ap}	0.31±0.03^{ap}	0.03±0.02^{q}	0.00±0.00^{q}	0.20±0.03	56.15	0.000	**
Day 0	0.06±0.02^{aq}	0.30±0.02^{abp}	0.28±0.01^{abp}	0.01±0.01^{qr}	0.00±0.00^{r}	0.17±0.03	64.58	0.000	**
Day 3	0.00±0.00^{br}	0.31±0.04^{abp}	0.23±0.02^{abq}	0.00±0.00^{r}	0.00±0.00^{r}	0.14±0.03	57.29	0.000	**
Day 7	0.01±0.01^{bq}	0.25±0.05^{bcp}	0.23±0.03^{abp}	0.00±0.00^{q}	0.00±0.00^{q}	0.13±0.03	18.44	0.000	**
Day 15	0.01±0.01^{bq}	0.18±0.04^{cdp}	0.20±0.02^{bp}	0.00±0.00^{q}	0.00±0.00^{q}	0.10±0.02	16.25	0.000	**
Day 28	0.00±0.00^{bq}	0.11±0.04^{dp}	0.08±0.04^{cp}	0.00±0.00^{q}	0.00±0.00^{q}	0.05±0.02	4.03	0.011	*
Day 35	0.00±0.00^{bq}	0.06±0.02^{dp}	0.05±0.03^{cpq}	0.00±0.00^{q}	0.00±0.00^{q}	0.03±0.01	3.27	0.027	*
Overall	0.02±0.01	0.23±0.02	0.20±0.02	0.01±0.00	0.00±0.00				
F value	4.42	8.48	9.97	1.66	0.00				
P value	0.001	0.000	0.000	0.158	0.000				
S / NS	**	**	**	NS	NS				

Table F-value for Days is 2.37 (5 %) and 2.75 (1 %); Table F value for Groups is 3.36 (5 %) and 4.17 (1 %).
* $p \leq 0.05$; ** $p \leq 0.01$ and NS – Non Significant
Means bearing superscripts p,q,r,s in rows show statistical significance between the groups while means bearing superscripts a,b,c,d in columns show the statistical significance within the groups.

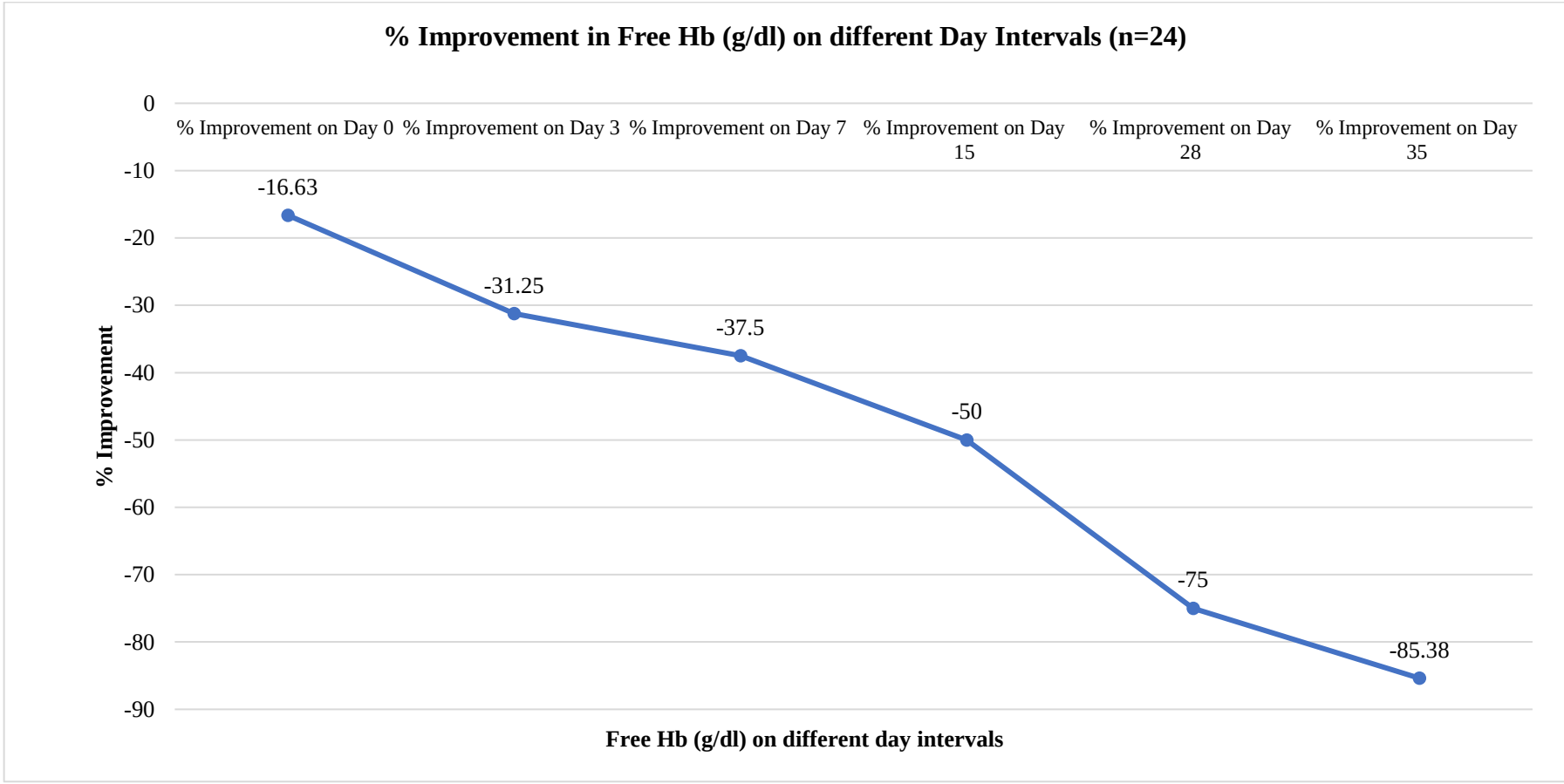


Figure 4.24 Percent Improvement in Free Hb (g/dl) in Anaemic Dogs on different Day Intervals (n=24)

and free Hb values resulting in a substantial and radical improvement in the clinical condition of the patient.

4.2.24 Serum Iron Profile in before treatment and after treatment Anaemic dogs:

On perusal of Table 4.61 (Figure 4.25) the Mean ± SE of Serum Iron (ug/dl), TIBC (ug/dl) and percent saturation (%) values in before-treatment (Anaemic) dogs and after-treatment dogs were (98.52 ± 3.15, 233.03 ± 7.95 and 42.80 ± 1.57) and (105.43 ± 2.56, 215.23 ± 7.23, 49.81 ± 1.89) respectively. There was a significant increase in the Serum Iron, a significant decrease in the TIBC and a Significant increase in percent Saturation in after-treatment anaemic dogs over the before-treatment dogs. The increased Iron levels on after treatment group could be attributed to the increase in iron stores after blood transfusion and administrations of intravenous Iron medications in the patients. The decrease in TIBC values could be credited to the improvement in hematological parameters due to blood transfusion. The increase in the percent saturation levels after treatment could be linked to the increase in Iron levels and correction of anaemia in the patients.

Table 4.61: Serum Iron (Mean ± SE) Profile in Before Treatment Group (n=19) and After Treatment Anaemic dogs (n=19)

Sr. No	Parameter	Before Treatment (n=19)	After Treatment (n=19)	t-stat
1	Serum Iron (µg/dl)	98.52 ± 3.15	105.43 ± 2.56	5.38*
2	TIBC (µg/dl)	233.03 ± 7.95	215.23 ± 7.23	3.02*
3	Percent Saturation (%)	42.80 ± 1.57	49.81 ± 1.89	4.79*

*: Value of Significance ($p \leq 0.05$)

Iron deficiency is a common cause of anaemia in dogs with renal disease, often resulting from chronic blood loss, impaired iron absorption, or inadequate dietary intake. Iron supplementation is often part of the therapeutic approach for managing anaemia in dogs, especially those receiving erythropoiesis-stimulating agents (ESAs) or undergoing treatment for iron deficiency. Monitoring iron parameters allows clinicians to assess the effectiveness of iron supplementation,

optimize dosing regimens, and ensure adequate iron availability for erythropoiesis (Devadevi *et al*. (2022).

Part II: Serum Protein Electrophoresis:

4.3 Analysis of Serum Protein Pattern in Healthy control and Hyperglobulinemic anaemic dogs:

Serum protein electrophoresis (SPE) shows the distribution of protein fractions, helping clinicians to characterize some pathologic processes. The results of the protein electrophoresis can be one of the most useful diagnostic aids in infectious and inflammatory diseases. Hence one of the objectives planned was to Analyse the Serum Protein Pattern in Hyperglobulinemic Dogs.

4.3.1 Overall Electrophoretic Pattern in Healthy control (n=6), Before Treatment (Day 0) and After Treatment (Day 20) in all anaemic dogs (n=24).

Table 4.62 (Figure 4.26) presents the Mean ± SE data for the overall electrophoretic pattern while Table 4.63 represents the disease-wise electrophoretic pattern in Healthy control, Before Treatment (Day 0) and After Treatment (Day 20) anaemic dogs.

The overall electrophoretic values (Mean ± SE) in Healthy control (n=6), Before Treatment i.e. Day 0 (n=24), and After Treatment i.e. Day 20 (n=24) of Total Protein (6393.33 ± 35.56, 6852.50 ± 280.45 and 6242.50 ± 158.95), Albumin (3193.33 ± 28.83, 1967.08 ± 97.39 and 2372.29 ± 109.59), Globulin (3200 ± 35.21, 4885.42 ± 247.45, 3870.21 ± 190.62), Alpha 1 (330 ± 22.06, 450.83 ± 41.86 and 360.42 ± 20.75), Alpha 2 (686.67 ± 21.40, 1122.92 ± 60.86 and 834.90 ± 48.33), Beta 1 (740.00 ± 24.36, 806.25 ± 34.18 and 731.03 ± 34.76), Beta 2 (795.00 ± 25.27, 1086.25 ± 46.60 and 845.42 ± 40.68) and Gamma Globulin (648.33 ± 35.35,1419.17 ± 192.16 and 1097.95 ± 143.56) were recorded, respectively. All sera protein electrophoresis of the dogs with hypergammaglobulinemia showed polyclonal gammopathies.

A comparison of all anaemic dogs (n=24) with healthy dogs (n=6) revealed hyperprotenemia, hypoalbuminemia and hyperglobulinemia. All globulin fractions viz. Alpha 1, Alpha 2, Beta 1, Beta 2 and Gamma globulins were

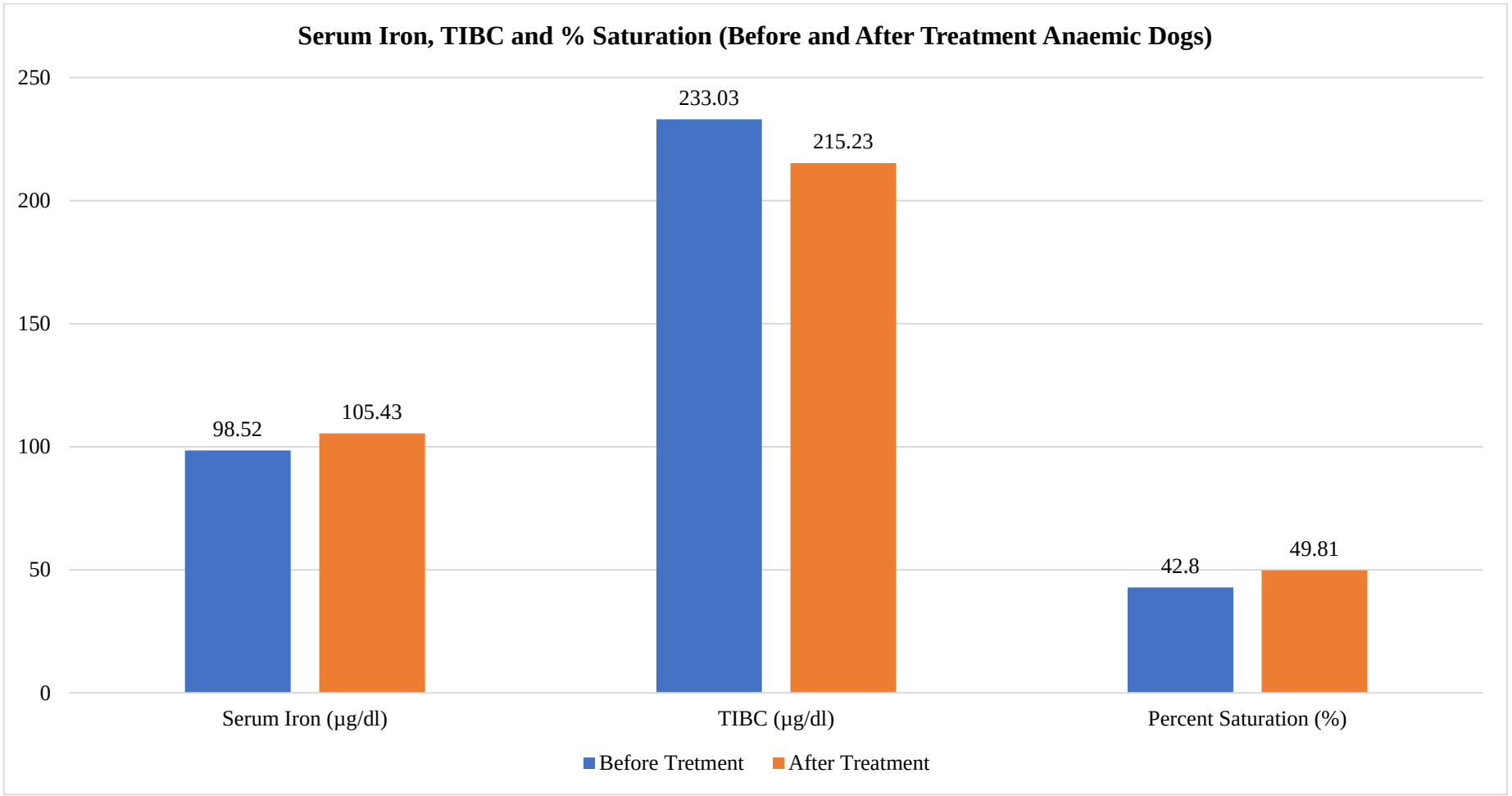


Figure 4.25- Serum Iron (µg/dl), TIBC(µg/dl) and % Saturation in Before Treatment and After Treatment Anaemic Dogs (n=24)

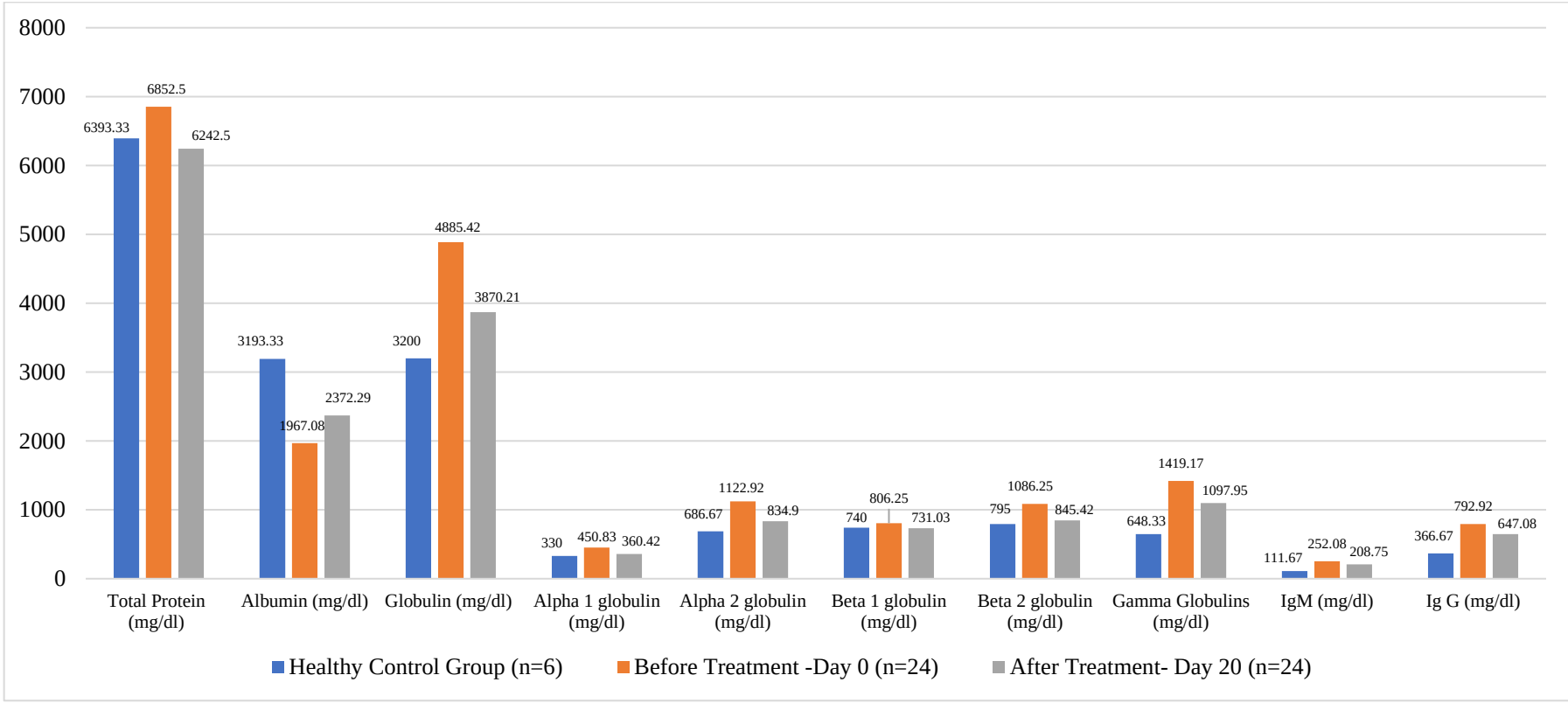


Figure 4.26 Overall Electrophoretic pattern in Healthy and anaemic dogs on Before Treatment (Day 0) and After Treatment (Day 20)

Table 4.62 Mean ± SE values of overall Electrophoretic pattern in Healthy, CKD, *E. canis*, *B. canis* and Pyometra anaemic dogs on Before Treatment (Day 0) and After Treatment (Day 20)

Sr. No	Parameter	Healthy Control Group (n=6)	Before Treatment -Day 0 (n=24)	After Treatment- Day 20 (n=24)
1	Total Protein (mg/dl)	6393.33 ± 35.56	6852.50 ± 280.45	6242.50 ± 158.95
2	Albumin (mg/dl)	3193.33 ± 28.83	1967.08 ± 97.39	2372.29 ± 109.39
3	Globulin (mg/dl)	3200.00±35.21	4885.42 ± 247.45	3870.21 ± 190.62
4	Alpha 1 globulin (mg/dl)	330.00 ± 22.06	450.83 ± 41.86	360.42 ± 20.75
5	Alpha 2 globulin (mg/dl)	686.67 ± 21.40	1122.92 ± 60.86	834.90 ± 48.33
6	Beta 1 globulin (mg/dl)	740.00 ± 24.36	806.25 ± 34.18	731.03 ± 34.76
7	Beta 2 globulin (mg/dl)	795.00 ± 24.36	1086.25 ± 46.60	845.42 ± 40.68
8	Gamma Globulins (mg/dl)	648.33 ± 35.35	1419.17 ± 192.16	1097.95 ± 143.56
9	IgM (mg/dl)	111.67 ± 5.43	252.08 ± 31.24	208.75 ± 25.23
10	Ig G (mg/dl)	366.67 ± 17.26	792.92 ± 102.29	647.08 ± 79.75

Table 4.63 Electrophoretic pattern in Healthy, CKD, *E. canis, B. canis,* and Pyometra anaemic dogs on Before Treatment (Day 0) and After Treatment (Day 20)

Parameter	Healthy	CKD		*E. canis*		*B. canis*		Pyometra	
		Day 0 (BT)	Day 20 (AT)	Day 0 (BT)	Day 20 (AT)	Day 0 (BT)	Day 20 (AT)	Day 0 (BT)	Day 20 (AT)
Total Protein (mg/dl)	6393.33 ± 35.56	7568.33 ± 763.33	6005.00 ± 295.05	7578.33 ± 510.23	6950.83 ± 283.29	5896.67 ± 89.39	6385.00 ± 232.36	6366.67 ± 396.73	5629.17 ± 229.53
Albumin (mg/dl)	3193.33 ± 28.83	1951.67 ± 149.48	2680.83 ± 230.44	2188.33 ± 155.27	2169.17 ± 257.68	1736.67 ± 72.00	2408.33 ± 232.85	1991.67 ± 319.86	2230.83 ± 125.30
Globulin (mg/dl)	3200.00± 35.21	5616.67 ± 667.26	3324.17 ± 286.50	5390.00 ± 571.05	4781.67 ± 420.68	4160.00 ± 40.91	3976.67 ± 269.25	4375.00 ± 196.04	3398.33 ± 253.83
Alpha 1 globulin (mg/dl)	330.00 ± 22.06	716.67 ± 70.03	348.33 ± 52.82	415.00 ± 34.03	393.33 ± 28.60	320.00 ± 27.45	373.33 ± 41.45	351.67 ± 75.16	326.67 ± 45.73
Alpha 2 globulin (mg/dl)	686.67 ± 21.40	1211.67 ± 71.90	712.92 ± 86.74	853.33 ± 60.97	765.00 ± 56.55	1138.33 ± 182.47	938.33 ± 51.15	1288.33 ± 65.80	923.33 ± 148.29
Beta 1 globulin (mg/dl)	740.00 ± 24.36	886.67 ± 90.69	599.14 ± 52.53	910.00 ± 36.51	838.33 ± 71.95	703.33 ± 23.62	801.67 ± 54.92	725.00 ± 67.07	685.00 ± 63.81
Beta 2 globulin (mg/dl)	795.00 ± 24.36	988.33 ± 93.61	655.00 ± 47.73	965.00 ± 63.65	903.33 ± 32.63	1058.33 ± 55.22	973.33 ± 88.98	1333.33 ± 82.85	850.00 ± 89.37
Gamma Globulins (mg/dl)	648.33 ± 35.35	1813.33 ± 391.47	1008.46 ± 143.40	2246.67 ± 424.43	1881.67 ± 361.99	940.00 ± 131.55	888.33 ± 128.46	676.67 ± 67.16	613.33 ± 175.87
IgM (mg/dl)	111.67 ± 5.43	315.00 ± 57.89	181.67 ± 19.90	390.00 ± 72.62	335.00 ± 62.70	176.67 ± 20.11	173.33 ± 25.78	126.67 ± 7.15	145.00 ± 48.22
Ig G (mg/dl)	366.67 ± 17.26	976.67 ± 194.54	576.67 ± 74.68	1266.67 ± 226.24	1098.33 ± 190.01	550.00 ± 71.13	543.33 ± 65.20	378.33 ± 24.69	370.00 ± 108.90

significantly increased in the anaemic dogs when compared with the healthy control dogs.

The immunological cascade activates in most tick-borne disease conditions, tick fever infections and inflammatory disease conditions such as pyometra resulting in overall increase in the globulins.

After-treatment values on Day 20 revealed decreased protein, increased albumin, and decreased globulin values when compared with before-treatment values (Day 0). However, these values were at par with the healthy control values indicating improvement in the general health and condition of the anaemic dogs.

The administration of immunosuppressive medications i.e. Prednisolone acetate might have resulted in breaking the immune complex reactions/ cascade and hence might have resulted in lowering the globulins in the present study.

4.3.2 Comparison of the overall Ratio of Albumin with Globulin (A: G Ratio), and Albumin with Globulin fractions in Healthy control Dogs, Anaemic Dogs and Day 20 (Treatment) group Dogs:

The overall ratio of Albumin with Globulin and Albumin with Globulin fractions is depicted in Table 4.64.

The overall Albumin: Globulin ratio in healthy control dogs (n=6) was 1.00 while in anaemic dogs (n=24) the skewed value i.e. 0.42 was noted. On treatment Day 20, the A: G ratio was increased to 0.66 indicating reduced globulin and increased albumin protein concentration indicating recovery of the dogs.

The observed alterations that demonstrated statistical significance ($p \leq 0.05$) were found to be connected with the levels of albumin and the albumin-to-globulin ratio (A/G).

The anaemic group exhibits suboptimal levels of albumin, which can be explained by the presence of comorbid conditions such as kidney dysfunction, tick fever, gastrointestinal issues, and trauma-related blood loss. These factors contribute to hypoalbuminemia in this subset of dogs, leading to a significant reduction in albumin levels ($p \leq 0.05$) which is akin with preset study findings. The

Table 4.64: Ratio of Albumin with Globulin and Albumin with Globulin fractions on Day 0, Day 20 and with healthy Dogs.

Protein Fraction	Albumin : Globulin	Albumin : Alpha 1 Globulin	Albumin: Alpha 2 Globulin	Albumin: Beta 1 Globulin	Albumin: Beta 2 Globulin	Albumin: Gamma Globulin	Albumin: Ig M (mg/dl)	Albumin: Ig G (mg/dl)
Day 0 (n=24)	0.42	5.15	1.89	2.54	1.88	1.86	9.85	3.25
Day 20 (n=24)	0.66	7.39	3.11	3.47	3.04	3.26	15.72	5.31
Healthy (n=6)	1.00	9.92	4.67	4.34	4.04	5.02	28.95	8.83

study conducted by Guadarrama-Olhovich *et al.* (2013) found that the ratio of albumin to globulin (A/G) is influenced by the levels of albumin and globulin. Specifically, a decrease in albumin levels caused by a primary illness results in elevated globulin levels. This relationship is particularly evident in inflammatory conditions. The researchers observed that these changes in albumin and globulin levels led to statistically significant reductions in the A/G ratio ($p \leq 0.05$).

