TRANSFUSION OF BLOOD AND ITS COMPONENTS IN DOGS WITH HAEMATOLOGICAL DISORDERS

Dissertation

Submitted to the Guru Angad Dev Veterinary and Animal Sciences University in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY
in
VETERINARY MEDICINE
(Minor Subject: Veterinary Parasitology)

By

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(L-2017-V-12-D)

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CERTIFICATE – I

This is to certify that the dissertation entitled, **"Transfusion of blood and its components in dogs with haematological disorders"** submitted for the degree of **Ph.D.** in the subject of **Veterinary Medicine** (Minor subject: **Veterinary Parasitology**) of the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, is a bonafide research work carried out by **Iqra Shafi Khan (L-2017-V-12-D)** under my supervision and that no part of this dissertation has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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ABSTRACT

Chronic Kidney disease was the most common cause of anaemia followed by liver disease and haemoprotozoan infections. Transfusion of whole blood/ pRBCs resulted in highest improvement in surgery/ trauma group followed by gastrointestinal diseases and haemoprotozoan infections, while the minimum improvement was noticed in pancytopenia group. Transfusion of whole blood resulted in mean increase of 2.27 g/dL in Hb level and 8.27 per cent in PCV level, while transfusion of pRBCs improved Hb level (2.64 g/dL) and PCV level (8.48%). Transfusion of whole blood or pRBCs improved survivability (69.23%) in comparison to non-transfusion group (34.62%). Mean platelet count increase of $13.50\times10^3/\mu l$ was recorded in thrombocytopenic dogs one hour after transfusion of platelet rich plasma. Transfusion of plasma proved to be more beneficial in enhancing survival in canine parvoviral enteritis as compared to dogs suffering from chronic liver disease and acute hepatitis. All the coagulation factors decreased in chronic liver diseases except vWF, while vWF factor was found to increase in diabetic patients. PT and APTT increased in dogs with chronic liver diseases, but remained within normal range or slightly higher in bleeding and thrombocytopenic groups. PCR analysis revealed haemoprotozoan infections in 25.0 per cent recipient dogs with *Babesia gibsoni* (11.25%), *Ehrlichia canis* (11.25%) and *Hepatozoon canis* (2.50%). Based on retrospective studies on 24,370 dogs, hospital prevalence of thrombocytopenia was 4.96 per cent. Based on 1257 suspected cases analyzed for total serum protein and albumin, 642 were found to have abnormal protein concentration (51.07%) with the maximum suffering from hypoproteinemia + hypoalbuminemia (31.26%).

Keywords: Anaemia, FFP**,** Hypoproteinemia, pRBCs, PRP, thrombocytopenia

Signature of Major Advisor **Signature of the Student**

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LIST OF ABBREVIATIONS

%	:	Percentage
@	:	At the rate
<	:	Less than
>	:	Greater than
±	:	Plus-minus
≤	:	Less than or equal to
≥	:	Greater than or equal to
°C	:	Degree Celsius
µl	:	Microliter
10^3/µL	:	Thousand per microliter
10^6/µL	:	Million per microliter
ACD	:	Acid citrate dextrose.
ADCAS	:	Anaemic dog clinical assessment score
AID	:	Anaemia of inflammatory disease
AKI	:	Acute kidney injury
ALKP	:	Alkaline phosphatase
ALT	:	Alanine aminotransferase
APTT	:	Activated partial thromboplastin time
AST	:	Aspartate aminotransferase
bp	:	Base pair
BUN	:	Blood urea nitrogen
Bwt	:	Body weight
CBC	:	Complete blood count
CKD	:	Chronic kidney disease
CPDA	:	Citrate phosphate dextrose adenine
DEA	:	Dog Erythrocyte Antigen
DIC	:	Disseminated intravascular coagulation
DLC	:	Differential leucocyte count
DNA	:	Deoxyribo nucleic acid
e.g.	:	For example

EDTA	:	Ethylene diamine tetra acetic acid
ELISA	:	Enzyme-linked immune sorbent assay
et al	:	Et alia (Latin-and others)
FFP	:	Fresh frozen plasma
Fig.	:	Figure
g/dl	:	Gram per deciliter
GI	:	Gastrointestinal
Hb	:	Haemoglobin
i.e	:	That is
IMHA	:	Immune mediated haemolytic anaemia
IMT	:	Immune mediated thrombocytopenia
IRIS	:	International renal interest society
IU/L	:	International units per liter
IV	:	Intravenous
kg	:	Kilogram
LFT	:	Liver function test
mg/dl	:	Milli-grams per deciliter
mg/kg	:	Milli-grams per kilogram
MODS	:	Multiple organ dysfunction syndrome
N	:	Number of cases
NSS	:	Normal saline solution
OPD	:	Outpatient department
PBS	:	Phosphate buffered saline
PCV	:	Packed Cell Volume
pRBCs	:	Packed red blood cells
PRP	:	Platelet rich plasma
PT	:	Prothrombin time
RBC	:	Red Blood cell
RBPT	:	Rose Bengal plate test
RFT	:	Renal function test
Rpm	:	Revolutions per minute