The presence of hyperglobulinemia indicates an excessive immunological response that is not sufficiently efficient (Woody and Hoskins, 1991; Moore and Williams, 2004). Furthermore, a simultaneous decrease in albumin levels may occur as a consequence of protein deficiency (caused by anorexia in our study), proteinuria, blood loss, peripheral loss of fluid owing to vasculitis (ascites) in all the anaemic group, and as a compensatory response to increased globulin levels (Varela *et al.*, 1997). Consequently, there was a substantial decrease in the A:G ratio between the healthy and sick groups. A decreased A:G ratio may indicate excessive synthesis of globulins, nephrotic syndrome, and sepsis. This indicates that dogs with CKD had a high level of pathogenesis, followed by *B. canis*, *E. canis*, and pyometra in anaemic dogs.

The overall ratio of Albumin with the Globulin fractions (Table 4.64) viz Alpha 1, Alpha 2, Beta 1, Beta 2, and Gamma globulin was (9.92, 4.67, 4.34, 4.04 and 5.02 in healthy dogs), (5.15, 1.89, 2.54, 1.88 and 1.86 in anaemic dogs) while (7.39, 3.11, 3.47, 3.04 and 3.26 in Day 20 treatment dogs), respectively. There was a decreased ratio of every globulin protein fraction in anaemic dogs when compared with healthy control group dogs. However, on treatment Day 20, the increased ratio in the respective globulin fraction, though not at par with the healthy control groups was noticed indicating a trend of improvement.

The overall ratio of Albumin with the Ig M (mg/dl) and Ig G (mg/dl) in healthy control (n=6), anaemic dogs (n=24) and Day 20 treatment dogs were (28.95, 8.83), (9.85, 3.25) and (15.72, 5.31) respectively. The value indicated a decreased ratio of both the immunoglobulins i.e. IgG and IgM in anaemic dogs when compared with healthy control dogs. There was an increased ratio on Day 20, treatment group dogs indicating the lowered inflammation as a result of

decreased hyperglobulinemia.

4.3.3 Comparison of Electrophoretic pattern in Healthy control, CKD Anaemic and Day 20 (Treatment) group Dogs:

The mean ± S.E. for serum protein electrophoretic fractions of six Healthy control dogs and six chronic kidney disease (CKD) dogs before treatment and on the 20th day of treatment are summarized in Table 4.65 (Figure 4.27).

The total proteins (6393.33 ± 35.55, 7568.33 ± 763.98, and 6005.00 ± 295.04), Albumin (3193.33 ± 28.83, 1951.66 ± 149.47 and 2680.83 ± 230.43), Alpha 1 Globulin (330.00 ± 22.06, 716.67 ± 70.03 and 348.33 ± 52.81), Alpha 2 Globulin (686.66 ± 21.39, 1211.67 ± 71.89 and 712.92 ± 86.73) Beta 1 Globulin (740.00 ± 24.35, 886.66 ± 90.68 and 599.14 ± 52.53) Beta 2 globulin (795.00 ± 25.26, 988.33 ± 93.61 and 655.00 ± 47.73) and gammaglobulin (648.33 ± 35.34, 1813.33 ± 391.47 and 1008.45 ± 143.39) concentrations were in the Healthy control group, CKD affected anaemic dogs and post-treatment (Day 20) dogs respectively.

This study presents the occurrence of significant alterations in the serum protein profile in dogs. Statistically significant ($p \leq 0.05$) hypoalbuminemia and hyperglobulinaemia were noted in CKD-affected anaemic dogs. Also, statistically Increased all globulin fractions viz. Alpha 1 (α 1), Alpha 2 (α 2), Beta 2 (β2) and Gamma (γ) globulins except non-significantly increased Beta 1 (β1) globulins in CKD-affected anaemic dogs when compared with Healthy Control dogs.

When treatment Day 20 protein values were compared with before treatment values, statistically ($p \leq 0.05$) elevated Albumin and decreased Alpha 1 (α 1), Alpha 2 (α 2), Beta 1 (β1), Beta 2 (β2) and Gamma (γ) globulins but at par with the healthy control group values were noted.

All sera protein electrophoresis of the dogs with hypergammaglobulinemia showed polyclonal gammopathies. The findings of the present study corroborate with the observations of Camacho *et al.* (2005) who studied that Infected dog with azotemia had shown a significant elevation of alpha-1 and alpha-2 globulins and significantly lower albumin levels than non-infected dogs. Among infected dogs, those presenting azotemia had significantly lower concentrations of total proteins,

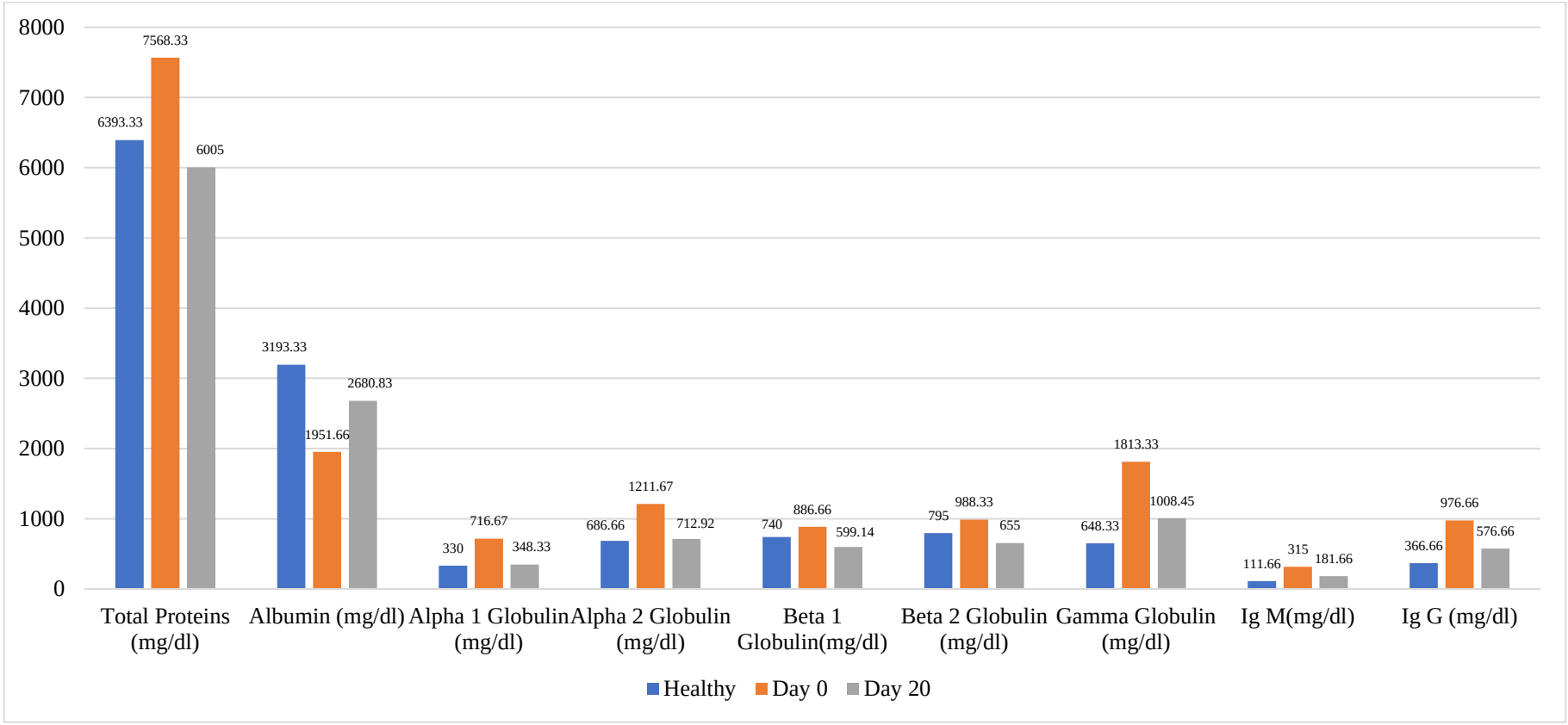


Figure 4.27 Electrophoretic pattern in Healthy control group, CKD anaemic dogs before Treatment (Day 0) and After Treatment (Day 20)

Table 4.65: Electrophoretic pattern in Healthy control group, CKD anaemic dogs before Treatment (Day 0) and After Treatment (Day 20)

Days/ Time Intervals	Groups (n=6)								
	Total Proteins (mg/dl)	**Albumin (mg/dl)**	**Alpha 1 Globulin (mg/dl)**	**Alpha 2 Globulin (mg/dl)**	**Beta 1 Globulin (mg/dl)**	**Beta 2 Globulin (mg/dl)**	**Gamma Globulin (mg/dl)**	**Ig M (mg/dl)**	**Ig G (mg/dl)**
Healthy	6393.33±35.55	3193.33±28.83^{a}	330.00±22.06^{b}	686.66±21.39^{b}	740.00±24.35^{b}	795.00±25.26^{b}	648.33±35.34^{b}	111.66±5.42^{b}	366.66±17.25^{b}
Day 0	7568.33±763.98	1951.66±149.47^{c}	716.67±70.03^{a}	1211.67±71.89^{a}	886.66±90.68^{a}	988.33±93.61^{a}	1813.33±391.47^{a}	315.00±57.89^{a}	976.66±194.53^{a}
Day 20	6005.00±295.04	2680.83±230.43^{b}	348.33±52.81^{b}	712.92±86.73^{b}	599.14±52.53^{b}	655.00±47.73^{b}	1008.45±143.39^{b}	181.66±19.90^{b}	576.66±74.68^{b}
Overall	6655.56±303.09	2608.61±150.82	465.00±51.65	870.42±68.71	741.94±44.11	812.78±47.37	1156.71±176.40	202.78±28.08	640.00±89.73
F value	2.95	15.31	17.45	19.96	5.35	7.19	6.09	8.47	6.59
P value	0.082	0.000	0.000	0.000	0.175	0.006	0.011	0.003	0.008
Result	NS	**	**	**	*	**	*	**	**

Table F-value is 3.68 (5 %); 6.35 (1 %).
* $p \leq 0.05$; ** $p \leq 0.01$ and NS – Non Significant
Means bearing different superscripts a and b in columns differ significantly.

albumin, beta, and gamma globulins, and significantly higher values of alpha-2 globulin.

The primary cause for the increase in Alpha-1 levels is the secretion of acute phase reactants, specifically alpha-1 antitrypsin (AT) and alpha 1-acid glycoprotein (AGP), as a response to the inflammatory process. The initial substance is an inhibitory enzyme created as a reaction to the release of collagenase by white blood cells. The precise biological function of the latter remains unknown.

Alpha-2 globulins increase due to the release of acute-phase reactants. Dogs that are afflicted and have azotemia exhibit elevated levels of alpha-2 globulins. This could indicate an increase in the levels of alpha-2 macroglobulin as a means of counteracting hypoalbuminemia. Elevations in the nephrotic syndrome have been observed as a means of preserving oncotic pressure, according to Stevenson *et al.* (1998).

Wozniak *et al.* (1997) noted that CKD infection is linked to an elevation in the levels of beta-globulins, which could indicate the existence of free-hemoglobin due to severe hemolytic anaemia. The observed band is most likely attributable to the binding of hemoglobin and haptoglobin. He further inferred that the absence of an increase in beta globulin levels in dogs with azotemia, compared to dogs without azotemia, could be attributed to the deposition of immune complexes and the subsequent depletion and elimination of C3 and C4 proteins from the blood serum. (ii) The excretion of transferrin through the kidney, as observed in cases of renal disease (Mackinnon *et al.*, 2003); or (iii) The decrease in transferrin levels during acute inflammatory processes, which is a characteristic of negative acute phase reactants and may be more common in dogs with azotemia.

4.3.4 Comparison of Electrophoretic pattern in Healthy control, *E. canis* Anaemic and Day 20 (Treatment) group Dogs:

The mean ± S.E. for serum protein electrophoretic fractions of six Healthy control dogs and six *E. canis* dogs on before treatment and on 20th day of treatment are summarized in Table 4.66 (Figure 4.28).

Table 4.66: Electrophoretic pattern in Healthy control group, *E. canis* anaemic dogs before Treatment (Day 0) and After Treatment (Day 20).

Days/ Time Intervals	Groups (n=6)								
	Total Proteins (mg/dl)	Albumin (mg/dl)	Alpha 1 Globulin (mg/dl)	Alpha 2 Globulin (mg/dl)	Beta 1 Globulin (mg/dl)	Beta 2 Globulin (mg/dl)	Gamma Globulin (mg/dl)	Ig M (mg/dl)	Ig G (mg/dl)
Healthy	6393.33±35.55	3193.33±28.83^{a}	330.00±22.06	686.66±21.39	740.00±24.35	795.00±25.26^{b}	648.33±35.34^{b}	111.67±5.42^{b}	366.66±17.25^{b}
Day 0	7578.33±510.23	2188.33±155.27^{b}	415.00±34.03	853.33±60.97	910.00±36.51	965.00±63.65^{a}	2246.67±424.43^{a}	390.00±72.61^{a}	1266.67±226.24^{a}
Day 20	6950.83±283.29	2169.16±257.67^{b}	393.33±28.59	765.00±56.55	838.33±71.94	903.33±32.62^{ab}	1881.67±361.98^{a}	335.00±62.70^{a}	1098.33±190.00^{a}
Overall	6974.17±217.48	2516.94±149.71	379.44±17.83	768.33±31.55	829.44±31.34	887.78±29.23	1592.22±241.12	278.89±41.92	910.56±132.53
F value	3.08	11.27	2.37	2.82	3.07	3.86	6.73	7.05	7.84
P value	0.075	0.001	0.126	0.090	0.075	0.044	0.008	0.006	0.004
Result	NS	**	NS	NS	NS	*	**	**	**

Table F-value is 3.68 (5 %); 6.35 (1 %).
* $p \leq 0.05$; ** $p \leq 0.01$ and NS – Non Significant
Means bearing different superscripts a and b in columns differ significantly.

The total proteins (6393.33 ± 35.55, 7578.33 ± 510.23 and 6950.83 ± 283.29), Albumin (3193.33 ± 28.83, 2188.33 ± 155.27 and 2169.16 ± 257.67), Alpha 1 Globulin (330.00 ± 22.06, 415.00 ± 34.03 and 393.33 ± 28.59), Alpha 2 Globulin, (686.66 ± 21.39, 853.33 ± 60.97 and 765.00 ± 56.55), Beta 1 Globulin (740.00 ± 24.35, 910.00 ± 36.51 and 838.33 ± 71.94) and β- 2 globulin (795.00 ± 25.26, 965.00 ± 63.65 and 903.33 ± 32.6) and gammaglobulin (648.33 ± 35.34, 2246.67 ± 424.43 and 1881.67 ± 361.98) were the concentrations in Healthy control group, *E. canis* affected dogs on Day 0 and Day 20, respectively.

This study presents the occurrence of significant alterations in the serum protein profile in dogs naturally infected with *E. canis*. Significant ($p \leq 0.05$) hypoalbuminemia, hyperglobulinaemia, Hyper β2-globulinemia, and hypergammaglobulinaemia were constantly observed in all *E. canis* infected dogs on before treatment when compared with healthy control group values. An insignificant increase was noted in α – 1 globulin, α – 2 globulin and β- 1 globulin protein fractions in anaemic dogs when compared with healthy control dogs.

On treatment day 20, the concentrations of β2 globulins and gamma globulin were decreased when compared with Day 0 values but the decrease was statistically non-significant. Similarly, the values of α-1 globulin, α-2 globulin, and β-1 globulin were non-significantly decreased on Day 20 when compared with Day 0 values.

In addition, all sera protein electrophoresis of the dogs with hypergammaglobulinemia showed polyclonal gammopathies. However, Breitschwerdt *et al.* (1987) noted monoclonal gammopathy associated with naturally occurring canine Ehrlichiosis characterized by the presence of monoclonal immunoglobulin in the serum or urine or both.

Asawakarn and Piyanan (2021) noted a monoclonal gammopathy in 35% of *E. canis* and 39% of *B. canis* single infections in 650 dogs affected with tick-borne infections i.e. *E. canis* and *B. canis* infections. Monoclonal gammopathies result from a single line of B lymphocytes or plasma cells, whereas polyclonal gammopathies are usually an indication of chronic inflammation and chronic liver damage (Jania, 2016).

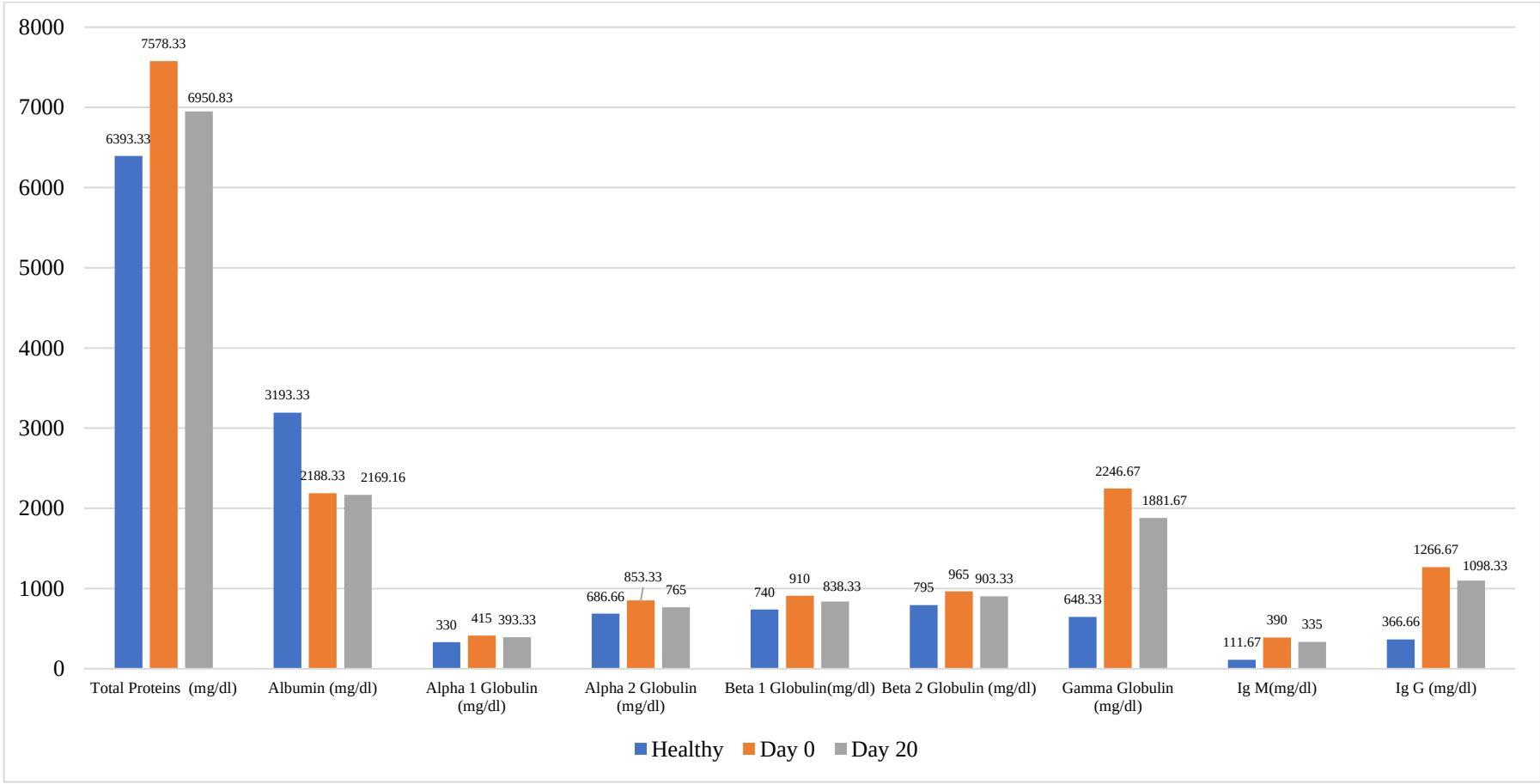


Figure 4.28 Electrophoretic pattern in Healthy control group, *E. canis* anaemic dogs before Treatment (Day 0) and After Treatment (Day 20)

The hypoalbuminemia observed in all stages of canine ehrlichiosis can be attributed to several factors. These include reduced protein intake due to anorexia, blood loss, loss of protein to inflammatory fluids caused by vasculitis, decreased protein production due to concurrent liver disease, or proteinuria. Codner *et al.* (1992) observed a temporary presence of albumin in the urine during the initial stage of ehrlichiosis in six beagles that were deliberately infected for the purpose of the experiment. Nevertheless, the affected dogs exhibited very minor alterations in the glomeruli (Hildebrandt *et al.*, 1970; Codner *et al.*, 1986). The decrease in albumin concentrations may serve as a compensatory mechanism for the hyperglobulinaemic state, ensuring that the oncotic pressure is maintained and preventing an increase in blood viscosity. This regulation of albumin synthesis is supported by Woody and Hoskins (1991).

The infected dogs exhibited a notable increase in protein levels, with gammaglobulins being the primary factor contributing to this change. The concentration of gammaglobulin increases during the phase of canine ehrlichiosis characterized by fever, and remains elevated during the subclinical and chronic phases of the disease (Ristic and Holland, 1993).

Harrus *et al.* (1999) noted hypergammaglobulinemia in the non-pancytopenic dogs while lower concentrations of gamma globulin fraction in the pancytopenic group and the results of the present study being pancytopenic corroborates with the findings of hypergammaglobulinemia. Harrus *et al.* (1999) hypothesized the cause of the reduced levels of the gammaglobulin percentage in the group with pancytopenia may be due to the significant decrease in white blood cells observed in these dogs. The activation, division, and differentiation of B-lymphocytes into antibody-forming cells (AFC) necessitates cytokines, primarily secreted by T-helper lymphocytes and antigen-presenting cells (Guy and Hodes, 1989). In pancytopenic dogs with severe leukopenia, there can be improper interactions among these cells, deficiencies in cytokines and AFC, and consequently lower concentrations of gammaglobulins.

A poor correlation between the gammaglobulin concentrations and the antibody titers of *E. canis* was recorded by Harrus *et al.* (1996). This finding was

in agreement with Weisiger *et al.* (1975) who followed anti-E, canis IFAT-titres and gammaglobulin concentrations in a beagle dog and described a lack of correlation between the two. Reardon and Pierce (1981) have also found no correlation between the two parameters. The poor correlation between the latter two parameters, and the polyclonal gammopathy recorded in most (41/42) sick dogs suggests that non-specific antibody production is induced by *E. canis* and that the anti-E, canis antibodies were not the main contributors to the hypergammaglobulinaemia. This phenomenon is known to occur in other diseases with prolonged antigenic stimulation (Tizard, 1982), and suggests an exaggerated immune response with inadequate effectiveness (Reardon and Pierce, 1981).

Recently, several articles have indicated that IL-6 serves as a valuable indicator of inflammation for different diseases and is likely to enhance the acute-phase response in cases of inflammation, particularly in tick-borne infections. Asawapattanakul *et al*., (2021) documented that the average CRP concentration in the Ehrlichia-infected group was approximately 8 times higher than that of healthy dogs.

4.3.5 Comparison of Electrophoretic pattern in Healthy control, *B. canis* Anaemic and Day 20 (Treatment) group Dogs:

The mean ± S.E. for serum protein electrophoretic fractions of Healthy control dogs (n=6) and *B. canis* anaemic dogs (n=6) on before treatment and on the 20th day of treatment are summarized in Table 4.67 (Figure 4.29).

The total proteins (6393.33 ± 35.55, 5896.66 ± 89.39 and 6385.00 ± 232.36), Albumin (3193.33 ± 28.83, 1736.66 ± 72.00, and 2408.33 ± 232.85), Alpha 1 Globulin (330.00 ± 22.06, 320.00 ± 27.44 and 373.33 ± 41.44), Alpha 2 Globulin (686.66 ± 21.39, 1138.33 ± 182.47 and 938.33 ± 51.14), Beta 1 Globulin (740.00 ± 24.35, 703.33 ± 23.61 and 801.66 ± 54.91), Beta 2 globulin (795.00 ± 25.26, 1058.33 ± 55.22 and 973.33 ± 88.98) and gamma globulin (648.33 ± 35.34, 940.00 ± 131.55 and 888.33 ± 128.46) concentrations in the Healthy control group, *B. canis* affected dogs on Day 0 and Day 20 respectively. All sera protein electrophoresis of the dogs with hypergammaglobulinemia showed polyclonal gammopathies.

Table 4.67: Electrophoretic pattern in Healthy control group, *B. canis* anaemic dogs before Treatment (Day 0) and After Treatment (Day 20)

Days/ Time Intervals	Groups (n=6)								
	Total Proteins (mg/dl)	**Albumin (mg/dl)**	**Alpha 1 Globulin (mg/dl)**	**Alpha 2 Globulin (mg/dl)**	**Beta 1 Globulin (mg/dl)**	**Beta 2 Globulin (mg/dl)**	**Gamma Globulin (mg/dl)**	**Ig M (mg/dl)**	**Ig G (mg/dl)**
Healthy	6393.33±35.55[a]	3193.33±28.83[a]	330.00±22.06	686.66±21.39[b]	740.00±24.35	795.00±25.26[b]	648.33±35.34	111.66±5.42	366.66±17.25
Day 0	5896.66±89.39[b]	1736.66±72.00[c]	320.00±27.44	1138.33±182.47[a]	703.33±23.61	1058.33±55.22[a]	940.00±131.55	176.66±20.11	550.00±71.13
Day 20	6385.00±232.36[a]	2408.33±232.85[b]	373.33±41.44	938.33±51.14[ab]	801.66±54.91	973.33±88.98[ab]	888.33±128.46	173.33±25.77	543.33±65.20
Overall	6225.00±96.81	2446.11±163.56	341.11±17.93	921.11±74.66	748.33±22.48	942.22±42.97	825.56±66.23	153.89±12.66	486.67±36.96
F value	3.86	26.47	0.81	4.22	1.77	4.66	2.07	3.66	3.37
P value	0.045	0.000	0.461	0.035	0.202	0.26	0.160	0.050	0.061
Result	*	**	NS	*	NS	**	NS	NS	NS

Table F-value is 3.68 (5 %); 6.35 (1 %).
* $p \leq 0.05$; ** $p \leq 0.01$ and NS – Non Significant
Means bearing different superscripts a and b in columns differ significantly.

In the present study, statistically significant ($p \leq 0.05$) reduced Albumin, elevated Alpha 2 (α 2), and Beta 2 (β2) globulins while non-significant increases in gamma globulin protein fractions in anaemic dogs were noted compared to Healthy Control dogs. The values of Alpha 1 (α 1), and Beta 1 (β1) globulins in anaemic dogs were at par with the healthy control dogs.

On treatment Day 20, statistically insignificant increased concentrations of Albumin, and decreased concentrations of Alpha 2 (α 2) and Beta 2 (β2) globulins were noted when compared with before-treatment values.

Further, it was also observed that on day 20, the values of Alpha 2 (α 2) and Beta 2 (β2) globulins were at par with the healthy control group values. There was a non-significant difference between Healthy Control, before treatment, and Day 20 values in the Alpha 1 and Beta -1 Globulin protein concentrations.

Asawakarn and Piyanan (2021) noted a monoclonal gammopathy in 35% of *E. canis* and 39% of *B. canis* single infections in 650 dogs affected with tick-borne infections i.e. *E. canis* and *B. canis* infections. Monoclonal gammopathies result from a single line of B lymphocytes or plasma cells, whereas polyclonal gammopathies are usually an indication of chronic inflammation and chronic liver damage (Jania, 2016).

The findings of Asawakarn and Piyanan (2021) regarding the serum protein profile of *B. canis* infected dogs which showed decreased albumin concentrations and A: G ratios but increased α- and β-globulin concentrations are in agreement with the present findings.

Elevated β2-globulin levels can occur due to heightened concentrations of C3a protein, which is a component of the complement system. Complements are implicated in the regulation of inflammatory processes, and this specific complement protein is involved in the progression of intravascular hemolysis, particularly in babesiosis (Tothova *et al.*, 2020)

A distorted pattern of alpha-2 region in electrophoresis is seen commonly in conditions of hemolysis, including *In-vivo* and *In-vitro*. The pathophysiology behind this pattern is the formation of hemoglobin-haptoglobin complexes in these conditions. This is a physiological adaptive response by human physiology

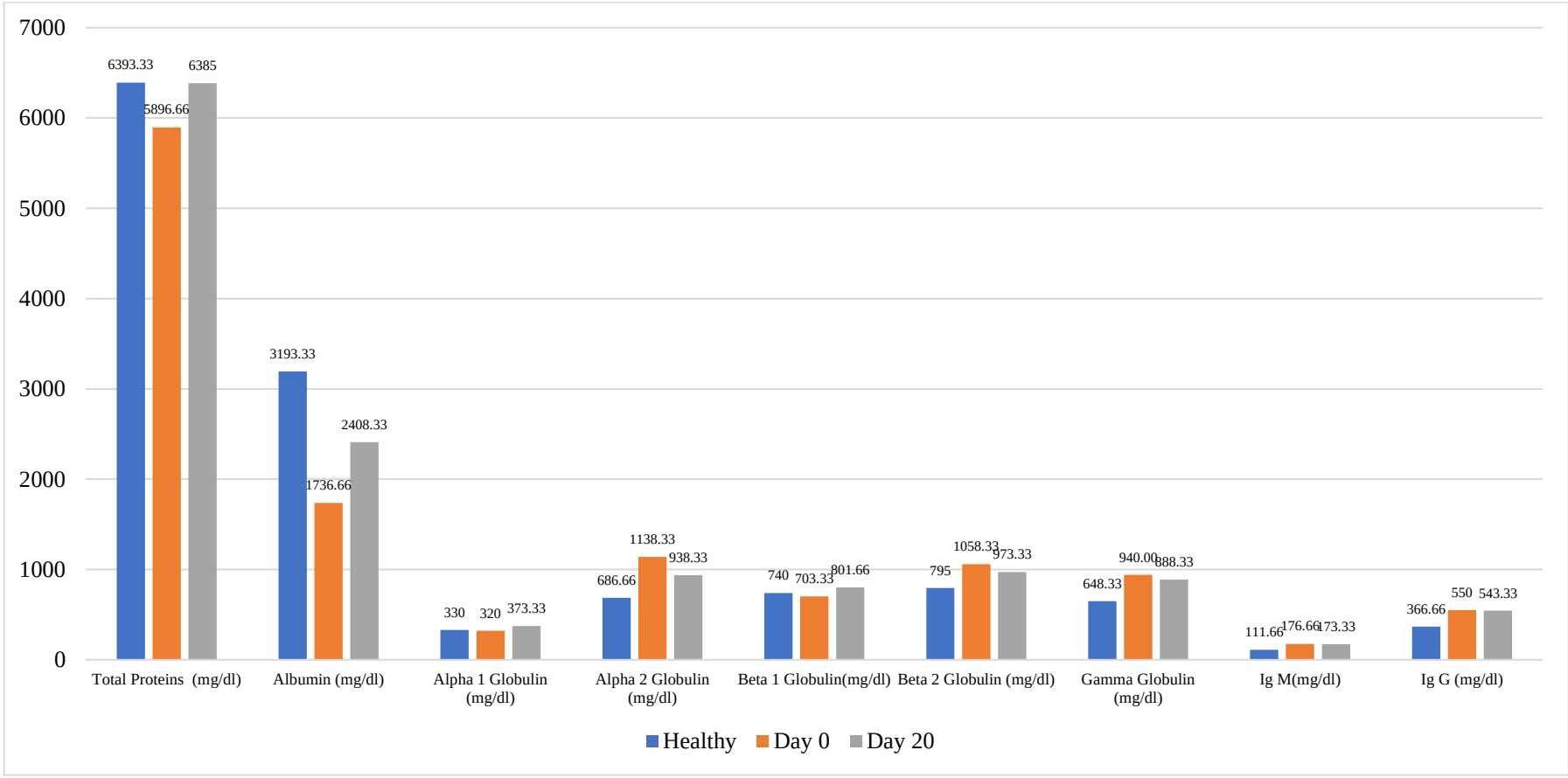


Figure 4.29 Electrophoretic pattern in Healthy control group, *B. canis* anaemic dogs before Treatment (Day 0) and After Treatment (Day 20)

to conserve hemoglobin released as a result of RBC breakdown into circulation and hemoglobin being a smaller globular protein is bound to be lost in urine. Hence to preserve it, haptoglobin is consumed to form a complex with hemoglobin which results in the formation of a macromolecular protein that is retained in circulation making hemoglobin available for the production of RBCs and prevention of anaemia. Haptoglobin and ceruloplasmin are acute-phase reactants and hence increased in acute inflammatory states.

CRP is a major acute-phase protein in dogs, mostly synthesized in the liver after tissue damage caused by infection, inflammation, or trauma. The acute-phase response is an innate host defense mechanism during tissue injury or immunological disorders and in the early stage of blood parasite infections. It is responsible for the accumulation and activation of granulocytes and mononuclear cells, releasing cytokines, interleukin (IL)-1, IL-16, and tumor necrosis factor-α. (Ceron,2005).

4.3.6 Comparison of Electrophoretic pattern in Healthy control, pyometra Anaemic and Day 20 (Treatment) group Dogs:

The Mean ± S.E. for serum protein electrophoretic fractions of Healthy control dogs (n=6) and pyometra-affected dogs (n=6) before treatment and on the 20^{th} day of treatment are summarized in Table 4.68 (Figure 4.30).

The total proteins (6393.33 ± 35.55, 6366.67 ± 396.73, 5629.17 ± 229.53), Albumin (3193.33 ± 28.83, 1991.66 ± 319.85 and 2230.83 ± 125.30, Alpha 1 Globulin (330.00 ± 22.06, 351.66 ± 75.16 and 326.66 ± 45.72), Alpha 2 Globulin (686.66 ± 21.39, 1288.33 ± 65.79 and 923.33 ± 148.29,) Beta 1 Globulin (740.00 ± 24.35, 725.00 ± 67.07 and 685.00 ± 63.81) Beta 2 globulin (795.00 ± 25.26, 1333.33 ± 82.85 and 850.00 ± 89.36) and gamma globulin (648.33 ± 35.34, 676.66 ± 67.16 and 613.33 ± 175.87) concentrations were in the Healthy control group, pyometra affected dogs on Day 0 and Day 20 respectively.

All sera protein electrophoresis of the dogs with hypergammaglobulinemia showed polyclonal gammopathies.

Table 4.68 Electrophoretic pattern in Healthy control group, Pyometra anaemic dogs before Treatment (Day 0) and After Treatment (Day 20)

Days/ Time Intervals	Groups (n=6)								
	Total Proteins (mg/dl)	**Albumin (mg/dl)**	**Alpha 1 Globulin (mg/dl)**	**Alpha 2 Globulin (mg/dl)**	**Beta 1 Globulin (mg/dl)**	**Beta 2 Globulin (mg/dl)**	**Gamma Globulin (mg/dl)**	**Ig M (mg/dl)**	**Ig G (mg/dl)**
Healthy	6393.33±35.55	3193.33±28.83^{a}	330.00±22.06	686.66±21.39^{b}	740.00±24.35	795.00±25.26^{b}	648.33±35.34	111.66±5.42	366.66±17.25
Day 0	6366.67±396.73	1991.66±319.85^{b}	351.66±75.16	1288.33±65.79^{a}	725.00±67.07	1333.33±82.85^{a}	676.66±67.16	126.66±7.14	378.33±24.68
Day 20	5629.17±229.53	2230.83±125.30^{b}	326.66±45.72	923.33±148.29^{b}	685.00±63.81	850.00±89.36^{b}	613.33±175.87	145.00±48.21	370.00±108.90
Overall	6129.72±167.62	2471.94±165.88	336.11±28.53	966.11±78.92	716.67±30.50	992.78±70.42	646.11±60.30	127.78±15.71	371.67±35.40
F value	2.67	10.21	0.06	10.29	0.26	16.99	0.08	0.34	0.008
P value	0.101	0.001	0.935	0.001	0.770	0.000	0.921	0.711	0.991
Result	NS	**	NS	**	NS	**	NS	NS	NS

Table F-value is 3.68 (5 %); 6.35 (1 %).
* $p \leq 0.05$; ** $p \leq 0.01$ and NS – Non Significant
Means bearing different superscripts a and b in columns differ significantly.

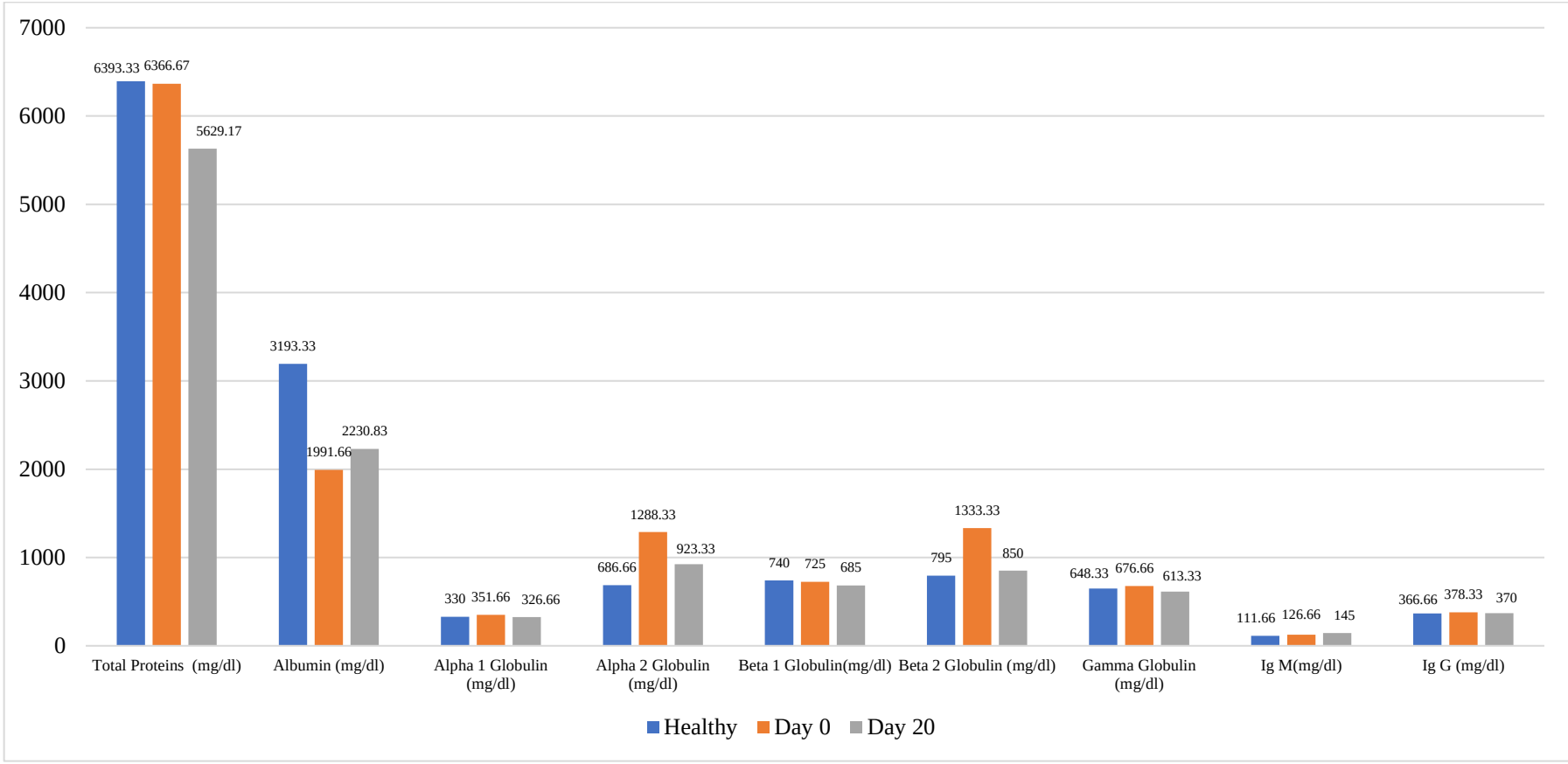


Figure 4.30 Electrophoretic pattern in Healthy control group, Pyometra anaemic dogs before Treatment (Day 0) and After Treatment (Day 20)

In the present study, statistically significant ($p \leq 0.05$) decreased Albumin, elevated Alpha 2 (α 2), and Beta 2 (β2) globulins in anaemic dogs were noted compared to Healthy Control dogs. Similarly, a non-significant increase was noted in α – 1 globulin, β-1 globulin, and gamma globulin protein fractions in anaemic dogs when compared with healthy control dogs.

On treatment Day 20, statistically insignificant increased concentrations of Albumin, and statistically significant decreased concentrations of Alpha 2 (α 2) and Beta 2 (β2) globulins were noted when compared with before-treatment values.

Further, it was also observed that on day 20, the values of Alpha 2 (α 2) and Beta 2 (β2) globulins were at par with the healthy control group values. Also, there was a non-significant difference between Healthy Control, before treatment, and Day 20 values in the Alpha 1 Globulin and Beta -1 Globulin protein concentrations.

The observations of Yoon *et al.* (2021) regarding canine pyometra i.e. decreased levels of albumin and elevated levels of α 2 globulin and β-globulin are akin to the present findings.

Reduced albumin and elevated α2- globulin are characteristic features of acute phase response. Alternatively, increased levels of β-globulin in dogs with pyometra may be associated with simultaneous infections. Inflammatory illnesses that occur simultaneously with infections can cause an increase in α- and β-globulins due to elevated levels of serum proteins, including β2-microglobulin, which possesses strong antibacterial properties. (Tothova *et al.*, 2016).

There is a possibility that other serum proteins, such as β2-microglobulins and immunoglobulins, might affect elevations of β-globulin in dogs with pyometra.

Yoon *et al.* (2021) in their electrophoretic studies on canine pyometra mentioned that serum amyloid A (SAA) was significantly higher in septic dogs with pyometra compared to non-septic dogs and could be a useful marker of sepsis.

In addition, Hp (heptaglobulin) is associated with the expressions of interleukin-6, interleukin-1, and tumor necrosis factor. Elevated Hp in pyometra has also been reported, suggesting Hp as a good candidate for monitoring inflammation in canine pyometra (Dabrowski *et al.* 2013).

In conclusion, elevated Acute phase proteins (APPs), and therefore, CRP, SAA, and Hp might be useful inflammatory indicators of canine pyometra (Hagman R., 2012)

4.3.7 IgM and IgG concentrations in Healthy control, Before Treatment (Day 0) and After Treatment (Day 20) anaemic dogs.

Table 4.69 presents the Mean ± SE data for overall IgM(mg/dl) and IgG (mg/dl) concentrations in anaemic and healthy dogs.

The overall IgM (mg/dl) concentrations in Healthy control (n=6), Before Treatment i.e. Day 0 (n=24), and After Treatment i.e. Day 20 (n=24) were (111.67 ± 5.43, 252.08 ± 31.24 and 208.75 ± 25.23) while overall IgG (mg/dl) concentrations were (366.67 ±17.26, 792.92 ± 102.29 and 647.08 ± 79.75) respectively.

The IgM and IgG concentrations in anaemic dogs were increased when compared with the healthy control dogs which after treatment i.e. on Day 20 were decreased and were at par with the healthy control group values.

The IgM(mg/dl) and IgG (mg/dl) concentrations in the Healthy control group, CKD-affected dogs and Day 20 were (111.66 ± 5.42, 315.00 ± 57.89 and 181.66 ± 19.90) and (366.66 ± 17.25, 976.66 ± 194.53 and 576.66 ±74.68), respectively. Significant ($p \leq 0.05$) increases in Ig M and IgG concentrations were noted in CKD-affected anaemic dogs which on post-treatment reached at par with the healthy control dogs.

The IgM(mg/dl) and IgG (mg/dl) concentrations in the Healthy control group, *E. canis* affected dogs on Day 0 and Day 20 were (111.67 ± 5.42, 390.00 ± 72.61 and 335.00 ± 62.70) and (366.66 ± 17.25, 1266.67 ± 226.24 and 1098.33 ± 190.00), respectively. Significant ($p \leq 0.05$) increases in Ig M and IgG concentrations were noted in *E. canis* affected anaemic dogs which on post-treatment reached at par with the healthy control dogs.

Table 4.69: IgM (mg/dl) and IgG (mg/dl) concentrations in Healthy control, Before Treatment (Day 0) and After Treatment (Day 20) anaemic dogs,

Group	Ig M(mg/dl)			Ig G (mg/dl)		
	Healthy	Day 0 (Before Treatment)	Day 20 (After treatment)	Healthy	Day 0 (Before Treatment)	Day 20 (After treatment)
CKD (n=6)	111.67 ± 5.43	315 ± 57.89	181.67 ± 19.90	366.67 ± 17.26	976.67 ± 194.54	576.67 ± 74.68
E. canis (n=6)	111.67 ± 5.43	390.00 ± 72.62	335.00 ± 62.70	366.67 ± 17.26	1266.67 ± 226.24	1098.33 ± 190.01
B. canis (n=6)	111.67 ± 5.43	176.67 ± 20.11	173.33 ± 25.78	366.67 ± 17.26	550.00 ± 71.13	543.33 ± 65.20
Pyometra (n=6)	111.67 ± 5.43	126.67 ± 7.15	145.00 ± 48.22	366.67 ± 17.26	378.33 ± 24.69	370.00 ± 108.90
Overall (n=24)	111.67 ± 5.43	252.08 ± 31.24	208.75 ± 25.323	366.67 ± 17.26	792.92 ± 102.29	647.08 ± 79.75

The IgM (mg/dl) and IgG (mg/dl) concentrations in the Healthy control group, *B. canis* affected dogs on Day 0 and Day 20 were (111.66 ± 5.42, 176.66 ± 20.11 and 173.33 ± 25.77) and (366.66 ± 17.25, 550.00 ± 71.13 and 543.33 ± 65.20), respectively. Non-significant increases in Ig M and IgG concentrations were noted in *B. canis* affected anaemic dogs which on post-treatment also at par with the healthy control dogs.

The IgM(mg/dl) and IgG (mg/dl) concentrations in the Healthy control group, pyometra-affected dogs on Day 0 and Day 20 were (111.66 ± 5.42, 126.66 ± 7.14 and 145.00 ± 48.21) and (366.66 ± 17.25, 378.33 ± 24.68 and 370.00 ± 108.90), respectively. Non-significant increases in Ig M and IgG concentrations were noted in pyometra-affected anaemic dogs which on post-treatment also at par with the healthy control dogs.

Slappendel (1979) emphasised the diagnostic importance of the direct antiglobulin test (DAT) in dogs with anaemia. Monospecific reagents (specifically Coomb's reagent) were employed in a study including 371 dogs. The Direct Antiglobulin Test (DAT) yielded positive results in 134 dogs (36.1%), whereas all 35 control dogs tested negative. The presence of antibodies and complement components in dogs who tested positive for direct antiglobulin test (DAT) were as follows: IgG alone was identified in 15 dogs (11.2%), IgG and C3b were discovered in 41 dogs (30.6%), C3b alone was detected in 74 dogs (55.2%), and IgM and C3b were detected in 2 dogs (1.5%). The study found a significant prevalence of positive Direct Antiglobulin Test (DAT) in dogs with anaemia and internal illnesses.

Immune-mediated reactions are crucial to the pathophysiology of infection with *E. canis*. The presence of anti-platelets antibodies (APA) was detected in dogs less than a week following an experimental infection with *E. canis*. Dogs with the infection have been observed to exhibit platelet aggregation abnormalities, anti-nuclear antibodies (ANA), RBC autoagglutination with positive Coombs' test, and circulating immune complexes, all of which are linked to the disease process.

Part III: RBC Survival Study

4.4 Assessment of RBC Survival by *In-vitro* Studies:

4.4.1 Comparison of Haemoglobin (g/dl) (*In-vitro)* between Healthy, Donor, Recipient, and Donor + Recipient on different day intervals:

The haemoglobin (g/dl) (*In-vitro*) values in Healthy, Donor, Recipient and Donor + Recipient anaemic dogs on different day intervals are depicted in Table 4.70 while the percentage of change on respective days is mentioned in Table 4.71 (Figure 4.31). The haemoglobin values of the Donor (*In-vitro*) on Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35 were 14.93 ± 0.41, 14.62 ± 0.41, 14.42 ± 0.41, 14.11 ± 0.40, 13.84 ± 0.41 and 13.58 ± 0.42 respectively. When these haemoglobin values were compared with the self-control values, it revealed a decrease in Haemoglobin (*In-vitro*) by 2.09 %, 3.43 %, 5.52 %, 7.32 % and 9.07 % on Day 3, Day 7, Day 15, Day 28 and Day 35 respectively.

The haemoglobin (g/dl) values of the Recipient (*In-vitro*) on Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35 were 3.96 ± 0.16, 3.64 ± 0.16, 3.38 ± 0.16, 3.06 ± 0.17, 2.74 ± 0.18 and 2.45 ± 0.16. When these haemoglobin values were compared with the self-control values on Day 0 it revealed a decrease in Haemoglobin by 8.10 %, 14.62 %, 22.82 %, 30.91 % and 38.28 % on Day 3, Day 7, Day 15, Day 28 and Day 35 respectively.

The haemoglobin (g/dl) values of the Donor + Recipient (*In-vitro*) on Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35 were 4.12 ± 0.22, 3.85 ± 0.22, 3.55 ± 0.22, 3.28 ± 0.22, 2.98 ± 0.22 and 2.68 ± 0.22. When these haemoglobin values were compared with the self-control values on Day 0 it revealed a decrease in Haemoglobin by 6.47 %, 13.85 %, 20.53%, 27.76 % and 34.98 % on Day 3, Day 7, Day 15, Day 28 and Day 35 respectively.

The overall decrease in the *In-vitro* Haemoglobin (g/dl) on Day 35 was highest in the Recipient (38.28 %) group followed by the Donor + Recipient (34.98 %) group and Donor (9.07 %) group of dogs.

Table 4.70:*In-vitro* Hb (g/dl), RBC (x 10^6/µl), PCV (%) and Free Hb (g/dl) values in Healthy, Donor, Recipient and Donor + Recipient Dogs on Different day intervals (n=24)

Different Day Intervals	Healthy (*In-vitro*)				Donor(*In-vitro*)				Recipient(*In-vitro*)				Donor + Recipient(*In-vitro*)			
	Hb (g/dl)	RBC (x 10^6/µl)	PCV (%)	Free Hb (g/dl)	Hb (g/dl)	RBC (x 10^6/µl)	PCV (%)	Free Hb (g/dl)	Hb (g/dl)	RBC (x 10^6/µl)	PCV (%)	Free Hb (g/dl)	Hb (g/dl)	RBC (x 10^6/µl)	PCV (%)	Free Hb (g/dl)
Day 0	12.17 ± 0.23	5.99 ± 0.39	38.62 ± 1.94	0.00 ± 0.00	14.93 ± 0.41	6.91 ± 0.21	42.60 ± 0.95	0.00 ± 0.00	3.96 ± 0.16	1.83 ± 0.09	12.98 ± 0.67	0.00 ± 0.00	4.12 ± 0.22	1.99 ± 0.12	13.15 ± 0.80	0.00 ± 0.00
Day 3	12.00 ± 0.24	5.86 ± 0.38	38.84 ± 1.94	0.00 ± 0.00	14.62 ± 0.41	6.78 ± 0.21	42.75 ± 0.96	0.00 ± 0.00	3.64 ± 0.16	1.78 ± 0.09	13.04 ± 0.67	0.00 ± 0.00	3.85 ± 0.22	1.92 ± 0.11	13.22 ± 0.81	0.00 ± 0.00
Day 7	11.63 ± 0.23	5.77 ± 0.37	39.13 ± 1.93	0.00 ± 0.00	14.42 ± 0.41	6.66 ± 0.20	43.45 ± 1.11	0.00 ± 0.00	3.38 ± 0.16	1.76 ± 0.09	13.12 ± 0.68	0.06 ± 0.02	3.55 ± 0.22	1.90 ± 0.11	13.21 ± 0.80	0.00 ± 0.00
Day 15	11.37 ± 0.23	5.36 ± 0.35	41.23 ± 2.07	0.03± 0.02	14.11 ± 0.40	6.22 ± 0.19	45.40 ± 1.02	0.02 ± 0.01	3.06 ± 0.17	1.63 ± 0.08	13.82 ± 0.70	0.15 ± 0.02	3.28 ± 0.22	1.77 ± 0.10	13.99 ± 0.85	0.12 ± 0.02
Day 28	11.10 ± 0.23	5.12 ± 0.34	40.27 ± 2.01	0.08± 0.02	13.94 ± 0.41	5.93 ± 0.18	44.34 ± 0.99	0.09 ± 0.01	2.74 ± 0.18	1.55 ± 0.08	13.51 ± 0.69	0.21 ± 0.02	2.98 ± 0.22	1.68 ± 0.10	13.74 ± 0.85	0.22 ± 0.02
Day 35	10.77 ± 0.21	4.13 ± 0.28	39.57 ± 2.15	0.13± 0.02	13.58 ± 0.42	4.80 ± 0.15	43.49 ± 0.97	0.12 ± 0.01	2.45 ± 0.16	1.25 ± 0.06	13.25 ± 0.68	0.28 ± 0.02	2.68 ± 0.22	1.36 ± 0.08	13.77 ± 0.78	0.31 ± 0.03

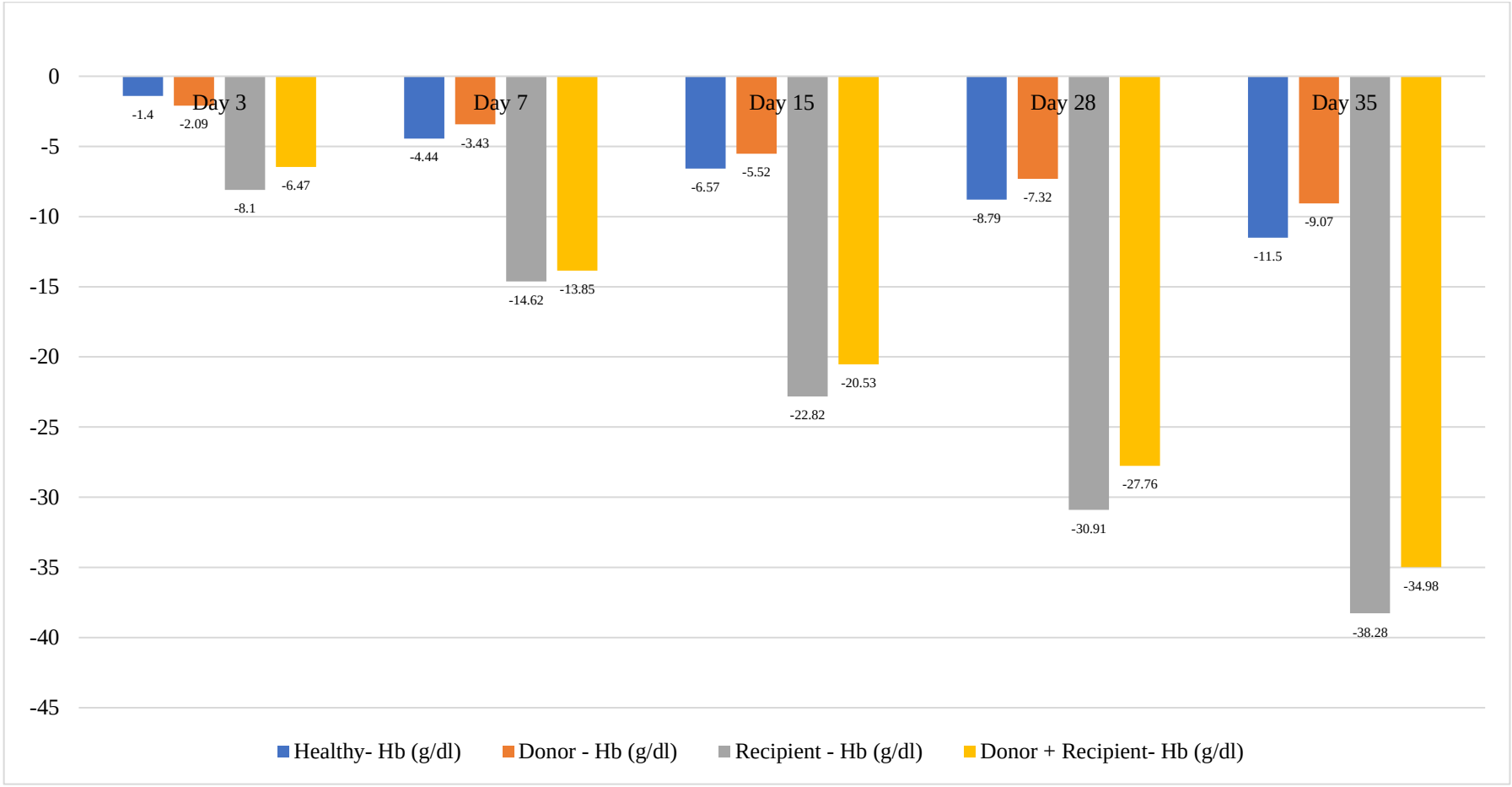


Figure 4.31 Comparison of Hb (g/dl) (*In-vitro)* between Healthy, Donor, Recipient, and Donor + Recipient on different day intervals

The higher decrease in the haemoglobin level in the stored blood of the recipient dog blood might be due to infected blood which releases certain haemolytic enzymes. *Babesia* spp parasites *are* intraerythrocytic and are commonly called piroplasms due to the pear-shaped forms found within infected red blood cells during the stored blood.

Arese and Flora (1990) reported that glucose-6-phosphate dehydrogenase occupies a central position to ensure the stability and viability of red blood cells. Harvey (1989) mentioned that Glutathione protects haemoglobin against oxidative stress. Poongodi (1997) noted the mean ± S.E. value of Haemoglobin (g/dl) in stored blood to be 12.30 ± 0.39, 12.25 ± 0.39, 12.18 ± 0.37, 11.87 ± 0.36, 11.65 ± 0.35, 11. 40 ± 0.29, 10.97 ± 0.33 on Day 0, 5,10,15,20,25 and 30 days. The decrease in the haemoglobin was in accordance with the present study findings.

Vertimalai (1992) studied that the haemoglobin level in the stored blood was maintained up to the tenth day of storage. After that a gradual decrease in haemoglobin was noticed on the fifteenth, twentieth and twenty-fifth day. A pronounced decrease was noticed on the 13th day of storage. According to Brar *et al*., (2014), the haemoglobin level gradually decreased as a result of a shift in the concentration of hydrogen ions. Up to Day 35 of storage, the haemoglobin level in the current investigation gradually decreased in the Donor, Recipient and Donor + Recipient blood which is akin to the writers mentioned above.

Refrigeration retards the rate of all chemical reactions but glycolysis continues throughout the storage with continuous consumption of high-energy phosphates. Glucose concentration falls and lactate rises bringing about a progressive fall in pH to a level which itself is rate limiting, possibly through inhibition of enzymes at some intermediary level (Bishop and Surgenor, 1964). Strumia (1954) reported that when blood is stored in plain isotonic sodium citrate, dextrose reaches critically low levels by the fifth day and at the same time, 2-3 diphosphoglycertate and adenosine triphosphate (ATP) fall rapidly.

During the storage of whole blood, the plasma undergoes progressive change, partly as a result of red cell metabolism because of the degradation of plasm proteins (Struma *et al.,* 1963).

Drawson *et al.,* (1970) mentioned that the normal functions of haemoglobin depend on adequate levels of 2,3 diphosphoglycertate (2,3 DPG) a compound that is better maintained at the higher pH afforded by citrate phosphate dextrose. Apart from 2,3, DPG other important variables which affect haemoglobin function during blood storage are pH, temperature and the ionic environment. Inorganic phosphate known to stimulate erythrocyte glycolysis, is present in one of the preservatives for blood storage i.e. citrate phosphate dextrose and the same is not present in ACD solution.

4.4.2 Comparison of Erythrocyte (x10^6/µl) (*In-vitro)* count between Healthy, Donor, Recipient, and Donor+Recipient on different day intervals:

The erythrocyte (X 10^6/µl) (*In-vitro*) values in Healthy, Donor, Recipient and Donor + Recipient anaemic dogs on different day intervals are depicted in Table 4.70 while the percentage of change on respective days is mentioned in Table 4.71 (Figure 4.32). The Erythrocyte values of the Donor (*In-vitro*) on Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35 were 6.91 ± 0.21, 6.78 ± 0.21, 6.66 ± 0.20, 6.22 ± 0.19, 5.93 ± 0.18 and 4.80 ± 0.15. When these Erythrocyte values were compared with the self-control values on Day 0 it revealed a decrease in erythrocytes by 1.92%, 3.66%, 9.91%, 14.16% and 30.47% on Day 3, Day 7, Day 15, Day 28 and Day 35 respectively.

The erythrocyte values of Recipient (*In-vitro*) on Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35 were 1.83 ± 0.09, 1.78 ± 0.09, 1.76 ± 0.09, 1.63 ± 0.08, 1.55 ± 0.08 and 1.25 ± 0.06. When these erythrocyte values were compared with the self-control values on Day 0 it revealed a decrease in erythrocytes by 2.49%, 3.72%, 10.85%, 15.25% and 31.74 % on Day 3, Day 7, Day 15, Day 28 and Day 35 respectively.

The erythrocyte values of Donor+ Recipient (*In-vitro*) on Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35 were 1.99 ± 0.12, 1.92 ± 0.11, 1.90 ± 0.11, 1.77 ± 0.10, 1.68 ± 0.10 and 1.36 ± 0.08. When these erythrocyte values were compared with self-control values on Day 0 it revealed a decrease in erythrocytes by 3.08%, 4.18%, 10.91%, 15.40% and 31.51% on Day 3, Day 7, Day 15, Day 28

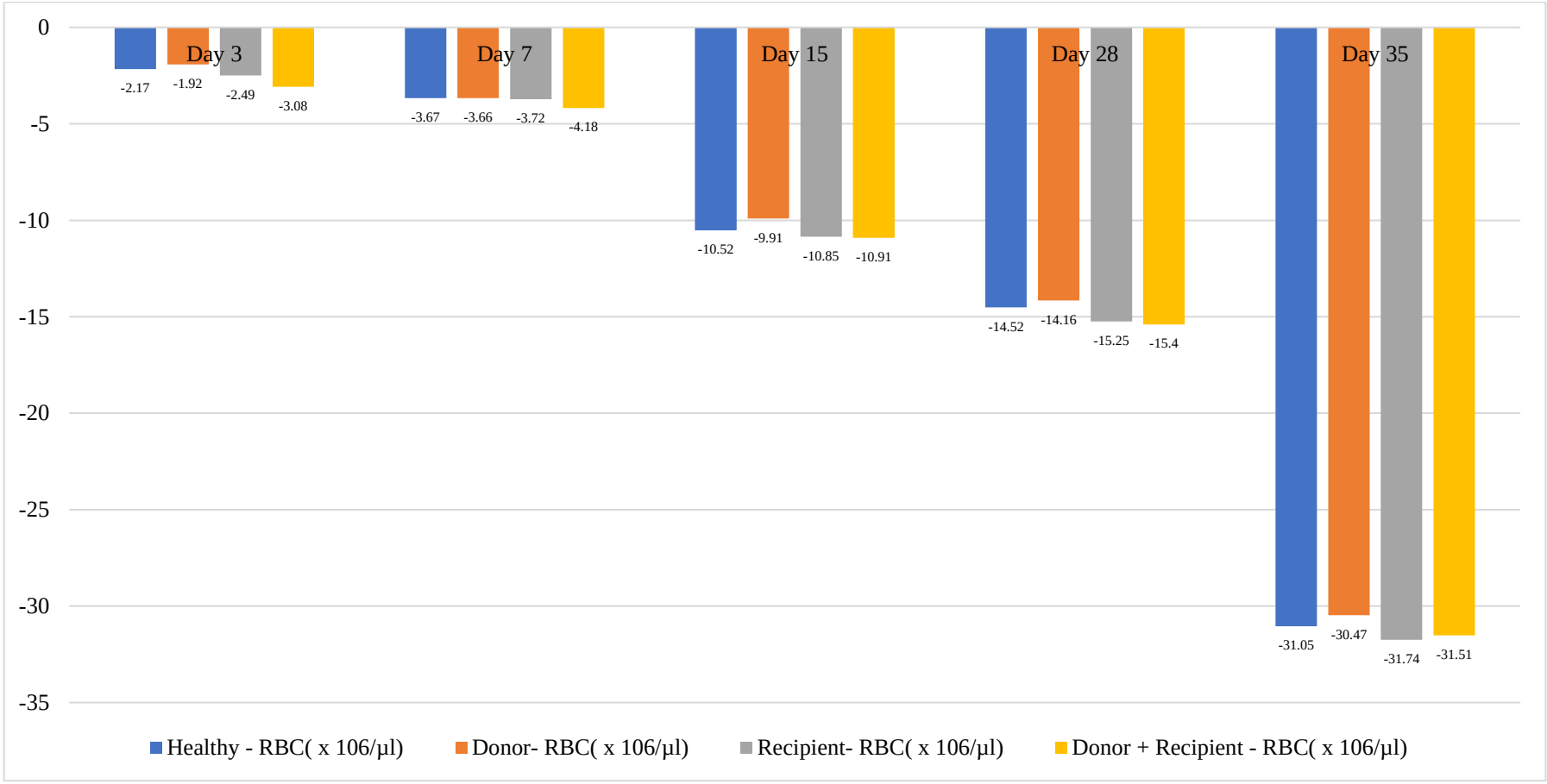


Figure 4.32 Comparison of RBC(x 10^6/µl) (*In-vitro)* between Healthy, Donor, Recipient, and Donor + Recipient on different day intervals

Table 4.71Percent difference of *In-vitro* Hb (g/dl), RBC (x 10^6/µl) and PCV (%) values in Donor, Recipient and Donor + Recipient Dogs on Different day intervals (n=24)

Different Day Intervals	**Healthy (*In-vitro*) (% Difference)**			**Donor (*In-vitro*) (% Difference)**			**Recipient(*In-vitro*) (% Difference)**			**Donor + Recipient (*In-vitro*) (% Difference)**		
	Hb(g/dl)	**RBC** (x 10^6/µl)	**PCV (%)**	**Hb(g/dl)**	**RBC** (x 10^6/µl)	**PCV (%)**	**Hb(g/dl)**	**RBC** (x 10^6/µl)	**PCV (%)**	**Hb(g/dl)**	**RBC** (x 10^6/µl)	**PCV (%)**
Day 0												
Day 3	-1.40	-2.17	0.57	-2.09	-1.92	0.37	-8.10	-2.49	0.47	-6.47	-3.08	0.51
Day 7	-4.44	-3.67	1.32	-3.43	-3.66	1.99	-14.62	-3.72	1.05	-13.85	-4.18	0.46
Day 15	-6.57	-10.52	6.76	-5.52	-9.91	6.59	-22.82	-10.85	6.46	-20.53	-10.91	6.37
Day 28	-8.79	-14.52	4.27	-7.32	-14.16	4.09	-30.91	-15.25	4.06	-27.76	-15.40	4.45
Day 35	-11.50	-31.05	2.46	-9.07	-30.47	2.10	-38.28	-31.74	2.09	-34.98	-31.51	4.67

and Day 35 respectively.

The overall decrease in the *In-vitro* erythrocyte count on Day 35 was highest in the Recipient (31.74 %) group followed by the Donor + Recipient (31.51%), and then the Donor (30.47 %) group of dogs. There might be minor blood incompatibility factors due to the mixing of donor and recipient blood causing increased lysis of the RBCs and hence sequestration of the RBCs was noted in the Donor + Recipient group of dogs.

Poongodi (1997) studied that the total red blood cell count was maintained up to the 10^{th} day, thereafter a significant decrease ($p \leq 0.01$) was noticed on the 5^{th}, 12^{th}, 25^{th} and 30^{th} day of storage while the lower value was recorded on the 13^{th} day was highly significant ($p \leq 0.01$) when compared with other groups.

Non-availability of donors in emergencies necessitates the storage of blood. It was believed that the progressive loss of erythrocyte viability in stored blood was in some way related to the simultaneous failure of cellular glycolysis (Rapoport and Marywing, 1947).

Vertimalai (1992) noted that the erythrocyte level in the stored blood was maintained up to the 7^{th} day and thereafter a gradual decrease was noticed on the twentieth, twenty-fifth and thirtieth day of storage.

4.4.3 Comparison of PCV (%) (*In-vitro)* values between Healthy, Donor, Recipient, and Donor+Recipient on different day intervals:

The PCV (%) (*In-vitro*) values in Donor, Recipient and Donor + Recipient anaemic dogs on different day intervals are depicted in Table 4.70 while the percentage of change on respective days is mentioned in Table 4.71 (Figure 4.33).

The PCV values (%) of the Donor (*In-vitro*) on Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35 were 42.60 ± 0.95, 42.75 ± 0.96, 43.45 ± 1.11, 45.40 ± 1.02, 44.34 ± 0.99 and 43.49 ± 0.97. When these PCV values were compared with the self-control values on Day 0 it revealed an increase in PCV by 0.37 %, 1.99%, 6.59%, 4.09% and 2.10% on Day 3, Day 7, Day 15, Day 28 and Day 35 respectively.

The PCV values of the Recipient (*In-vitro*) on Day 0, Day 3, Day 7, Day

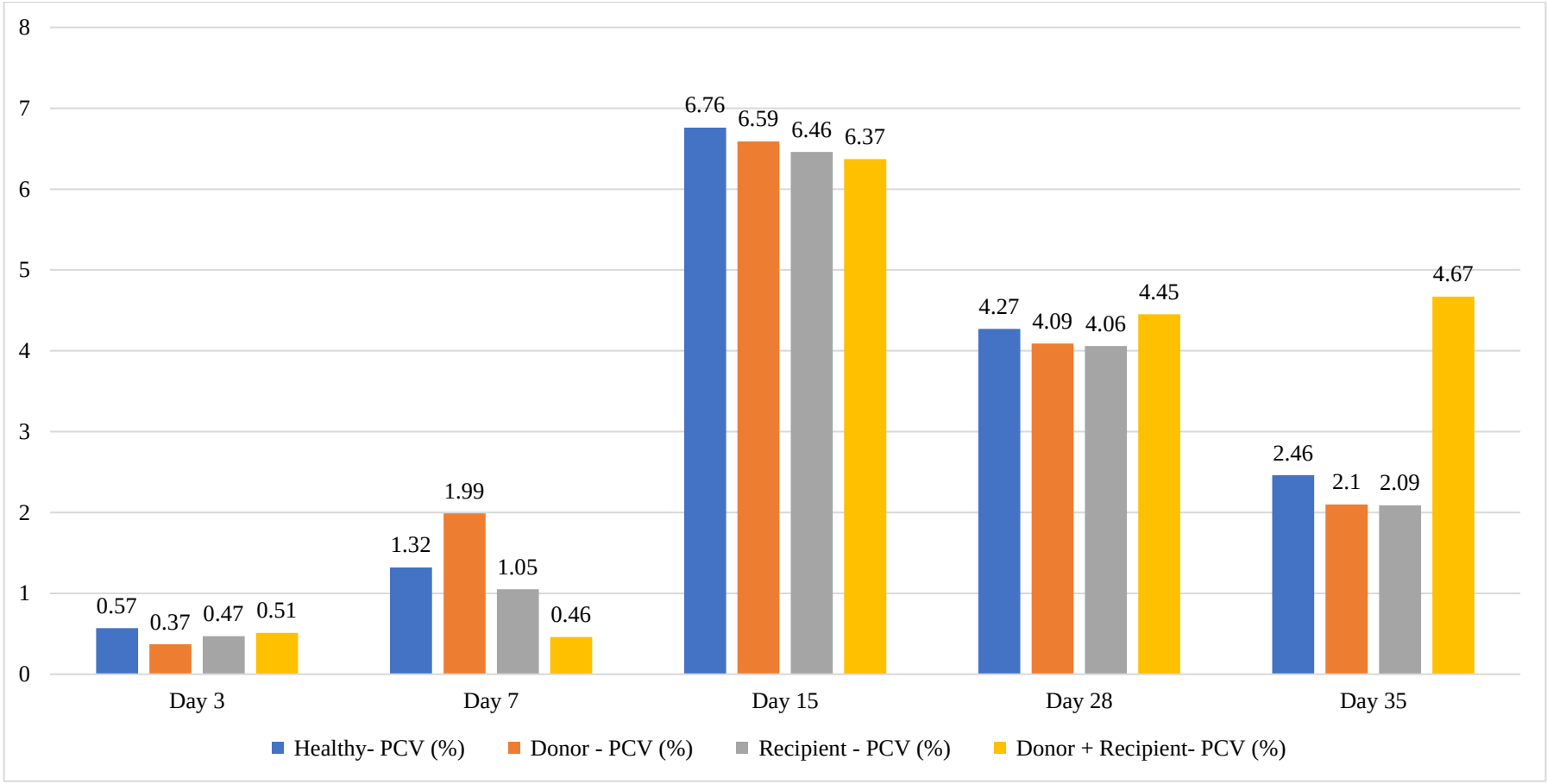


Figure 4.33 Comparison of PCV (%) (*In-vitro)* between Healthy, Donor, Recipient, and Donor + Recipient on different day intervals

15, Day 28 and Day 35 were 12.98 ± 0.67, 13.04 ± 0.67, 13.12 ±0.68, 13.82 ± 0.70, 13.51± 0.69 and 13.25 ± 0.68. When these PCV values were compared with the self-control values on Day 0 it revealed an increase in PCV by 0.47%, 1.05%, 6.46%, 4.06% and 2.09% on Day 3, Day 7, Day 15, Day 28 and Day 35 respectively.

The PCV values of the Donor + Recipient (*In-vitro*) on Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35 were 13.15 ± 0.80,13.22 ± 0.81. 13.21 ± 0.80, 13.99 ± 0.85, 13.74 ± 0.85 and 13.77 ± 0.78. When these PCV values were compared with the self-control values it revealed an increase in PCV by 0.51%, 0.46%, 6.37%, 4.45% and 4.67% on Day 3, Day 7, Day 15, Day 28 and Day 35 respectively.

The overall increase in PCV (*In-vitro)* values on Day 35 was highest in the Donor + Recipient (4.67 %) group followed by the Donor (2.10 %) and Recipient (2.09 %) group of dogs.

In all the groups it was observed that there was a gradual rise in the PCV values from Day 0 to Day 15 followed by a consistent decrease till Day 35. The erythrocytes swell due to the osmotic fragility of the RBC cell wall resulting in increased PCV.

The findings of the present study corroborate with Poongodi (1997) who noted that the packed cell volume value progressively increased (Statistically significant) up to the 20^{th} day and then reduced up to the 13^{th} day. A statistically increased value was observed on the 20^{th} day ($p \leq 0.01$).

Rapaport (1964) confirmed that there was a progressive increase in the packed cell volume and a marked swelling of cells as a result of an increase in sodium ion and entry of water in the cell and a decrease in potassium ion with a net increase in blood cell total base and water. In the present study also, a progressive increase in packed cell volume was noticed during the entire storage period. Hanswinter (1968) noticed that the mean increase in packed cell volume was 14.9%.

According to Venkataraman and Rajamani (1974), the canine blood began exhibiting hemolysis (a change in plasma colour) within 21.43+1.96 days in an

ACD solution. After 20 days of storage, Vetrimalai (1992) observed a decrease in the volume of packed cells. After 15 days of storage, the packed cell volume value in the current study dropped, which is consistent with Venkataraman and Rajamani (1974) and Vetrimalai (1992).

In the stored blood there is a marked swelling of cells as a result of an increase in sodium and a decrease in potassium with a net increase in blood cell total base and water as evidenced by the MCHC and cell diameter.

As storage progresses there is a progressive loss of osmotic resistance as shown by Rapaport (1964) and there is rough correlation exists between the increased fragility and post-transfusion survival of red cells. Gibson *et al.,* (1957) observed that there is no haemolysis in 0.6% solution of sodium chloride in CPD when stored from 0 to Day 35, whereas 50 % of the initially collected ACD cells were haemolysed.

In the study made by Venkataraman and Rajamani (1974), it was found that the keeping quality of canine blood stored at 4^0C in ACD solution was for 21 days. David *et al.,* (1974) stated that canine blood could be stored for 56 days.

4.4.4 Comparison of Free Hb (*In-vitro)* values between Donor, Recipient, and Donor + Recipient on different day intervals:

The Free Hb (g /dl) (*In-vitro*) values in Donor, Recipient and Donor + Recipient anaemic dogs on different day intervals are depicted in Table 4.70.

The Free Hb (g/dl) values of the Donor (*In-vitro*) were 0.00 ± 0.00, 0.00 ± 0.00, 0.00 ± 0.00, 0.02 ± 0.01, 0.09 ± 0.01 and 0.12 ± 0.01, Recipient (*In-vitro*) were 0.00 ± 0.00, 0.00 ± 0.00, 0.06 ± 0.02, 0.15 ± 0.02, 0.21 ± 0.02 and 0.28 ± 0.02 and in Donor + Recipient (*In-vitro*) were 0.00 ± 0.00, 0.00 ± 0.00, 0.00 ± 0.00, 0.12 ± 0.02, 0.22 ± 0.02 and 0.31 ± 0.03 on Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35 respectively.

The overall *In-vitro* Free Hb values on Day 35 were highest in the Donor + Recipient (0.31 ± 0.03) group followed by the Recipient (0.28 ± 0.02) and Donor (0.12 ± 0.01) group of dogs.

In the present study, it was noted that there is an increase in the free haemoglobin % in all three groups, as there is a progressive increase in the fragility rate with the storage period. The slightly higher level of *In-vitro* Free Hb in the Donor + Recipient (0.31 ± 0.03) group might be due to a few, donor-recipient blood incompatibilities which could not have been detected in the cross-match reactions. Also, the positive correlation of *In-vitro PCV* values and *In-vitro* Free Hb values in the Donor+ Recipient group of dogs was noted. The donors were healthy and hence caused less sequestration of blood, erythrolysis and release of free Hb.

The present findings of the study are in accordance with Rapaport (1964) who stated that in preserved human blood, the fragility rate of red cells increased and haemolysis occurred in the hypotonic saline solutions near to isotonic concentration as the storage period advanced. It was due to the increased swelling of the red cell reacting to the critical bursting point. Breazile *et al.,* (1971) stated that the red cell membrane is not absolutely unpermeable to Na+ and K+ so that these ions move across the cell membrane continuously and are transported.

Struma *et al.,* (1963) reported that very few red cells undergo haemolysis during storage. After 28 days, the plasma haemoglobin may be 150 mg per 100 ml representing lysis of about 1 % of the red cells. After transfusion, this haemoglobin is rapidly cleared from the circulation and is of no clinical significance.

4.4.5 Comparison of ratios between Donor (*In-vitro*) and Recipient (*In-vitro*) of Hb, RBC and PCV values on different day intervals:

Table 4.72 represents the ratios between Donor (*In-vitro*) and Recipient (*In-vitro*) of Hb, RBC and PCV values on different day intervals. The ratio between Donor (*In-vitro*) and Recipient (*In-vitro*) of Haemoglobin (g/dl) were 3.77, 4.01,4.26, 4.21, 5.06 and 5.55, erythrocyte (x 10^6/µl) ratios were 3.78, 3.80, 3.78, and 3.82. 3.83 and 3.85 respectively on Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35, respectively.

The data revealed an increasing trend of haemoglobin ratio and RBC ratio between Donor (*In-vitro*) and Recipient (*In-vitro*) from Day 0 to Day 35

Table 4.72 Comparison of ratios between Donor (*In-vitro*) and Recipient (*In-vitro*) of Hb, RBC and PCV values on different day intervals (n=24)

Different Day Intervals	Haemoglobin (g/dl)			RBC (x 10^6/µl)			PCV (%)		
	IVD	IVR	Ratio of IVD to IVR	IVD	IVR	Ratio of IVD to IVR	IVD	IVR	Ratio of IVD to IVR
Day 0	14.93 ± 0.41	3.96 ± 0.16	3.77	6.91 ± 0.21	1.83 ±0.09	3.78	42.60 ± 0.95	12.98 ± 0.67	3.28
Day 3	14.62 ± 0.41	3.64 ± 0.16	4.01	6.78 ± 0.21	1.78 ±0.09	3.80	42.75 ± 0.96	13.04 ± 0.67	3.28
Day 7	14.42 ± 0.41	3.38 ± 0.16	4.26	6.66 ± 0.20	1.76 ±0.09	3.78	43.45 ± 1.11	13.12 ± 0.68	3.31
Day 15	14.11 ± 0.40	3.06 ± 0.17	4.61	6.22 ± 0.19	1.63 ±0.08	3.82	45.40 ± 1.02	13.82 ± 0.70	3.29
Day 28	13.94 ± 0.41	2.74 ± 0.18	5.06	5.93 ± 0.18	1.55 ±0.08	3.83	44.34 ± 0.99	13.51 ± 0.69	3.28
Day 35	13.58 ± 0.42	2.45 ± 0.16	5.55	4.80 ± 0.15	1.25 ±0.06	3.85	43.49 ± 0.97	13.25 ± 0.68	3.28

indicating increased haemolysis and RBC sequestration in recipient dogs than in donor dogs.

The ratio between Donor (*In-vitro*) and Recipient (*In-vitro*) of PCV (%) were 3.28, 3.28, 3.31, 3.29, 3.28 and 3.28 respectively. The PCV ratio reveals that there is an almost constant ratio throughout all the days except the mild increase in the ratio on Day 7 and Day 15. It reflects that the change in PCV in Donor (*In-vitro*) and Recipient (*In-vitro*) values was constant till Day 35.

4.4.6 Percent Improvement (Hb, RBC, and PCV) in the Donor + Recipient (*In-vitro*) group over the Recipient (*In-vitro)* group on different day intervals

Table 4.73 (Figure 4.34) represents percent improvement (Hb, RBC, and PCV) in the Donor + Recipient (*In-vitro*) group over the Recipient (*In-vitro)* group on different day intervals.

Comparison of Haemoglobin (g/dl) between Recipient (*In-vitro*) and Donor + Recipient (*In-vitro*) values revealed 4.00 %, 5.84 %, 4.93 %, 7.08 %, 8.75 % and 9.54 % improvement in Donor + Recipient group on Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35, respectively when respective days recipient (*In-vitro*) values were treated as self-control values.

Comparison of erythrocytes (X 10^6/µl) between Recipient (*In-vitro*) and Donor + Recipient (*In-vitro*) values revealed 8.66 %, 8.00 %, 8.15 %, 8.59 %, 8.47 % and 9.02 % improvement in Donor + Recipient group on Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35, respectively when respective days recipient (*In-vitro*) values were treated as self-control values.

While a comparison of PCV (%) between Recipient (*In-vitro*) and Donor + Recipient (*In-vitro*) values revealed 1.35 % and 1.40 %. 0.76 %, 1.26 %, 1.73 % and 3.92 % improvement in the Donor + Recipient group on Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35, respectively when respective days recipient (*In-vitro*) values were treated as self-control values.

There is an increase in the % improvement in Hb, RBC and PCV *In-vitro* values from Day 0 to Day 35 which revealed that the Hemolysis and RBC sequestration is comparatively less in Donor + Recipient (*In-vitro*) group as

Table 4.73 Percent improvement (Hb, RBC, and PCV) in the Donor + Recipient (*In-vitro*) group over the Recipient (*In-vitro)* group on different day intervals.

Different Day Intervals	Haemoglobin (g/dl) *(In-vitro)*			RBC (x 10^6/µl) *(In-vitro)*			PCV (%) *(In-vitro)*		
	Hb (IVR)	Hb (IVDR)	% Improvement of Hb in IVDR	RBC (IVR)	RBC (IVDR)	% Improvement of RBC in IVDR	PCV (IVR)	PCV (IVDR)	% Improvement of PCV in IVDR
Day 0	3.96 ± 0.16	4.12 ± 0.22	4.00	1.83 ± 0.09	1.99 ± 0.12	8.66	12.98 ± 0.67	13.15 ± 0.80	1.35
Day 3	3.64 ± 0.16	3.85 ± 0.22	5.84	1.78 ± 0.09	1.92 ± 0.11	8.00	13.04 ± 0.67	13.22 ± 0.81	1.40
Day 7	3.38 ± 0.16	3.55 ± 0.22	4.93	1.76 ± 0.09	1.90 ± 0.11	8.15	13.12 ± 0.68	13.21 ± 0.80	0.76
Day 15	3.06 ± 0.17	3.28 ± 0.22	7.08	1.63 ± 0.08	1.77 ± 0.10	8.59	13.82 ± 0.70	13.99 ± 0.85	1.26
Day 28	2.74 ± 0.18	2.98 ± 0.22	8.75	1.55 ± 0.08	1.68 ± 0.10	8.47	13.51 ± 0.69	13.74 ± 0.85	1.73
Day 35	2.45 ± 0.16	2.68 ± 0.22	9.54	1.25 ± 0.06	1.36 ± 0.08	9.02	13.25 ± 0.68	13.77 ± 0.78	3.92

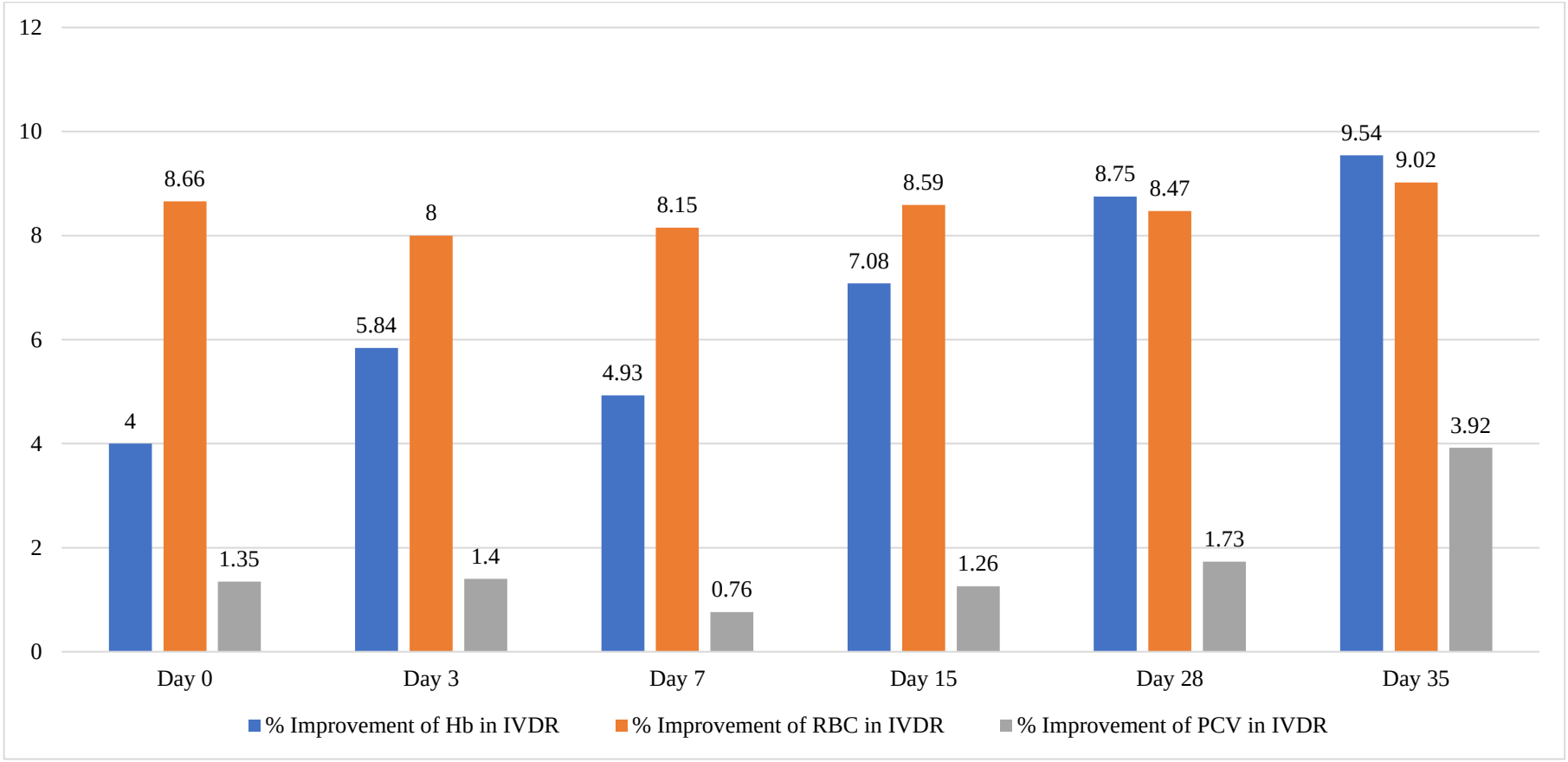


Figure 4.34 Percent improvement (Hb, RBC, and PCV) in the Donor + Recipient (*In-vitro*) group over the Recipient (*In-vitro)* group on different day intervals

compared to the Recipient (*In-vitro)* group. This decreased ratio of RBC sequestration and Hb values might be due to effective blood transfusion and disease-specific treatment.

4.4.7 Ratio and proportion (RBC) between the Donor + Recipient (*In-vivo*) group over the Donor + Recipient (*In-vitro)* group on different day intervals:

Table 4.74 (Figure 4.35) depicts the ratio (RBC) between the Donor + Recipient (*In-vivo*) group over the Donor + Recipient (*In-vitro)* group on different day intervals that showed 1.00, 1.09, 1.20,1.59 1.78 and 2.36 values. There is an increasing trend in the ratio from Day 0 to Day 35 where the lowest ratio was noted on Day 0 while the highest ratio was noted on Day 35.

In the present study, it was noted that approximately 70.25 % of *In-vitro* (Donor + recipient blood sample in CPDA) erythrocytes corresponding to 2.25 million/µl (70.25 % of 3.21) erythrocytes survived while 29.75 % of *In-vitro* erythrocytes corresponding to 0.96 million/µl (29.75 % of 3.21) erythrocytes lysed in *In-vitro* Donor + recipient (CPDA) blood sample on Day 35.

Further, it was noted that approximately 69.46 % of *In-vitro* (Donor blood sample in CPDA) erythrocytes corresponding to 4.80 million/µl (69.46 % of 6.91) erythrocytes survived while 30.54 % of *In-vitro* erythrocytes corresponding to 2.11 million/µl (30.54 % of 6.91) erythrocytes lysed in *In-vitro* Donor (CPDA) blood sample on Day 35.

Further, it was noted that approximately 68.3 % of *In-vitro* (Recipient blood sample in CPDA) erythrocytes corresponding to 1.25 million/µl (68.3 % of 1.83) erythrocytes survived while 31.7 % of *In-vitro* erythrocytes corresponding to 0.58 million/µl (31.7 % of 1.83) erythrocytes lysed in *In-vitro* Recipient (CPDA) blood sample on Day 35.

The data reflects that the rate of decreasing haemoglobin level and RBC sequestration is higher in *In-vitro* blood samples and it goes on increasing with the advancement of time. The RBCs are in a dynamic phase in *In-vivo* blood samples

Table 4.74 Ratio and Proportion Calculations (Hb, RBC, and PCV) between the Donor + Recipient (*In-vivo*) group over the Donor + Recipient (*In-vitro)* group on different day intervals.

Different Day Intervals	Haemoglobin (g/dl)					RBC (x 10^6/µl)					PCV (%)				
	Hb (IVDR) *(In-vivo)*	**Hb (IVDR) *(In-vitro)***	**Hb Ratio *(In-vivo to In-vitro)***	**Hb Prop. (*In-vitro*)**	**Hb Prop. (*In-vivo*)**	**RBC (IVDR) *(In-vivo)***	**RBC (IVDR) *(In-vitro)***	**RBC Ratio *(In-vivo to In-vitro)***	**RBC Prop. (*In-vitro*)**	**RBC Prop. (*In-vivo*)**	**PCV (IVDR) *(In-vivo)***	**PCV (IVDR) *(In-vitro)***	**PCV Ratio *(In-vivo to In-vitro)***	**PCV Prop. (*In-vitro*)**	**PCV Prop. (*In-vivo*)**
Day 0	4.12	4.12	1.00	**50.00**	**50.00**	1.99	1.99	1.00	**50.00**	**50.00**	13.15	13.15	1.00	**50.00**	**50.00**
Day 3	4.58	3.85	1.19	**54.28**	**45.72**	2.09	1.92	1.09	**52.09**	**47.91**	14.44	13.22	1.09	**52.20**	**47.80**
Day 7	5.06	3.55	1.42	**58.76**	**41.24**	2.28	1.90	1.20	**54.52**	**45.48**	16.10	13.21	1.22	**54.92**	**45.08**
Day 15	5.85	3.28	1.79	**64.13**	**35.87**	2.81	1.77	1.59	**61.36**	**38.64**	19.19	13.99	1.37	**57.84**	**42.16**
Day 28	6.52	2.98	2.19	**68.66**	**31.34**	2.98	1.68	1.78	**63.98**	**36.02**	19.82	13.74	1.44	**59.05**	**40.95**
Day 35	7.17	2.68	2.67	**72.79**	**27.21**	3.21	1.36	2.36	**70.25**	**29.75**	21.19	13.77	1.54	**60.61**	**39.39**

$$\text{Ratio} = \frac{\textit{In-vivo}}{\textit{In-vitro}} \qquad \text{Proportion} = \frac{\textit{In-vivo}}{\textit{In-vivo} + \textit{In-vitro}} \times 100$$

(Ref: Module 1.3, David Castellan, FAO regional Veterinary Epidemiologist, Basic measures and Tools of Descriptive epidemiology, Veterinary field epidemiology in action, course notes, 4 to 29 January 2010, Bangkok Thailand)

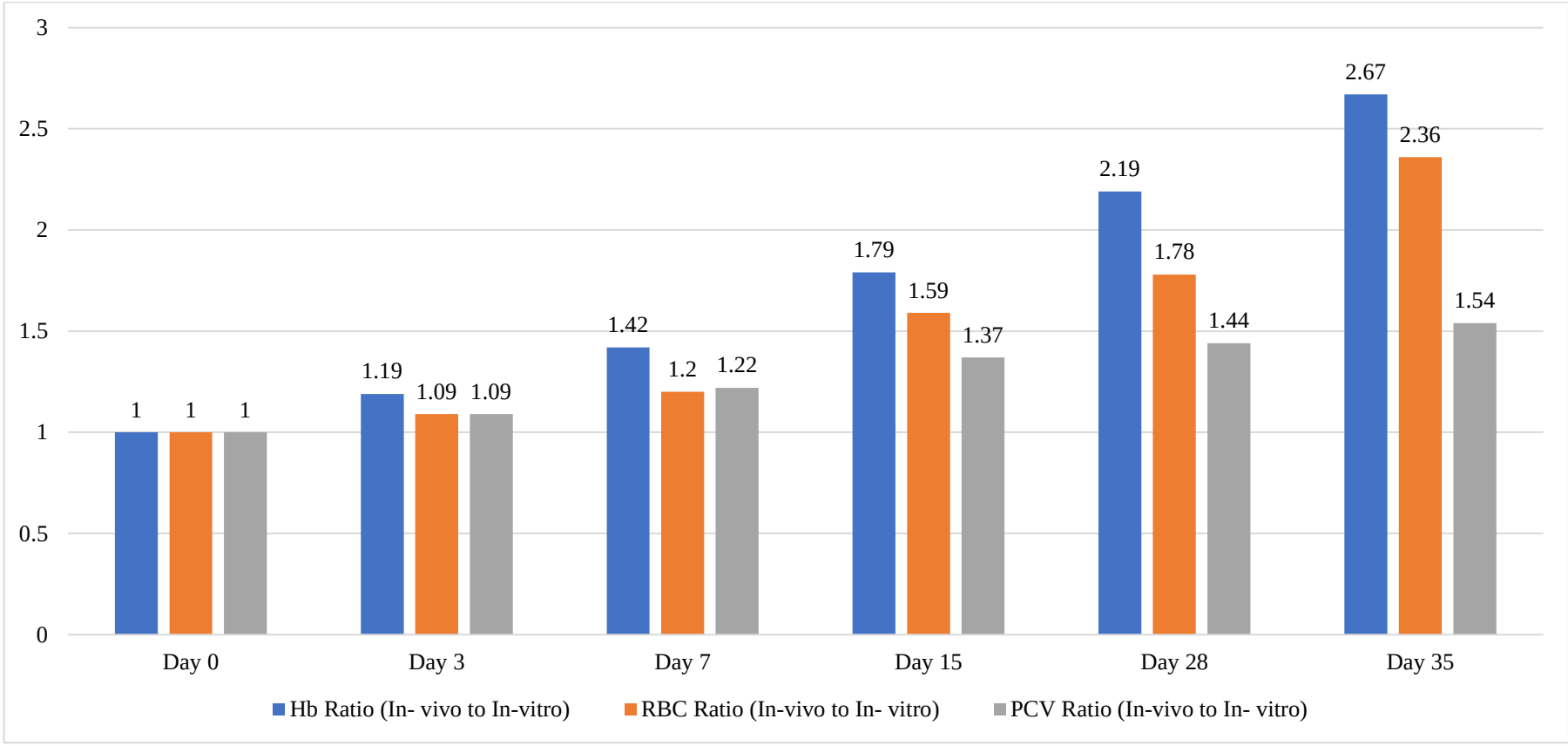


Figure 4.35 Ratio Calculations (Hb, RBC, and PCV) between the Donor + Recipient (*In-vivo*) group over the Donor + Recipient (*In-vitro)* group on different day intervals.

and hence body fluids, enzymes and oxygenation processes might be contributing in decreasing the haemolysis and RBC sequestration in *In-vivo* samples which on the contrary is absent in the *In-vitro* blood samples. There is a continuous release of oxidizing agents and haemo-destructive agents which cause damage to the αRBC storage and cause physiologic alterations that can be assessed using *In-vitro* measures. These alterations include decreased pH, ATP, and 2,3-DPG, lactic acid accumulation, heightened red cell membrane inflexibility, and impaired nitric oxide (NO) metabolism. These metabolic alterations collectively are referred to as the "storage lesion," and they may be a factor in reduced oxygen intake and delivery (Roback, 2011). Also during storage, there is the release of Tissue Necrosis Factor (TNFα) and interleukin (IL 1 and IL 6) which causes the cell destruction or cell lysis. Glucose and Adenine, also cause RBC deformity during storage. The RBC deformity can be observed by rheological studies where there is cell rigidity and Intravenous destruction at the capillary end.

4.4.8 Percent Haemolysis in Donor + Recipient (*In-vivo*), Donor (*In-vitro*), Recipient (*In-vitro*) and Donor + Recipient (*In-vitro*) blood

Table 4.75 (Figure 4.36) represents the percent haemolysis in Donor + Recipient (*In-vivo*) dogs while Table 4.76 depicts the percent haemolysis in Donor (*In-vitro*), Recipient (*In-vitro*) and Donor + Recipient (*In-vitro*) dogs on different day intervals. The values in Table 4.76 about Donor + recipient (*In-vivo*) revealed that there is no haemolysis till Day 7 however the percentage of haemolysis goes on increasing as 2.05 %, 3.37 % and 4.31 % respectively on Day 15, Day 28 and Day 35 respectively.

Table 4.76 about *In-vitro* haemolysis revealed a similar pattern of onset of haemolysis in Donor (*In-vitro*), Donor + Recipient (*In-vitro*) dog blood where there was no haemolysis till Day 7 however it was initiated and was in increasing pattern on Day 15, Day 28 and Day 35. In Recipient *In-vitro* dog blood the haemolysis started earlier comparatively i.e. From Day 7 onwards and it was in an increasing pattern as in other haemolysis trends.

Table 4.75 – Percent Haemolysis in Donor + Recipient (*In-vivo*) dogs (n=24)

Day	Donor + Recipient (*In-vivo*)				
	Hb (g/L)	RBC (x 10^{12}/L)	PCV(L/L)	Free Hb (g/L)	% Haemolysis
Day 0	41.21	1.99	0.13	0.00	0.00
Day 3	45.80	2.09	0.14	0.00	0.00
Day 7	50.60	2.28	0.16	0.00	0.00
Day 15	58.50	2.81	0.19	1.20	2.05
Day 28	65.20	2.98	0.20	2.20	3.37
Day 35	71.70	3.21	0.21	3.10	4.31

Values of Hb, RBC, PCV and Free Hb are in SI Units.

Table 4.76 – Percent Haemolysis in Donor (*In-vitro*), Recipient (*In-vitro*), and Donor + Recipient (*In-vitro*) dogs (n=24)

Day	Donor (*In-vitro*)					Recipient (*In-vitro*)					Donor + Recipient (*In-vitro*)				
	Hb (g/L)	RBC (x 10^{12}/L)	PCV (L/L)	Free Hb (g/L)	% Haemolysis	Hb (g/L)	RBC (x 10^{12}/L)	PCV (L/L)	Free Hb (g/L)	% Haemolysis	Hb (g/L)	RBC (x 10^{12}/L)	PCV (L/L)	Free Hb (g/L)	% Haemolysis
Day 0	149.33	6.91	0.43	0.00	0.00	39.63	1.83	0.13	0.00	0.00	41.21	1.99	0.13	0.00	0.00
Day 3	146.21	6.78	0.43	0.00	0.00	36.42	1.78	0.13	0.00	0.00	38.54	1.92	0.13	0.00	0.00
Day 7	144.21	6.66	0.43	0.00	0.00	33.83	1.76	0.13	0.60	1.77	35.50	1.90	0.13	0.00	0.00
Day 15	141.08	6.22	0.45	0.20	0.14	30.58	1.63	0.14	1.50	4.90	32.75	1.77	0.14	1.20	3.66
Day 28	138.42	5.93	0.44	0.90	0.65	27.38	1.55	0.14	2.10	7.66	29.96	1.68	0.14	2.20	7.33
Day 35	135.79	4.80	0.43	1.20	0.88	24.46	1.25	0.13	2.80	11.43	26.79	1.36	0.14	3.10	11.55

Values of Hb, RBC, PCV and Free Hb are in SI Units.

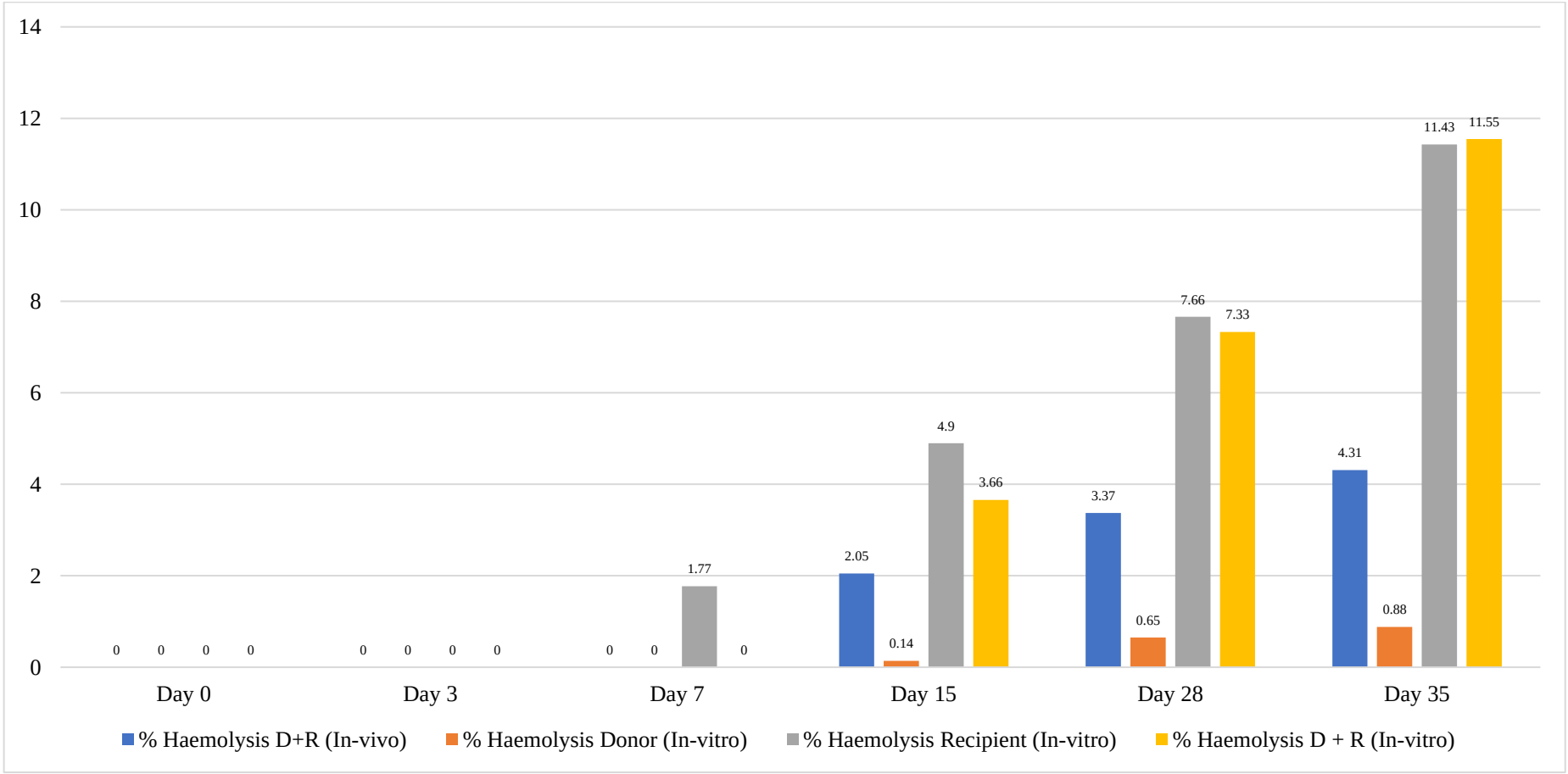


Figure 4.36 % Haemolysis in Donor + Recipient (*In-vivo*), Donor (*In-vitro*), Recipient (*In-vitro*), and Donor + Recipient (*In-vitro*) dogs (n=24)

In-vitro studies revealed that On Day 35, the maximum haemolysis was noted in Donor+Recipient (*In-vitro*) dogs as 11.55 % followed by Recipient (*In-vitro*) as 11.43 % and Donor (*In-vitro*) as 0.88 %. Clavería (2021) noted that red blood cells in most anaemias are more heterogeneous in their physical properties than healthy red blood cells, spanning adhesiveness, rigidity, density, size, and shape. On a similar analogy, the maximum red blood cell sequestration was noted in Recipient (*In-vitro*) blood affected with CKD, *E. canis*, *B. canis* and pyometra anaemias as compared to Donor (*In-vitro*) blood.

The highest % haemolysis in Donor+ Recipient (*In-vitro*) blood might be due to the blood incompatibilities addressed on mixing two bloods. It has also been documented that certain enzymes and metabolites are liberated on the mixing of blood which generally is taken care of by the body's immune system in *In-vivo*. However in *In-vitro* blood chemical oxidation of iron in haemoglobin is the central reaction that initiates oxidative stress, the major element for the development of the storage lesion.

Comparison of Donor + Recipient (*In-vivo*) and Donor+ Recipient (*In-vitro*) values revealed higher grade RBC sequestration in the *In-vitro* blood of dogs. Yoshida (2019) documented that Red blood cells (RBCs) have developed mechanisms to deal with oxidative and mechanical pressures they face during their crucial role as transporters of oxygen in living organisms. Nevertheless, even in a condition of isolation and subjected to hypothermic storage outside of the body, red blood cells encounter a distinct array of chemical and mechanical pressures. Due to the absence of evolutionary pressure, red blood cells (RBCs) have not developed any physiological mechanisms to deal with the storage lesions caused by these circumstances. The underlying factors contributing to the formation of the RBC storage lesion can be broadly categorised into two groups: (i) those that occur due to the isolation of RBCs, dilution of plasma with an additive solution, and prolonged hypothermic storage in a sealed bag; and (ii) those that arise from the storage of RBCs outside the body in the presence of oxygen, leading to oxidative stress and the depletion of biochemical defence mechanisms that were active *In-vivo*. Both factors lead to physical harm and metabolic dysfunction in stored RBCs.

Summary and Conclusions

CHAPTER – V

SUMMARY AND CONCLUSIONS

The present study entitled “Therapeutic Management of Anaemia Associated Hyperglobulinemia with Special Reference to Erythrocyte Survival in Dogs” encompassed twenty-four anaemic dogs (n=24) having hyperglobulinemia and six dogs (n=6) as the healthy control group. The inclusion criteria outlined in the material and procedure section, which required Haemoglobin levels below 5g/dl and serum globulin levels above 4g/dl. A full array of initial tests, encompassing clinical, haematological, biochemical, and urine analysis, was conducted on the canines that were brought for examination. The purpose of these tests was to evaluate the extent of anaemia and the body's capacity for erythropoiesis.

Protein electrophoresis (SPE) was performed on the serum samples both before treatment and on the 20th day of therapy to measure the fractions of globulins. There were six cases each of CKD, *E. canis*, *B. canis*, and pyometra, which were identified as the underlying causes of hyperglobulinemia and anaemia in dogs. Blood transfusion and disease-specific therapy were provided to all the canines until their recovery or release. The *In-vitro* tests involved measuring the levels of Hb, RBC, PCV, and Free Hb in the Donor, Recipient, and Donor + Recipient blood on before treatment, Day 1, 3, 7, 15, 28, and 35 of blood storage.

In the present investigation, data analyzed for the clinical assessment of anaemic dogs, based on different gradations and histograms revealed severely pale/ icteric mucus membrane (7.87 %), anorexia (5.36%), Dull and dehydrated skin coat with mild pruritus (8.33%), severely lethargic (6.02%), moderate exercise tolerance (5.09%) and melena (6.02 %) as the most consistent clinical findings. Urine analysis showed yellow-coloured urination (5.83 %), clear appearance (8.33 %), proteinuria (5.83) and acidic (10.00 %) as well as alkaline (10.00 %) pH. Hematuria was noted in 8 dogs (6.66 %). The blood coagulation evaluation of the anaemic dogs showed positive autoagglutination in 9 dogs (18.75%) and 7 dogs (14.58 %) showed the blood clotting time as 6 minutes or less than 6 minutes. There was an increased body temperature in 11 (41.7 %) in

the range of 102.7^{0}F to 104.3^{0}F followed by 6 (25.02 %) in the range of 104.3^{0}F, Tachycardia in 9 (37.53 %) in more than 129.7/min range followed by 7 (29.19 %) in the range of 114.6/min to 129.7/min and polypnea in 5 (20.85 %) in the range of 46.6/min to 55.2/min, 3 (12.51 %) in the range of more than 55.2/ min anaemic dogs.

The highest prevalence of anaemia in the present study was noted in Geriatric dogs i.e. in the age group of 74 to 109 months old 8 (33.33 %) and age group between 39 to 74 months old 7 (29.16 %) followed by more than 109 months old 4(16.66 %) and 5 to 39 months old 4 (16.66 %). The prevalence of anaemia due to *E. canis* and *B. canis* was higher in the middle-aged group of animals. A higher percent prevalence of anaemia was observed in males 15 (62.50%) than in females 9 (37.50%). Per cent prevalence of anaemia was found highest in the Labrador (33.33%) breed of dog, followed by Non-descript (16.67 %), Golden retriever (12.50%) then Boxer (8.33 %) and German shepherd (8.33 %).

In the present study, 70.83 % (17/24) of anaemic dogs were found to exhibit normocytic normochromic anaemia whereas 25.00% (6/24) showed macrocytic hypochromic anaemia followed by single (4.17%) case of microcytic hypochromic anaemia. Normocytic normochromic blood picture was evident in CKD, *E. canis* and pyometra dogs while *B. canis*-caused anaemia showed macrocytic hypochromic anaemia type of blood picture. The distribution of the anaemic dogs based on bone marrow activity exhibited maximum cases i.e. 18 (75%) of non-regenerative anaemia type while 6 (25%) cases of regenerative anaemia.

The histogram showed 50% (12/24) cases having albumin concentration between 1.5 to 2 (g/dl) followed by 7 (29.17%) having albumin concentration of 2 to 2.5 (g/dl) and 3 (12.50%) more than 2.5 (g/dl). Each single case having albumin concentration of 1 (g/dl) and 1 to 1.5 (g/dl) was noted in the present study. The 17 out of 24 (70.89%) anaemic cases contributed maximum number of anaemic dogs having globulin concentration between 3.4 to 5.0 (g/dl). The histogram showed 3 (12.51%) anaemic dogs each in globulin concentration

having between 5.1 to 6 g/dl and more than 6 g/dl. Only a single case (4.17 %) of globulin 3.99 (g/dl) was noted. Haematological analysis of the anaemic dogs (n=24) revealed a significant decrease in Hb, TEC and PCV values and a significant increase in MCV when compared with healthy dogs (n=6). Non-significant difference was noted in MCH and MCHC values between anaemic and healthy dogs. Individual disease-wise data showed a significant decrease in Hb, TEC and PCV in all the anaemic groups over the healthy control group. An insignificant difference in MCV, MCH and MCHC in CKD and *E. canis* groups while significant MCV, MCHC in *B. canis* and non-significant MCV, MCHC in pyometra groups were recorded.

The overall Hb values showed 3.99%, 15.45%, 27.65%, 47.74%, 64.57% and 80.86% increment, overall erythrocyte count showed 8.66%, 14.50%, 24.80%, 53.75%,63.31% and 75.69% increment while overall PCV values showed 1.35%, 11.26%, 24.05%, 47.89%, 52.68% and 63.23% increment respectively on Day 1, Day 3, Day 7, Day 15, Day 28 and Day 35.

The MCV showed an overall decrease of 5.69 % on Day 1, an increase of 1.80 %, 2.03 % on Day 3 and Day 7 respectively and a decrease of 2.96 %, 5.50% and 6.71 % on Day 15, Day 28 and Day 35 respectively compared to before transfusion MCV values. MCH values revealed an overall decrement of 3.60%, an increment of 5.97 %, an increment of 3.90 %, a decrement of 3.49 %, an increment of 1.47 % and an increment of 2.10 % respectively on Day 1, Day 3, Day 7, Day 15, Day 28 and Day 35 respectively in anaemic dogs. The overall MCHC values revealed percent increment in MCHC as 2.67, 4.10, 1.90, (Decrement 0.27),7.24 and 8.94 on Day 1, Day 3, Day 7, Day 15, Day 28 and Day 35 respectively.

Comparative analysis of the Hb, erythrocyte and PCV values between CKD, *E. canis*, *B. canis* and pyometra groups on BT, Day 1, 3, 7,15, 28 and 35 revealed significant differences when compared individually with the healthy control groups on respective days.

Comparative analysis of the MCV values between CKD, *E. canis*, *B. canis* and pyometra groups on Day 3, Day 15 and Day 28 revealed significant

differences while non-significant differences in before treatment, Day 1, Day 7 and Day 35 when compared individually with the healthy control groups on respective days.

Comparative analysis of the MCH values between CKD, *E. canis*, *B. canis* and pyometra groups on Day 3, Day 7, Day 15, Day 28 and Day 35 revealed significant ($p \leq 0.01$) differences while non-significant differences in before treatment and Day 1 when compared individually with the healthy control groups on respective days.

Comparative analysis of the MCHC values between CKD, *E. canis*, *B. canis* and pyometra groups on before treatment, Day 15, Day 28 and Day 35 revealed significant differences while non-significant differences in Day 1, Day 3 and Day 7 when compared individually with the healthy control groups on respective days.

Individual groups of anaemic dogs revealed the highest percentage of improvement in haemoglobin in *B. canis* (137.70) followed by *E. canis* (82.52), CKD (77.19) and pyometra (64.71) anaemic dogs. The order of percent improvement in erythrocyte count was *B. canis* (90.93) followed by *E. canis* (86.65), CKD (68.76) and pyometra (60.55) anaemic dogs while in PCV it was *E. canis* (75.21) followed by *B. canis* (66.69), CKD (59.38) and pyometra (10.37) anaemic dogs. The highest percentage of difference in MCV was noted in *B. canis* (9.77) followed by *E. canis* (6.81), pyometra (5.78) and CKD (4.25) anaemic dogs. Individual MCH values revealed an increment of 1.79% (CKD), a decrease of 3.31% (*E. canis),* an increment of 13.59% (*B. canis)* and decrement of 2.50% (pyometra) in anaemic dogs. The MCHC values on Day 35 revealed highest percentage of improvement in *B. canis* (25.32) followed by CKD (5.86), pyometra (3.70) and *E. canis* (3.35) anaemic dogs.

A statistically significant increase in the Hb, erythrocyte and PCV values of CKD, *E. canis*, *B. canis* and pyometra anaemic groups (except a non-significant increase in erythrocyte and PCV in the pyometra group) was noted when Day 35 values of respective diseases were compared with before-treatment values.

When MCV, MCH and MCHC values of Day 35 were compared with the respective values of before treatment group it revealed a statistically significant increase in *E. canis* group (In MCH) and *B. canis* group (In MCHC) while the rest of the comparisons were non-significant.

Leucocyte studies of all anaemic dogs (n=24) with healthy dogs (n=6) revealed leucocytosis, at par values of Neutrophils and Eosinophils, lymphopenia and monocytosis however, the statistical difference between them was non-significant. Individual disease-wise data observed non-significant leucocytosis in CKD and *E. canis* and leucopenia in *B. canis* anaemic dogs while significant leucocytosis in pyometra anaemic dogs over healthy control dogs. There was a significant difference in neutrophil count in *B. canis* and pyometra groups while monocytosis was noted among all the dogs infected with *E. canis* and *B. canis.*

Post-treatment overall total leucocyte count showed percent increment of 7.35, and 54.51 on Day 1 and Day 3 respectively and later on percent decrement as 19.86, 20.63, 27.43 and 48.16 on Day 7, Day 15, Day 21 and Day 35 respectively.

Post-treatment leucogram analysis revealed significant neutrophilia in CKD, *E. canis* and *B. canis* while significant neutropenia in pyometra anaemic dogs on Day 1 and Day 7 while non-significant difference was noted on Day 15, Day 28 and Day 35 when compared individually with the healthy control groups on respective days. The eosinophilic count revealed non-significant differences throughout all the day intervals in all the anaemic groups. Lymphopenia was noted in *E. canis* anaemic dogs on Day 1, Day 3 and Day 28. Monocytosis was noted in *E. canis* and *B. canis* anaemic dogs throughout all the study intervals.

There was a statistically non-significant difference in the whole leucogram, i.e. neutrophil, eosinophil, lymphocyte and monocyte values of CKD, *E. canis* and pyometra anaemic groups except statistically significant neutrophilia in the *B. canis* group when Day 35 values of respective disease were compared with before treatment values.

Platelet values were significantly decreased in overall anaemic as well as individual diseased groups of dogs over the healthy control group. The overall

platelet values were found to be increased by 17.30 %, 28.08%, 51.82%, 62.67%, 106.37% and 106.63% on Day 1, Day 3, Day 7, Day 15, Day 28 and Day 35, respectively. Marked thrombocytopenia was noticed in *B. canis* followed by *E. canis* and CKD and anaemic groups while moderate thrombocytopenia was recorded in the pyometra group in the before-treatment group. The highest percentage of improvement (Increase) in platelets was noted in *B. canis* (529.78) followed by *E. canis* (119.25), CKD (84.81) and pyometra (33.46) anaemic dogs. Statistically significant increase in the platelet count of CKD, *E. canis* and *B. canis* anaemic groups while the non-significant increase in the pyometra group was noted when Day 35 values of respective disease were compared with before treatment values.

The overall study revealed a significant difference of increment in ESR in anaemic dogs (41.96±2.87) over healthy (4.50±0.22) control dogs. The individual disease-wise study observed a significant increase in values of ESR (mm/hr.) in CKD (43.00 ± 4.69,) *E. canis* (40.00 ±5.64), *B. canis* (43.00 ±7.18) and pyometra (41.83 ± 6.72) anaemic dogs over the healthy control group. Post-treatment ESR values revealed decrement as 25.22%, 38.63%, 42.80%, 59.88%, 67.92% and 78.85% values on Day 1, Day 3, Day 7, Day 15, Day 28 and Day 35 respectively.

Comparative analysis of the ESR values between CKD, *E. canis, B. canis* and pyometra groups on BT, Day 1, 3, 7,15, 28 and 35 revealed significant differences when compared individually with the healthy control groups on respective days, except Day 35 where the non-significant difference was noted.

The highest percentage of improvement i.e. decrease in ESR was noted in *B. canis* (79.84) followed by *E. canis* (79.17), CKD (78.30) and pyometra (78.09) in anaemic dogs.

There was a statistically significant decrease in the ESR values of CKD, *E. canis, B. canis* and pyometra anaemic groups on Day 35 values of respective diseases when compared with before-treatment values.

There was statistically significant increase in reticulocyte while an insignificant increase in reticulocyte production index in anaemic dogs when compared with healthy control dogs. Individual disease-wise data of reticulocyte

count revealed significant difference of increment in *B. canis* anaemic group of dogs. The reticulocyte production index showed a significant increase in the *B. canis*, CKD and pyometra groups.

Post-treatment overall reticulocyte revealed a percent decrement of 6.34, 7.30, 21.24, 10.14 and 7.44 on Day 1, Day 7, Day 15, Day 28 and Day 35 except for Day 3 which witnessed percent increment of 31.08. The reticulocyte production index values revealed percent increments of 13.76, 68.53, 49.33, 48.57, 58.87 and 63.70 on Day 1, Day 7, Day 15, Day 28 and Day 35.

Comparative analysis of the reticulocyte and reticulocyte production index values between CKD, *E. canis*, *B. canis* and pyometra groups revealed significant differences throughout all the study intervals when compared individually with the healthy control groups on respective days.

Overall reticulocyte and reticulocyte production index revealed higher values in *B. canis* anaemic group followed by *E. canis*, pyometra and CKD groups.

The reticulocyte counts before treatment showed an increment of 67.86%, decrement of 2.61%, decrement of 20.06% and increment of 102.43% while reticulocyte production index values showed precent increment of 174.44 %, 62.54%, 42.32% and 247.13% respectively in respective anaemic groups in CKD, *E. canis*, *B. canis* and pyometra anaemic groups respectively.

The highest percentage of improvement in reticulocyte count was in pyometra, followed by CKD, *E. canis* and *B. canis* while the percentage of improvement in reticulocyte production index was in pyometra (247.13) followed by CKD (174.44), *E. canis* (62.54) and *B. canis* (42.32) anaemic dogs.

There was statistically non-significant increase in the reticulocyte and reticulocyte production index values of *E. canis*, *B. canis* and pyometra anaemic groups while significant increase in CKD when Day 35 values of respective diseases were compared with before treatment values.

Overall biochemical alterations all anaemic dogs (n=24) revealed a significant increase in total bilirubin, direct bilirubin, Indirect bilirubin, Globulin,

BUN and Creatinine while a significant decrease in albumin and A: G ratio in anaemic dogs when compared with healthy control dogs. Non-significant increases in the SGOT, SGPT, alkaline phosphatase and total protein values were noted in anaemic groups over healthy control dogs.

Individual disease-wise data revealed significantly increased BUN in CKD and *B. canis* anaemic dogs while an insignificant difference of increment was recorded in *E. canis* and pyometra groups. Serum creatinine values were statistically increased in CKD anaemic dogs while an insignificant difference of increment was noted in *E. canis, B. canis* and pyometra anaemic dogs. Also, there was a significant increase in total bilirubin and Indirect Bilirubin in the *B. canis* group while a significant decrease in direct bilirubin in the CKD group and a significant increase in *B. canis* group. The alkaline phosphatase was found to be significantly decreased in the CKD group while significantly increased in the *B. canis* anaemic group over healthy control values. There was a nonsignificant increase in total protein values in CKD and *E. canis* anaemic dogs while a non-significant decrease in pyometra anaemic dogs however statistically significant decrease was noted in the *B. canis* group of dogs. Significantly decreased Albumin, increased Globulin and decreased Albumin to Globulin ratio (A: G ratio) in all the anaemic groups viz, CKD, *E. canis, B. canis* and pyometra was recorded when compared with the healthy control dogs.

Post-treatment biochemical analysis revealed as follows: Percent change in serum BUN was 34.16, 12.11, 5.44, -28.18, -51.76 and -54.76), percent increment in serum creatinine was 21.08, 11.02, and 25.42, and a decrement of 9.34, 31.46 and 37.76 on Day 1, Day 3, Day 7, Day 15, Day 28 and Day 35, respectively. The overall increment of 6.02 % and decrement of 9.63 %, 11.10 %, 18.77%, 33.49% and 65.12% in Total Bilirubin, an overall increment of 2.76 % and decrement of 15.08 %, 12.61%, 22.07%, 36.00% and 71.67% in direct bilirubin while an increment of 9.74 % and a decrement of 3.44%, 9.44%, 15.02%, 30.63% and 57.35% in indirect bilirubin was noted on Day 1, Day 3, Day 7, Day 15, Day 28 and Day 35, respectively. SGOT values revealed an increment of 3.93 % and a decrement of 31.40 %, 47.11 %, 49.19 %, 50.75 % and 61.26 % on Day 1, Day 3, Day 7, Day 15, Day 28 and Day 35 SGOT values respectively.

A similar trend was observed in SGPT values wherein an increment of 35.05 % on Day 1 was followed by a decrement of 32.46 %, 39.44 %, 43.57 %, 45.90 % and 57.13 % on Day 3, Day 7, Day 15, Day 28 and Day 35 respectively. The percent values of alkaline phosphatase were found to be decreased by 20.50%., 11.04 %, 4.95%, 33.20 %and 39.20, % on Day 1, Day 3, Day 15, Day 28 and Day 35 respectively except an increment of 48.12 % noted on Day 7. The overall total protein values showed a percent increment of 2.69 on Day 1 followed by a decrement of 4.08 %, 4.35 %, 7.41 %, 10.40% and 10.27 % on Day 3, Day 7, Day 15, Day 28 and Day 35 respectively. The overall albumin values exhibited percent increments of 9.29, 12.34, 13.99, 15.27, 25.83 and 28.12 on Day 1, Day 3, Day 7, Day 15, Day 28 and Day 35 respectively. The overall globulin values showed a percent increment of 0.67 on Day 1 followed by a decrement of 10.16%, 11.21 %, 16.06 %, 24.34 % and 25.26 % on Day 3, Day 7, Day 15, Day 28 and Day 35 respectively. The overall A: G ratio values showed percent increments as 10.78, 24.31, 31.86, 38.20, 67.78 and 75.99 on Day 1, Day 3, Day 7, Day 15, Day 28 and Day 35, respectively

Comparative analysis of the serum BUN values between CKD, *E. canis*, *B. canis* and pyometra groups on Day 3, Day 7, Day 15, Day 28 and Day 35 revealed significant differences while in before treatment, Day 1, Day 3, Day 7 and Day 28 non-significant difference in Day 15 and Day 35 when compared individually with the healthy control groups on respective days.

The serum creatinine values were found to be significant in all the day intervals in all the anaemic dogs when compared individually with the healthy control groups on respective days.

Comparative analysis of the Total Bilirubin, Direct Bilirubin and Indirect Bilirubin values between CKD, *E. canis*, *B. canis* and pyometra groups revealed significant differences throughout all the Day intervals except on Day 7 when compared individually with the healthy control groups on respective days.

Comparative analysis of the total protein values between CKD, *E. canis*, *B. canis* and pyometra groups revealed significant differences throughout all the day intervals except Day 7 and Day 15 where non-significant difference was

noted when compared individually with the healthy control groups on respective days.

Comparative analysis of the albumin and globulin values between CKD, *E. canis*, *B. canis* and pyometra groups revealed significant differences throughout all the study intervals when compared individually with the healthy control groups on respective days.

Comparative analysis of the A: G ratio values between CKD, *E. canis*, *B. canis* and pyometra groups on BT, Day 1, 3, 7,15, 28 and 35 revealed significant skewed differences when compared individually with the healthy control groups on respective days.

The highest percentage of improvement in serum BUN i.e. decrease was noted in CKD (73.50), followed by *B. canis* (63.12), *E. canis* (21.77) and pyometra (20.58) respectively in anaemic dogs while the order of percentage of improvement i.e. decrease in serum creatinine was noted in CKD (45.21) followed by *B. canis* (37.43), pyometra (35.94) and *E. canis* (7.74), respectively in anaemic dogs.

The highest percentage of decrement in the Total Bilirubin, Direct Bilirubin and indirect Bilirubin on Day 35 was noted in the *E. canis* group followed by the *B. canis*, pyometra and CKD group of dogs.

The highest percentage of improvement (Decrease) in SGOT was documented in *E. canis,* followed by *B. canis*, pyometra, and CKD while in SGPT highest percentage (Decrease) was in *E. canis,* pyometra, CKD and *B. canis* anaemic groups. The order of percent improvement in ALP values was *B. canis*, pyometra, *E. canis* and CKD anaemic groups.

There was an overall percent decrement of 16.53, 13.47 and 17.81 in CKD, *E. canis* and pyometra groups respectively except for the *B. canis* group which showed an increment of 10.02 % on Day 35.

The highest percentage of improvement in albumin was noted in *B. canis* (78.03) followed by CKD (32.30), *E. canis* (5.96) and pyometra (5.00) anaemic dogs.

The highest percentage of decrement in globulin was noted in CKD (32.29,) followed by pyometra (28.11), *E. canis* (21.24), *B. canis* (18.15) anaemic dogs.

The highest percentage of increment in the A: G ratio was noted in *B. canis* (123.74) followed by CKD (99.44), pyometra (59.40) and *E. canis* (27.91) anaemic dogs. There was statistically significant increase in the A: G ratio values of CKD, *E. canis* and *B. canis* anaemic groups while non-significant increase in the pyometra group when Day 35 values of respective disease were compared with before treatment values.

There was a statistically significant decrease in the serum BUN values of CKD, *B. canis* and pyometra anaemic groups while non-significant decrease in *E. canis* group on Day 35 when respective diseases were compared with before-treatment values. Statistically significant decrease was noted in serum creatinine values of CKD and *B. canis* values groups while a non-significant decrease in *E. canis* and pyometra groups on Day 35 when respective diseases were compared with before-treatment values.

There was statistically non-significant decrease in the Total Bilirubin and Direct Bilirubin values on Day 35 in all the anaemic groups when respective diseases were compared with before treatment values. There was also statistically non-significant decrease in the Indirect Bilirubin on Day 35 in all the anaemic group except *B. canis* group when respective diseases were compared with before treatment values.

There was statistically non-significant decrease in the SGOT, SGPT and Alkaline phosphatase values of CKD, *E. canis. B. canis* and pyometra anaemic groups on Day 35 when respective disease values were compared with before treatment values.

Total protein values were found to be significantly decreased in pyometra while non-significantly increased in *B. canis* and non-significantly decreased in CKD and *E. canis* anaemic groups when Day 35 values of respective disease were compared with before treatment values.

There was statistically significant ($P \leq 0.05$) increase in the albumin values

of *B. canis* while non-significant increase in CKD, *E. canis* and pyometra anaemic groups when Day 35 values of respective disease were compared with before treatment values.

There was statistically significant decrease in the globulin values of CKD and pyometra groups while non-significant decrease in *E. canis* and *B. canis* anaemic groups when Day 35 values of respective diseases were compared with before treatment values.

The overall values of blood lactate levels in healthy control and anaemic dogs were 0.86± 0.14 and 4.80 ± 0.31, respectively where a significant increase in the anaemic dogs was recorded. Significant increment was noted in blood lactate throughout all the anaemic groups when compared with the healthy control group. Post treatment values revealed percent decrement as 8.19, 14.50, 20.20, 28.74, 37.96 and 51.44 on Day 1, Day 3, Day 7, Day 15, Day 28 and Day 35, respectively. The highest percentage of decrement in lactate was noted in *B. canis* (68.60) followed by pyometra (57.39), *E. canis* (56.72), and CKD (30.90) anaemic dogs. There was statistically significant decrease in the lactate values of *E. canis*, *B. canis* and pyometra anaemic groups while a non-significant decrease in CKD when Day 35 values of respective disease were compared with before treatment values.

The analysis of electrolytes in individual anaemic group showed as follows: The sodium values were found to be significantly decreased in *B. canis*, significantly increased in Pyometra and non-significantly decreased in CKD and *E. canis* anaemic dogs. The potassium values were significantly increased in all anaemic groups except a significant decrease in CKD group. There was a non-significant difference in chloride values in all the anaemic groups except *B. canis* (Significant increase) when compared with healthy control values.

The overall percent change in Sodium revealed a decrement of 1.61, 0.44 and 1.21 on Day 1, Day 3 and Day 7 respectively followed by respective increments of 2.56, 3.62 and 6.89 on Day 15, Day 28 and Day 35. The Potassium values witnessed a decrement of 1.33, 0.42, 4.64, 2.57 and 1.91 on Day 1, Day 7, Day 15, Day 28 and Day 35 respectively except for an increment of 0.18 on Day

3.

The percent change in Chloride revealed decrement of 0.13 on Day 1 followed by an increment of 0.18, 0.83, 1.17, 1.75 and 3.40 on Day 3, Day 7, Day 15, Day 28 and Day 35 respectively.

Comparative analysis of the Sodium Potassium and Chloride values between CKD, *E. canis*, *B. canis* and pyometra groups on BT, Day 1, 3, 7, 15, 28 and 35 revealed statistically significant sodium values throughout all the days except non-significant on Day 28 and Day 35 significant potassium values throughout all the days except non-significant on Day 3 and Day 35, statistically non-significant chloride values throughout all the days except significant on Day 1 and Day 7 when compared individually with the healthy control groups on respective days.

The overall per cent difference in Sodium values were 1.61 (decrement), 0.44 (decrement), 1.21 (decrement), 2.56 (increment), 3.62 (increment) and 6.89 (increment), Potassium were 1.33 (decrement), 23.61 (increment), 0.42 (decrement), 4.64 (decrement), 2.57 (decrement) and 1.91 (decrement) while Chloride were 0.13 (decrement), 0.18 (increment), 0.83 (increment), 1.17 (increment), 1.75 (increment) and 3.40 (increment) on Day 1, Day 3, Day 7, Day 15, Day 28 and Day 35 respectively.

Sodium level was found to be significantly increased in *E. canis* and *B. canis*, and significantly decreased in pyometra while at par values in the CKD anaemic group when Day 35 values of respective disease were compared with before treatment values.

Potassium level was found to be significantly decreased in *E. canis* and *B. canis*, while non-significantly increased in CKD and pyometra anaemic dogs when Day 35 values of respective disease were compared with before treatment values.

Chloride level was found to be non-significantly increased throughout all the anaemic groups when Day 35 values of respective disease were compared with before treatment values.

Urinary protein was found to be statistically increased while Urinary creatinine and UPC ratio was non significantly increased in anaemic dogs when compared with healthy control dogs. The individual disease-wise analysis of Urinary protein, Urinary Creatinine and UPC ratio revealed a significant increase in Urinary protein and UPC ratio while a non-significant increase in Urinary Creatinine in the CKD group, non-significant increase in Urinary protein, Urinary creatinine and UPC ratio in the rest of the anaemic dog groups viz. *E. canis*, *B. canis* and pyometra.

The overall UPC ratio values showed percent difference ratio as 7.32, 65.10, 6.81, 53.33, 21.83 (decrement), 29.15 (decrement) on Day 1, Day 3, Day 7, Day 15, Day 28 and Day 35 respectively.

Comparative analysis of the UPC ratio values between CKD, *E. canis*, *B. canis* and pyometra groups revealed significant differences throughout all the day intervals except Day 15 where the non-significant difference was noted when compared individually with the healthy control groups on respective days.

The highest percentage of improvement in UPC ratio was noted in pyometra, followed by CKD, *E. canis* and *B. canis* anaemic dogs.

There was a non-significant decrease in the UPC ratio values of CKD, *E. canis B. canis* and pyometra anaemic groups when Day 35 values of respective diseases were compared with before-treatment values.

The significant difference in increment in Free Hb values in anaemic dogs was noted when compared with healthy control dogs. Individual group analysis found a significant increase of free haemoglobin in *E. canis* and *B. canis* groups followed by CKD anaemic dogs while non-significant increase was noted in pyometra anaemic dogs when compared with healthy control dogs. Comparative analysis of the free Hb values between CKD, *E. canis*, *B. canis* and pyometra groups on BT, Day 1, 3, 7,15, 28 and 35 revealed significant difference when compared individually with the healthy control groups on respective days. Post-treatment free Hb revealed a statistically significant decrease in the free Hb values of CKD, *E. canis* and *B. canis* anaemic groups while a non-significant decrease in the pyometra group when Day 35 values of respective disease were compared

with before-treatment values. The per cent decrement in free Hb values on Day 35 compared to before treatment was 100, 82.51, 84.23 and 100 for CKD, *E. canis*, *B. canis* and pyometra anaemic groups respectively.

There was a nonsignificant decrease in the Serum Iron, nonsignificant increase and Significant decrease in percent Saturation in anaemic dogs over the healthy control dogs. However, post treatment findings in the anaemic dogs revealed significant increase in the Serum Iron, significant decrease in the TIBC and Significant increase in percent Saturation over the before treatment dogs.

Part II: Protein Electrophoresis

A comparison of all anaemic dogs (n=24) with healthy dogs (n=6) revealed hyperprotenemia, hypoalbuminemia and hyperglobulinemia. All globulin fractions viz. Alpha 1, Alpha 2, Beta 1, Beta 2 and Gamma globulins were significantly increased (Polycloncal gammopathy) in the anaemic dogs when compared with the healthy control dogs. After-treatment values on Day 20 revealed decreased protein, increased albumin, and decreased globulin values when compared with before-treatment values (Day 1). However, these values were at par with the healthy control values indicating improvement in the general health and condition of the anaemic dogs.

The overall Albumin: Globulin ratio in healthy control dogs (n=6) was 1.00 while in anaemic dogs (n=24) the skewed value i.e. 0.42 was noted. On treatment Day 20, the A: G ratio was increased to 0.66 indicating reduced globulin and increased albumin protein concentration. The overall ratio of Albumin with the Globulin fractions viz Alpha 1, Alpha 2, Beta 1, Beta 2, and Gamma globulin was (9.92, 4.67, 4.34, 4.04 and 5.02 in healthy dogs), (5.15, 1.89, 2.54, 1.88 and 1.86 in anaemic dogs) while (7.39, 3.11, 3.47, 3.04 and 3.26 in Day 20 treatment dogs), respectively. There was a decreased ratio of every globulin protein fraction in anaemic dogs when compared with healthy control group dogs. However, on treatment Day 20, the increased ratio in the respective globulin fraction, though not at par with the healthy control groups was noticed indicating a trend of improvement.

The overall ratio of Albumin with the Ig M (mg/dl) and Ig G (mg/dl) in

healthy control (n=6), anaemic dogs (n=24) and Day 20 treatment dogs were (28.95, 8.83), (9.85, 3.25) and (15.72, 5.31) respectively. The value indicated a decreased ratio of both the immunoglobulins i.e. IgG and IgM in anaemic dogs when compared with healthy control dogs however there was an increased ratio on Day 20, treatment group dogs indicating the lowered inflammation as a result of decreased hyperglobulinemia.

Electrophoretic patterns in CKD anaemic dogs revealed statistically significant hypoalbuminemia and hyperglobulinaemia. Significantly increased all globulin fractions viz. Alpha 1 (α 1), Alpha 2 (α 2), Beta 2 (β2) and Gamma (γ) globulins except non-significantly increased Beta 1 (β1) globulins were noted when compared with Healthy Control dogs. Post treatment Day 20 protein fractions were at par with the healthy control group values. All sera protein electrophoresis of the CKD-affected dogs with hypergammaglobulinemia showed polyclonal gammopathies.

Electrophoretic patterns in *E. canis* anaemic dogs revealed significant hypoalbuminemia, hyperglobulinaemia, Hyper β2-globulinemia, and hypergammaglobulinaemia. An insignificant increase was noted in α – 1 globulin, α – 2 globulin and β- 1 globulin protein fractions in anaemic dogs when compared with healthy control dogs. On treatment day 20, the concentrations of β2 globulins and gamma globulin were decreased when compared with Day 1 values but the decrease was statistically non-significant. Similarly, the values of α-1 globulin, α-2 globulin, and β-1 globulin were non-significantly decreased on Day 20 when compared with Day 1 values. All sera protein electrophoresis of the *E. canis* affected dogs with hypergammaglobulinemia showed polyclonal gammopathies.

Electrophoretic patterns in *B. canis* anaemic dogs showed statistically significant reduced Albumin, elevated Alpha 2 (α 2), and Beta 2 (β2) globulins while non-significant increases in gamma globulin protein fractions in anaemic dogs. Further, it was also observed that on Day 20, the values of Alpha 2 (α 2) and Beta 2 (β2) globulins were at par with the healthy control group values.

Electrophoretic patterns in pyometra anaemic dogs showed statistically significant decreased Albumin, elevated Alpha 2 (α 2), and Beta 2 (β2) globulins

in anaemic dogs. Non-significant increase was noted in α – 1 globulin, β-1 globulin, and gamma globulin protein fractions in anaemic dogs. Further, on day 20, the values of Alpha 2 (α 2) and Beta 2 (β2) globulins were at par with the healthy control group values.

The overall IgM (mg/dl) concentrations in Healthy control (n=6), Before Treatment i.e. Day 1 (n=24), and After Treatment i.e. Day 20 (n=24) were (111.67 ± 5.43, 252.08 ± 31.24 and 208.75 ± 25.23) while overall IgG (mg/dl) concentrations were (366.67 ±17.26, 792.92 ± 102.29 and 647.08 ± 79.75) respectively. The IgM and IgG concentrations in anaemic dogs (n=24) were increased when compared with the healthy control dogs which after treatment i.e. on Day 20 were decreased and were at par with the healthy control group values.

Significant increases in Ig M and IgG concentrations were noted in CKD-affected anaemic and *E. canis*-affected anaemic dogs which on post-treatment reached at par with the healthy control dogs. Non-significant increases in IgM and IgG concentrations were noted in *B. canis-affected* anaemic dogs and pyometra-affected anaemic dogs which on post-treatment also at par with the healthy control dogs.

Part III: RBC Survival Study

RBC survival study of haemoglobin (*In-vitro*) revealed a decrease in values of Donor by 2.09 %, 3.43 %, 5.52 %, 7.32 % and 9.07 %, decrease in values of Recipient by 8.10 %, 14.62 %, 22.82 %, 30.91 % and 38.28 %. decrease in values of Donor + Recipient by 6.47 %, 13.85 %, 20.53%, 27.76 % and 34.98 % on Day 3, Day 7, Day 15, Day 28 and Day 35 respectively. The overall decrease in the *In-vitro* Haemoglobin (g/dl) on Day 35 was highest in the Recipient (38.28 %) group followed by the Donor + Recipient (34.98 %) group and Donor (9.07 %) group of dogs.

RBC survival study of Erythrocyte (*In-vitro*) revealed a decrease in values of Donor by 1.92%, 3.66%, 9.91%, 14.16% and 30.47%, decrease in values of Recipient by 2.49%, 3.72%, 10.85%, 15.25% and 31.74 %. decrease in values of Donor + Recipient by 3.08%, 4.18%, 10.91%, 15.40% and 31.51% on Day 3, Day 7, Day 15, Day 28 and Day 35 respectively. The overall decrease in the *In-vitro*

erythrocyte count on Day 35 was highest in the Donor + Recipient (31.51 %) group followed by the Donor (30.47 %) group followed by the Donor + Recipient (31.74%) group of dogs.

RBC survival study of PCV (*In-vitro*) revealed an increase in values of Donor by 0.37 %, 1.99%, 6.59%, 4.09% and 2.11%, increase in values of Recipient by 0.47%, 1.05%, 6.46%, 4.06% and 2.09%. increase in values of Donor + Recipient by 0.51%, 0.46%, 6.37%, 4.45% and 4.67% on Day 3, Day 7, Day 15, Day 28 and Day 35 respectively. The overall increase in PCV (*In-vitro)* values on Day 35 was highest in the Donor + Recipient (4.67 %) group followed by the Recipient (2.09 %) and Donor (2.10 %) group of dogs. In all the groups it was observed that there was a gradual rise in the PCV values from Day 1 to Day 15 followed by a consistent decrease till Day 35.

The overall *In-vitro* Free Hb values on Day 35 were highest in the Donor + Recipient (0.31 ± 0.03) group followed by the Recipient (0.28 ± 0.02) and Donor (0.12 ± 0.01) group of dogs.

The ratio between Donor (*In-vitro*) and Recipient (*In-vitro*) of Haemoglobin (g/dl) were 3.77, 4.01,4.26, 4.21, 5.06 and 5.55, erythrocyte (X 10^6/µl) ratios were 3.78, 3.80, 3.78, and 3.82. 3.83 and 3.85 respectively on Day 1, Day 3, Day 7, Day 15, Day 28 and Day 35, respectively. The data revealed an increasing trend of haemoglobin ratio and RBC ratio between Donor (*In-vitro*) and Recipient (*In-vitro*) from Day 1 to Day 35 indicating increased haemolysis and RBC sequestration in recipient dogs than in donor dogs. The ratio between Donor (*In-vitro*) and Recipient (*In-vitro*) of PCV (%) were 3.28, 3.28, 3.31, 3.29, 3.28 and 3.28 respectively. The PCV ratio reveals that there is an almost constant ratio throughout all the days except the mild increase in the ratio on Day 7 and Day 15. It reflects that the change in PCV in Donor (*In-vitro*) and Recipient (*In-vitro*) values were constant till Day 35.

The approximate proportion of Hb that might present in *In-vivo* blood is 50.00 %, 54.28 %, 58.76 %, 64.13%, 68.66 % and 72.79 % while in *In-vitro* blood it is 50.00 %, 45.72%, 41.24 %, 35.87 %, 31.34 % and 27.21 % on Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35 *In-vitro* storage respectively.

Similarly, the approximate proportion of RBCs that might present in *In-vivo* blood are 50.00 %, 52.09 %, 54.52 %, 61.36%, 63.98 % and 70.25 % while in *In-vitro* blood it is 47.91 %, 45.48%, 38.64 %, 36.02 %, 29.75 % on Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35 *In-vitro* storage respectively.

Also, the approximate proportion of PCV that might present in *In-vivo* blood is 50.00 %, 52.20 %, 54.92 %, 57.84%, 59.05 % and 60.61% while in *In-vitro* blood it is 50.00 %, 47.80%, 45.08 %, 42.16 %, 40.95 % and 39.39 % on Day 0, Day 3, Day 7, Day 15, Day 28 and Day 35 *In-vitro* storage respectively.

Percent Improvement (Hb, RBC, and PCV) in the Donor + Recipient (*In-vitro*) group over the Recipient (*In-vitro) group* revealed that there is an increase in the % improvement in Hb, RBC and PCV (*In-vitro)* values from Day 0 to Day 35 which showed that the Hemolysis and RBC sequestration is comparatively less in Donor + Recipient (*In-vitro*) group as compared to the Recipient (*In-vitro)* group.

Comparison of *In-vivo* Vs *In-vitro* studies in the Donor + Recipient group revealed an increasing trend in *In-vivo* Hb, RBC and PCV values from Day 0 to Day 35 while the opposite trend of decrement in the *In-vitro* Hb, RBC and PCV values was noted.

Percent Haemolysis study revealed that there is no haemolysis in Donor+ recipient (*In-vivo*) till Day 7 however the percentage of haemolysis goes on increasing as 2.05 %, 3.37 % and 4.31 % respectively on Day 15, Day 28 and Day 35 respectively. In -vitro studies revealed that there was no haemolysis till Day 7 however the percentage of haemolysis goes on increasing and On Day 35, the maximum haemolysis was noted in Donor+Recipient (*In-vitro*) dogs as 11.55 % followed by recipient (*In-vitro*) as 11.43 % and Donor (*In-vitro*) as 0.88 %.

The following conclusions can be drawn from the study:

1. Anorexia, lethargy, exercise intolerance, melena and pale mucus membrane were most common clinical signs in anaemic dogs.
2. On Haematological examination, mean values of Hb, TEC, PCV and platelets were significantly reduced whereas MCV, ESR, Free Hb and Reticulocyte % were significantly increased.
3. Biochemical studies showed a significant reduction in serum Albumin, A:G ratio and serum Iron while significant increase in BUN, Creatinine, Total Bilirubin, Direct Bilirubin and Indirect Bilirubin; and Lactate levels in anaemic dogs.
4. Electrophoretic studies revealed significant hypoalbuminemia and hyperglobulinemia with predominant polyclonal gammopathy in anaemic dogs.
5. The sequestration of RBC increases with an increase in IgG and IGM levels. IgM levels were comparatively higher in pyometra followed by *B canis*, CKD and *E canis* while IgG levels were comparatively higher in pyometra followed by CKD, *B canis* and *E canis* anaemic dogs.
6. There was continuous and progressive sequestration of RBCs and Hb in CPDA-stored blood where Donor + recipient (*In-vitro*) blood sequestration was more as compared to Donor + recipient (*In-vivo*) blood. The rate of haemolysis in the *In-vitro* group was 0, 0, 0, 3.66, 7.33 and 11.55 % on Days 0, 3, 7, 15, 28 and 35 respectively.

Suggestions for future research work:

1. In the present study we documented the *In-vitro* RBC survival study. However, the radiolabeled RBC *In-vivo* is encouraged us to plan the future research work.
2. The serum protein electrophoresis studies were conducted in the present study in different anaemic dogs. We also came across with the references

of urine sample protein electrophoresis in nephropathies. Hence the same studies are encouraged as the future studies.

3. The diagnostics in anaemia, blood cross matching, blood or blood components transfusion, cross matching, RBC survival studies in Large ruminants is need of the time and hence encouraged as the better prospects for future studies.

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Appendix

APPENDIX I

Case record sheet

Case No:		Patient Name:		Owner's name:	
Age:		Breed:		Sex (M/F):	
Weight-					
Medical History:					
Vaccination Status:			Deworming status:		
Primary Complaint:			Diagnosis:		

Past Illness:

Ongoing medication:

Clinico-Physical Examination:

	BT	Day 0	Day 3	Day 7	Day 15	Day 28	Day 35
Temp (^{0}F)							
Heart Rate (Per minute)							
Respiration Rate (Per minute)							
Blood Clotting Time							
Pulse Quality							
Auto-agglutination							
Appetence							
Body Condition							
Mucus Membrane							
Skin and Hair coat							
Posture and Gait							
Behavior							
Lethargy							
Exercise Tolerance							
Melena							

Blood coagulation Evaluation:

Parameter	Gradation	Before Treatment	After Treatment
Autoagglutination	Negative		
	Positive		
Blood Clotting	6 or less than 6		
	More than 6		

Routine Urine Examination:

Urine Examination	BT	Day 0	Day 3	Day 7	Day 15	Day 28	Day 35
Color							
Appearance							
pH							
Protein in Urine							
Occult blood in urine							

Hematological examination:

Parameter	**BT**	**Day 0**	**Day 3**	**Day 7**	**Day 15**	**Day 28**	**Day 35**	**Unit**
Hb								g/dl
TEC								(X 10^6/µl
PCV								%
MCV								fl
MCH								pg
MCHC								g/dl
TLC								X 10^3/cmm
DLC								
Neutrophils								%
Eosinophils								%
Lymph								%

oocytes								
Monocytes								%
Basophils								%
Platelets								X 10^3/µl
Reticul ocytes								%
Absolute Retic.count								Cells/ µl
Corrected Retic. count								Cells/ µl
RPI								
ESR								mm/hr
FreeHb								g /dl

Biochemical examination

Parameter	**BT**	Day 0	Day 3	Day 7	Day 15	Day 28	Day 35	**Unit**
Total Bilirubin								mg/dl
Direct Bilirubin								mg/dl
Indirect Bilirubin								mg/dl
SGOT								IU/L
SGPT								IU/L
ALP								IU/L
TP								g /dl
Albumin								g /dl
Globulin								g /dl
A/G								-
BUN								mg/dl
Creatinine								mg/dl
Blood lactate								mmol/l
Sodium								mEq/L
Potassium								mEq/L
Chloride								mEq/L

Urinary Proteins and Creatinine Ratio:

Parameter	**BT**	**Day 0**	**Day 3**	**Day 7**	**Day 15**	**Day 28**	**Day 35**	**Unit**
Urinary Protein								mg/dl
Urinary Creatinine								mg/dl
Urinary Pro/ Creat Ratio								< 0.2 Non Proteinuric

Part II (Protein Electrophoresis)

Parameter	Before Treatment (BT)	Day 20	Units
Total Protein			g /dl
Albumin			g /dl
Globulin			g /dl
Alpha 1			g /dl
Alpha 2			g /dl
Beta 1			g /dl
Beta 2			g /dl
Gamma Globulins			g /dl
IgG			mg/dl
IgM			mg/dl

Part III (RBC Survival Study)

1. *In-vitro* Donor Blood examination:

	Day 0	Day 3	Day 7	Day 15	Day 28	Day 35	Unit
Hb							g /dl
TEC							(X 10^6/µl
PCV							%

2. *In-vitro* Recipient Blood examination

	Day 0	Day 3	Day 7	Day 15	Day 28	Day 35	Unit
Hb							g /dl
TEC							(X 10^6/µl
PCV							%

3. *In-vitro* Donor + Recipient Blood examination

	Day 0	Day 3	Day 7	Day 15	Day 28	Day 35	Unit
Hb							g /dl
TEC							(X 10^6/µl
PCV							%

APPENDIX – II

MUMBAI VETERINARY COLLEGE PAREL, MUMBAI - 400 012.

Phone No:022-24131180, 24137030 Ext. 143/132

DEPARTMENT OF VETERINARY CLINICAL MEDICINE, ETHICS AND JURISPRUDENCE

Date: / /

Title of the project: Therapeutic Management of Anaemia Associated Hyperglobulinemia with Special Reference to Erythrocyte Survival in Dogs.

CONSENT FORM OF DONOR DOGS

I, Mr/Mrs._____am willing to donate blood of my pet hereby authorize Dr._____and his/her representative to perform jugular puncture for blood collection of my Male/Female dog_____, age_____, belonging to_____breed and having_____ color.

All possible due care will be taken while examining, collection of blood etc. I am aware of all possible complications like hematoma and phlebitis occurring because of puncturing of the jugular vein and the doctor in charge or his/her associates shall not be responsible for any kind of such reaction.

I herewith declare that my dog is healthy to the best of my knowledge.

Date: Sign of the owner/ Guardian
Contact number:
Email ID:

Clinical examination/ Medical Examination of donor dog:

Color of mucus membrane: Pink/ Pale/ Cyanotic, Other:_____.
Temperature:_____, Heart rate:_____, Resp Rate:_____.
Mental Status and behaviour:_____.
Findings of PE:_____.
Comments: Clinically fit/Unfit for blood donation
Blood sent for lab investigation: Yes/No. Laboratory Findings of Donor Dog:
HB:__gm/dl, PCV:_____%, Platelet on smear:_____. Hemoprotozoan examination: E.canis/ H. canis/ B. canis/Microfilaria/ Trypanosoma, others_____.

Medical Fitness Certificate to donate canine blood

I have examined and verified the records of the donor dog. He/She is fit to donate_____ml of blood.

Doctor Sign

APPENDIX - III

DEPARTMENT OF VETERINARY CLINICAL MEDICINE, ETHICS AND JURISPRUDENCE

Date: / /

MUMBAI VETERINARY COLLEGE
PAREL, MUMBAI - 400 012.

Title of the project : Therapeutic Management of Anaemia Associated Hyperglobulinemia with Special Reference to Erythrocyte Survival in Dogs.

CONSENT FORM FOR RECIPIENT

Owner's Name:_____ Contact No : _ Email ID:_____

Address:_____ Animal : Breed : _____

Age :_____ Sex : M/F Colour:

Referred By :_____

I,_____, hereby authorize the Department ____ to collect biological sample_____ and_____ procedure (Specify name of procedure) for_____ of my animal as per the standard procedure explained to me.

The procedure, its requirements and the risks associated with the procedure, if any; have been explained to me and I have understood the same. I understand that this is a part of the routine diagnostic protocol/ therapeutic protocol and will aid in diagnosis, prognosis and treatment of my pet/ animal.

I have also been informed by the doctors that there could be certain risks and complications associated with such procedure(s). The limitations of the procedures have been explained to me as well and for which I will not create any legal issue.

I further understand that during the procedure, any unforeseen complications may arise, which necessitate administration of medicines or performance of additional procedures. I authorize the use of appropriate medications or additional procedures as needed by the patient; before, during or after the procedure.

I allow and do not object to use the data generated through these procedures for education, research and/or publication purposes.

(Sign of Veterinarian) **(Signature Owner)**

APPENDIX - IV

Transfusion Monitoring sheet

Recipient details **Product Details**

Name:	**Blood product ID:**
Signalment:	**Date of collection:**
Case no:	**Expiry date:**
PCV/TS:	**PCV/TS:**
BW:	**Unit Volume:**

Previous transfusions: _____
Reason for transfusion: _____
Clinician: _____
Signature:

Administration plan (volume and rate):

(Please circle)	pRBC	FFP	FWB

Crossmatched?	Compatible/Incompatible/Not evaluated	
Start time: _____	Finish time: _____	Volume infused:

	Time	Infusion rate (ml/hr)	RR	HR	MM color and CRT	Temp (^{0}C)	Mentation	Pruritis/ Erythema (Y/N)	Other findings
Pre-Transfusion									
5 mins									
15 mins									
30 mins									
60 mins									
2 hours									
4 hours									
15 mins post transfusion									
1 hour post transfusion									
24-hour post transfusion									
Comments:									

APPENDIX - V

Details of the Dogs under study having Anaemia with Hyperglobulinemia (n=24)

Case No	Name of Owner	Diagnosis	Age (Years)	Age (Months)	Sex	Breed
1	Mr.Niranjan Wagh	*E. canis*	2 years	24	Male	Labrador
2	CISF, RCF Alibagh	CKD	9 Years	108	Male	Labrador
3	CISF Mumbai Airport	*E. canis*	5 Years	60	Male	Labrador
4	Railway Police, Kalyan	*E. canis*	10 Years	120	Male	Labrador
5	Mrs.Shrushti Sontakke	*B. canis*	7 years	84	Male	Crocker Shannel
6	Mr. Kaustub Bhoi	*B. canis*	7 months	7	Male	Golden Retriever
7	Mr. Robin Clyde	CKD	6 Years	72	Male	Boxer
8	Mr. Sandeep Sawant	Pyometra	6 Years	72	Female	Labrador
9	Mr. Mahesh Dhakaliya	*E. canis*	4 Years	48	Male	Boxer
10	Mr. Vishal Chacha	CKD	7.5 years	90	Male	German Shepherd
11	Mr. Rayogi Patil	CKD	7 Years	84	Male	Golden Retriever
12	Mrs.Vimcent Anthony	*E. canis*	6 Years	72	Male	Doberman
13	Mr. Amol Doiphode	*B. canis*	1 Year	12	Female	German Shepherd
14	MRs.Varsha Prashant Salvi	*B. canis*	5 Months	5	Male	Non-Descript
15	Mr.Siddhesh Surve	Pyometra	10 Years	120	Female	Non-Descript
16	Paws Paradise Foundation	CKD	6 Years	72	Female	Non-Descript
17	Mr. Thomas D. Parokkaran	Pyometra	6.5 years	78	Female	Labrador
18	Mr. Prasad Sawant	Pyometra	7 Years	84	Female	Schitzu
19	Mrs. Pooja Vargeese	Pyometra	8 Years	96	Female	Labrador

20	Mr. Nitin Tiwari	CKD	3.5 Years	42	Male	Siberian Huskey
21	Mr. Vaijanath Khandare	*B. canis*	6 Months	6	Male	Non-Descript
22	Mrs. Sonal Gavade	*B. canis*	10 years	120	Female	Labrador
23	Mr. Vaibhav Pawar	*E. canis*	7 Years	84	Male	Golden Retriever
24	Mr.Abhishek Pawar	Pyometra	12 Years	144	Female	Rotviller
25	Mr.Amar	Healthy	3 Years	36	Male	Labrador
26	Mr.Kushal	Healthy	2 Years	24	Male	Non-Descript
27	Mr.Kadam	Healthy	4 Years	48	Male	Labrador
28	Mr.Thomas	Healthy	6 Years	72	Male	Labrador
29	Mr.Yogesh	Healthy	4 Years	48	Male	Labrador
30	Mr.Farah	Healthy	3 Years	36	Male	Labrador
	Average			**65.6**		

Thesis Abstract

Appendix – G

THESIS ABSTRACT

a)	Title of the thesis (in Capital letters)	:	**THERAPEUTIC MANAGEMENT OF ANAEMIA ASSOCIATED HYPERGLOBULINEMIA WITH SPECIAL REFERENCE TO ERYTHROCYTE SURVIVAL IN DOGS**
b)	Full name of student	:	**Hase Pankaj Bhanudas**
c)	Name and address of Major Advisor	:	**Dr. Rajiv Vasantrao Gaikwad** Professor and Head Department of Veterinary Medicine, Mumbai Veterinary College, Parel, Mumbai- 400 012
d)	Degree to be awarded	:	**Ph.D.**
e)	Year of award of degree	:	**2024**
f)	Major subject	:	**Veterinary Clinical Medicine, Ethics and Jurisprudence.**
g)	Total number of pages in the thesis	:	290
h)	Number of words in the abstract	:	497
i)	Signature of Student	:	
j)	Signature, Name and address of forwarding authority (HOD/ SH)	:	**Dr. R. V. Gaikwad** Professor and Head Department of Veterinary Clinical Medicine, Ethics and Jurisprudence
	Signature of the Associate Dean	:	

Dr. Rajiv V. Gaikwad M.V.Sc., Ph.D.
Professor, Hospital Superintendent and In charge,
Department of Veterinary Medicine, Veterinary Nuclear Medicine Including Radio Isotope Laboratory,
Teaching Veterinary Clinical Complex,
Bombay Veterinary College (MAFSU), Parel, Mumbai-12

Associate Dean
Mumbai Veterinary College
Parel, Mumbai - 400 012.

ABSTRACT

The present study entitled “Therapeutic Management of Anaemia Associated Hyperglobulinemia with Special Reference to Erythrocyte Survival in Dogs” was conducted in Department of Veterinary Clinical Medicine, Mumbai Veterinary College, Mumbai encompassing 24 hyperglobulinemic anaemic dogs having 6 cases each of CKD, *E canis*, *B. canis* and pyometra as etiology and 6 dogs as healthy control.

Clinical assessment of anaemic dogs, showed severely pale/ icteric mucus membrane (7.87 %), anorexia (5.36%), Dull and dehydrated skin coat with mild pruritus (8.33%), severely lethargic (6.02%), moderate exercise tolerance (5.09%) and melena (6.02 %) as the most consistent findings. Geriatric dogs i.e. in the age group of 74 to 109 months old 8 (33.33 %), males 15 (62.50%) and Labrador (33.33 %) breed was the most prevalent. 50% dogs noted albumin between 1.5 to 2 g/dl while 70.89% showed globulin between 3.4 to 5.0 g/dl. Normocytic normochromic anaemia was recorded as a major type of anaemia.

Hematology revealed a significantly lower values of Hb, TEC, PCV, and platelet values and significant increase in MCV, ESR, Free Hb, Reticulocyte and RPI in anaemic dogs. Serum biochemistry revealed significantly decreased Albumin, A:G ratio and Iron values while significantly increased BUN, Creatinine, Total Bilirubin, Direct Bilirubin, Indirect Bilirubin, Globulin and Lactate values in anaemic dogs. Serum electrolytes showed significantly increased Potassium.

Post treatment values revealed improvement by 80.86%, 75.69 % and 63.23 % in Hb, TEC and PCV on Day 35. The order of post treatment percent improvement in Hb and TEC was *B. canis*, *E. canis*, CKD and pyometra anaemic dogs while in PCV it was *E. canis*, *B. canis*, CKD and pyometra anaemic dogs. The highest percentage of difference in MCV was noted in *B. canis* (9.77) followed by *E. canis* (6.81), pyometra (5.78) and CKD (4.25) anaemic dogs.

Protein Electrophoretic studies revealed hyperprotenemia, hypoalbuminemia and hyperglobulinemia in anaemic dogs where all globulin fractions viz. Alpha 1, Alpha 2, Beta 1, Beta 2 and Gamma globulins were

significantly increased (Polycloncal gammopathy) in anaemic dogs when compared with healthy dogs. Post-treatment values on Day 20 revealed decreased protein and globulin and increased albumin when compared with before-treatment values. However, these values were at par with the healthy control values indicating improvement in the general health and condition of the anaemic dogs.

Electrophoretic patterns in CKD anaemic dogs revealed significant hypoalbuminemia, hyperglobulinaemia, increased all globulin fractions viz. Alpha 1 , Alpha 2, Beta 2 and Gamma globulins except non-significantly increased Beta 1 globulins when compared with Healthy Control dogs. Electrophoretic patterns in *E. canis* anaemic dogs revealed significant hypoalbuminemia, hyperglobulinaemia, Hyper β2-globulinemia, and hypergammaglobulinaemia. An insignificant increase was noted in α – 1 globulin, α – 2 globulin and β- 1 globulin protein fractions in anaemic dogs when compared with healthy control dogs. Electrophoretic patterns in *B. canis* anaemic dogs showed statistically significant reduced Albumin, elevated Alpha 2 (α 2), and Beta 2 (β2) globulins while non-significant increases in gamma globulin protein fractions in anaemic dogs. Electrophoretic patterns in pyometra anaemic dogs showed statistically significant decreased Albumin, elevated Alpha 2 (α 2), and Beta 2 (β2) globulins in anaemic dogs. Non-significant increase was noted in α – 1 globulin, β-1 globulin, and gamma globulin protein fractions in anaemic dogs. Post treatment Day 20 protein fractions in all anaemic groups were at par with the healthy control group values.

The overall Albumin: Globulin ratio in healthy dogs was 1.00 while in anaemic dogs the skewed value i.e. 0.42 was noted. On treatment Day 20, the A: G ratio was increased to 0.66 indicating reduced globulin and increased albumin protein concentration.

Significant increases in Ig M and IgG concentrations were noted in CKD-affected anaemic and *E. canis*-affected anaemic dogs which on post-treatment reached at par with the healthy control dogs.

RBC survival study revealed haemolysis in order as Donor + Recipient (*In-vitro*) 11.55%, Recipient (*In-vitro*)11.43%, Donor + Recipient (*In-vivo*) 4.31 % and Donor (*In-vitro*) 0.88 %.

Appendix – G

प्रबंध सारांश

१.	प्रबंधाचे शीर्षक	:	**श्वानांमध्ये लाल रक्त पेशीचे जगणे, या विशेष संदर्भासह रक्तक्षयाशी (अनेमिया) संबंधित हायपरग्लोबुलिनेमियाचे उपचारात्मक व्यवस्थापन**
२.	विद्यार्थ्याचे पूर्ण नाव	:	**पंकज भानुदास हासे**
३.	प्रमुख मार्गदर्शकाचे नाव व पत्ता	:	**डॉ. राजीव गायकवाड** प्राध्यापक आणि विभाग प्रमुख चिकित्सालयीन औषधवैद्यक शास्त्र, नीतिशास्त्र आणि न्यायवैद्यक शास्त्र विभाग मुंबई पशुवैद्यकीय महाविद्यालय, मुंबई
४.	पदवी दिली जाईल	:	**आचार्य**
५.	पदवी प्रदान करण्याचे वर्ष	:	**२०२४**
६.	मुख्य विषय	:	**चिकित्सालयीन औषधवैद्यक शास्त्र, नीतिशास्त्र आणि न्यायवैद्यक शास्त्र विभाग**
७.	प्रबंधातील एकूण पृष्टांची संख्या	:	२९०
८.	सारांशाचे एकूण शब्द	:	४९४
९.	विद्यार्थ्याची सही	:	
१०.	विभाग प्रमुखाचे नाव सही आणि पत्ता; दिनांक	:	**डॉ. राजीव गायकवाड** प्राध्यापक आणि विभाग प्रमुख चिकित्सालयीन औषधवैद्यक शास्त्र, नीतिशास्त्र आणि न्यायवैद्यक शास्त्र विभाग मुंबई पशुवैद्यकीय महाविद्यालय, मुंबई परळ, मुंबई – ४०० ०१२
११.	सहयोगी अधिष्ठाता, मुंबई पशुवैद्यकीय महाविद्यालय, परळ, मुंबई ४०० ०१२	:	Associate Dean Mumbai Veterinary College Parel, Mumbai - 400 012.

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सारांश

"श्वानांमध्ये लाल रक्त पेशीचे जगणे, या विशेष संदर्भासह रक्तक्षयाशी (अनेमिया) संबंधित हायपरग्लोबुलिनेमियाचे उपचारात्मक व्यवस्थापन" या शीर्षकाचा अभ्यास चिकित्सालयीन औषधवैद्यक शास्त्र, नितीशास्त्र आणि न्यायवैद्यक शास्त्र विभाग, मुंबई पशुवैद्यकीय महाविद्यालय, मुंबई येथे आयोजित करण्यात आला होता. यामध्ये २४ हायपरग्लोब्युलिनेमिक रक्तक्षय बाधित श्वानांचा, की ज्यामध्ये प्रत्येकी ६ श्वान असलेल्या सि.के.डी, ई कॅनिस,बी. कॅनिस आणि पायोमेट्रा या आजारांनी बाधित आणि ६ सशक्त श्वानांचा समावेश करण्यात आला होता.

रक्तक्षय बाधित श्वानांमध्ये चिकित्सालयीन मूल्यांकन केले असता, अतिजास्त फिकट गुलाबी / पिवळसर श्लेष्मा पडदा (७.८७%), खाणे बंद करणे (५.३६ %), निस्तेज आणि निर्जलित त्वचा (८.३३ %), अतिशय सुस्त (६.०२ %), मध्यम व्यायाम सहनशीलता (५.०९ %) आणि गडद विष्ठा (६.०२ %) सर्वात सुसंगत निष्कर्ष म्हणून दिसून आले. वयोरुद्ध श्वान की ज्यांचे वय ७४ ते १०९ महिने वयोगटातील ८ (३३.३३ %) आहे, नर श्वान १५ (६२.५० %) आणि लॅब्राडोर (३३.३३%) जात सर्वाधिक प्रचलित होत्या. ५० % श्वानांनी १.५ ते २.0 g/dl दरम्यान अल्ब्युमिन नोंदवले. तर ७०.८९ % श्वानांनी ३.४ ते ५.0 g/dl दरम्यान ग्लोब्युलिन दर्शविले. नॉर्मोसाइटिक नॉर्मोक्रोमिक पंडुरोगाचा प्रकार अधिक आढळून आला.

रक्त तपासणी मध्ये रक्तक्षय बाधित श्वानांमध्ये हिमोग्लोबिन, टोटल एरिथ्रोसाइट काउंट, पॅक्ड सेल व्हॉल्यूम आणि प्लेटलेट्स यांच्या संखेत लक्षणीय कमी तर एमसीव्ही, ईएसआर, फ्री एचबी, रेटिक्युलोसाइट आणि आरपीआयमध्ये लक्षणीय वाढ दिसून आली. सीरम बायोकेमिस्ट्रीने, रक्तक्षय (ॲनिमिक) श्वानांमध्ये अल्ब्युमिन, ए:जी प्रमाण आणि लोह संखे मध्ये लक्षणीय घट झाल्याचे दिसून आले, तर बीयूएन, क्रिएटिनिन, एकूण बिलीरुबिन, डायरेक्ट बिलीरुबिन, अप्रत्यक्ष बिलीरुबिन, ग्लोब्युलिन आणि लॅक्टेट मूल्यांमध्ये लक्षणीय वाढ झाली. रक्तजल क्षार प्रमाण (सीरम इलेक्ट्रोलाइट्स) मध्ये पोटॅशियममध्ये लक्षणीय वाढ दर्शविली.

उपचारानंतरच्या मूल्यांमध्ये ३५ व्या दिवशी हिमोग्लोबिन , टोटल एरिथ्रोसाइट काउंट आणि पॅक्ड सेल व्हॉल्यूम मध्ये ८०.८६, ७५.६९ % आणि ६३.२३ % ची सुधारणा दिसून आली. हिमोग्लोबिन आणि टोटल एरिथ्रोसाइट काउंट मध्ये रक्तक्षय उपचारानंतरच्या टक्के सुधारणांचा क्रम बी. कॅनिस, ई कॅनिस, सि.के.डी **आणि** पायोमेट्रा असा कुत्र्यांमध्ये होता. पॅक्ड सेल व्हॉल्यूम मध्ये ते ई कॅनिस, बी. कॅनिस, सि.के.डी आणि पायोमेट्रा असा होता. एमसीव्ही मधील फरकाची सर्वाधिक टक्केवारी बी. कॅनिस (९.७७) त्यानंतर ई कॅनिस (६.८१), पायोमेट्रा (५.७८) आणि सि.के.डी (४.२५) अशी नोंदवली गेली.

प्रथिने इलेक्ट्रोफोरेटिक अभ्यासात रक्तक्षय बाधित श्वानांमध्ये हायपरप्रोटेनेमिया, हायपोअल्ब्युमिनिमिया आणि हायपरग्लोब्युलिनमिया दिसून आला, जेथे निरोगी कुत्र्यांच्या तुलनेत रक्तक्षय बाधित श्वानांमध्ये सर्व ग्लोब्युलिन उदा. अल्फा १, अल्फा २, बीटा १, बीटा २ आणि गॅमा ग्लोब्युलिनमध्ये लक्षणीय वाढ झाली आहे (पॉलीक्लोन्सल गॅमोपॅथी).

२०व्या दिवशी उपचारानंतरच्या मूल्यांमध्ये प्रथिने आणि ग्लोब्युलिन कमी झाल्याचे आणि उपचारापूर्वीच्या मूल्यांच्या तुलनेत अल्ब्युमिनमध्ये वाढ झाल्याचे दिसून आले. तथापि, ही मूल्ये रक्तक्षय बाधित श्वानांच्या सामान्य आरोग्य आणि स्थितीत सुधारणा दर्शविणारी निरोगी नियंत्रण मूल्यांच्या बरोबरीची होती.

सि.के.डी. रक्तक्षय श्वानांमधील इलेक्ट्रोफोरेटिक पॅटर्नमुळे लक्षणीय हायपोअल्ब्युमिनिमिया, हायपरग्लोबुलिनेमिया, सर्व ग्लोब्युलिन वाढले आहेत. अल्फा १, अल्फा २, बीटा २ आणि गॅमा ग्लोब्युलिन वगळता बीटा १ ग्लोब्युलिन सशक्त श्वानाच्या तुलनेत लक्षणीयरीत्या वाढलेले नाहीत. ई कॅनिस बाधित रक्तक्षय श्वानांमधील इलेक्ट्रोफोरेटिक पॅटर्नमध्ये लक्षणीय हायपोअल्ब्युमिनिमिया, हायपरग्लोबुलिनेमिया, हायपर बीटा २-ग्लोब्युलिनेमिया आणि हायपरगामाग्लोबुलिनेमिया दिसून आले. निरोगी श्वानांच्या तुलनेत अल्फा १ ग्लोब्युलिन, अल्फा २ ग्लोब्युलिन आणि बीटा १ ग्लोब्युलिन प्रोटीन फ्रॅक्शन्समध्ये रक्तक्षय श्वानांमध्ये क्षुल्लक वाढ नोंदवली गेली. बी कॅनिस बाधित रक्तक्षय श्वानांमधील इलेक्ट्रोफोरेटिक पॅटर्नमध्ये सांख्यिकीयदृष्ट्या लक्षणीय घटलेले अल्ब्युमिन, वाढलेले अल्फा २, आणि बीटा २ ग्लोब्युलिन दिसून आले, तर रक्तक्षय श्वानांमध्ये गॅमा ग्लोब्युलिन प्रोटीन अंशांमध्ये लक्षणीय वाढ झाली नाही. पायोमेट्रा बाधित रक्तक्षय श्वानांमधील इलेक्ट्रोफोरेटिक पॅटर्नमध्ये सांख्यिकीयदृष्ट्या लक्षणीय घटलेली अल्ब्युमिन, वाढलेले अल्फा २, आणि बीटा २ ग्लोब्युलिन दिसून आले. अशक्त / रक्तक्षय कुत्र्यांमधील अल्फा १ ग्लोब्युलिन, बीटा १ ग्लोब्युलिन आणि गॅमा ग्लोब्युलिन प्रोटीन अंशांमध्ये लक्षणीय वाढ नोंदवली गेली.उपचारानंतर २० व्या दिवशी सर्व रक्तक्षय गटांमध्ये प्रथिने फ्रॅक्शिअस हे निरोगी नियंत्रण गट मूल्यांच्या बरोबरीने होते.

निरोगी कुत्र्यांमध्ये एकूण अल्ब्युमिन: ग्लोब्युलिनचे प्रमाण १.०० होते तर रक्तक्षय श्वानांमध्ये तिरपे मूल्य म्हणजेच 0.४२ नोंदवले गेले. उपचाराच्या २० व्या दिवशी, A: G गुणोत्तर 0.६६ वाढवले गेले जे ग्लोब्युलिन कमी झाल्याचे आणि अल्ब्युमिन प्रोटीन वाढल्याचे दर्शविते.

आय. जी. एम. आणि आय. जी. जी. एकाग्रता मध्ये सी के डी. प्रभावित रक्तक्षय आणि ई कॅनिस प्रभावित रक्तक्षय कुत्र्यांमध्ये लक्षणीय वाढ नोंदवली गेली जी उपचारानंतर निरोगी नियंत्रण कुत्र्यांच्या बरोबरीने पोहोचली.

लाल रक्त पेशीचे जगणे अभ्यासामध्ये हेमोलायसिसचा क्रम दाता + प्राप्तकर्ता (इन-विट्रो) ११.५५ %, प्राप्तकर्ता (इन-विट्रो) ११.४३ %, दाता + प्राप्तकर्ता (इन-व्हिवो) ४.३१ % आणि दाता (इन-व्हिट्रो) 0.८८ % असा होता.

Vitae

VITAE

The author **Mr. Hase Pankaj Bhanudas** was born on 12th October, 1981 at Sangamaner, Dist. Ahmednagar of Maharashtra State.

He completed his primary education, S.S.C. and H.S.C. from New English School and Junior College of Science, Parner. Dist Ahmednagar. He secured distinction in S.S.C. and first class in H.S.C. examinations in the year 1997 and 1999 respectively.

As he has a special interest towards animal welfare initiation activities, he admitted to College of Veterinary & Animal Sciences, MAFSU, Parbhani and obtained B.V.Sc.& A.H. degree certificate with first class in July 2004.

The author has special attitude towards clinical subjects and knows animal suffering, therefore joined the Department of Veterinary Medicine at the College of Veterinary and Animal Sciences, MAFSU, Parbhani where he completed the Master's Degree course securing first class in the year 2006.

He joined Maharashtra Animal and Fishery Sciences University, Nagpur as an Assistant Professor, at College of Veterinary and Animal Sciences Udgir in April 2008. Later he transferred to COVAS Parbhani, PGIVAS Akola and again COVAS Udgir. He then transferred and presently working in Mumbai Veterinary College, Parel, Mumbai as an Assistant Professor to accomplish his In-service Ph. D.

He had participated in many National Integration Camps in NCC and completed the NCC "C" certificate examination in the year 2004. He voluntarily participated in National Service Scheme (NSS) and Animal health camps held during his undergraduate and postgraduate degree programme. Up till now, he has 48 research papers in national and international journals and 190 extension articles to his credit.