SAGM	:	Saline adenine glucose mannitol
SE	:	Standard error
TAE	:	Tris-acetate-EDTA
TB	:	Total bilirubin
TEC	:	Total erythrocyte count
TLC	:	Total leucocyte count
TP	:	Total protein
TR	:	Transfusion reactions
TRALI	:	Transfusion related acute lung injury
vWD	:	von Willebrand disease
vWF	:	von Willebrand factor
WB	:	Whole blood

CHAPTER I

INTRODUCTION

Haematological disorders mainly comprise of decreased/ increased RBC count (anaemia/polycythemia), increased/decreased platelet count (thrombocytopenia /thrombocytosis) and coagulation disorders. However, erythrocytosis and thrombocytosis are very rare in dogs and have less clinical significance. Anaemia in dogs may occur due to loss of blood, destruction of RBCs or decreased production from bone marrow. Thrombocytopenia mainly occurs as a result of *Ehrlichia canis*, immune-mediated disorders or bone marrow depression. The primary haemostatic disorders include coagulation factor deficiencies, von Willebrand disease and acquired coagulation disorders.

Blood transfusion in haematological disorders is an integral part of a life-saving procedure and saves life in many critically ill canine patients (Howard et al., 1992). The practice of transfusion medicine has undergone marked change over the last decade, and advancement in blood typing, component therapy and infectious pathogen screening have increased the safety of transfusion therapy for canine patients (Feldman and Kristensen, 1995; Lucas et al., 2004; Wardrop et al., 2005)

The first successful transfusion of blood from one dog to another was reported in 1665 by Richard Lower. However, blood transfusion became more popular in veterinary medicine in the last few decades. (Cotter, 1991; Davidow, 2013). The 20th century is known for discovering anticoagulants and preservatives for blood products, the description of human blood groups, and the development of compatibility assays (Bird, 1971). In humans, donor day is celebrated worldwide every year on 14th June by World Health Organization (WHO). Blood transfusion and blood product transfusions are routine practice in human medicine and blood banks are compulsory at each human hospital.

Anaemia is defined as an absolute decrease in thepacked cell volume (PCV), heamoglobin (Hb) concentration and RBC count. The clinical signs of anaemic patients are pale mucus membranes, exercise intolerance, weak pulse, tachypnea, tachycardiaand hypotension. These signs of anaemia are associated with decreased oxygen delivery and hypotensionbecause Hb plays an essential role in delivering oxygen to cells (Davidow, 2013).

Anaemia accompanied by reticulocytosis is termed as regenerative. It is typical of blood loss (haemorrhage) or erythrocyte destruction (haemolysis). In contrast, anaemia without reticulocytosis is termed as non-regenerative and occurring due to reduced erythropoiesis in bone marrow (Tvedten, 2010). The severity of anaemia is variable can be life-threatening and occurs commonly in diseases such as haemorrhagic gastroenteritis, trauma, parasitic gastroenteritis, immune-mediated haemolytic anaemia, haemoprotozoan diseases, septicaemia, chronic kidney disease and liver diseases.

Transfusion is indicated in anaemic patients when PCV is 15 percent or less and haemoglobin is less than 5 gm/dL of blood (Choudhary et al., 2017). Low haematocrit and haemoglobin concentration are important transfusion indicators in haemolytic and subacute to chronic blood-loss disorders. Red cells can be transfused in the form of packed red cells (pRBCs) or whole blood transfusion. Whole blood transfusion is indicated in several clinical conditions including anaemia of various causes like acute/chronic haemorrhage, coagulopathies, poisonings, hypoproteinemia and burn patients (Bhikane & Kawitker, 2002). Whole blood transfusions are not recommended to correct haemorrhagic syndromes and usually crystalloid or colloid solutions in addition to pRBCs are sufficient (Freireich, 2011).

The suitability of the donor animal and method of collection and storage of blood and its components should be supervised to minimize the life-threatening complications of transfusions. The ideal canine donor should be thin, 2-8 years of age, weigh at least 25 kg, be in good body condition, have a PCV of at least 40 percent and adequately immunized. The donor dog should not have received prior blood transfusions. Donor females should be neutered as estrogen influences platelet number and function (Pichler & Turnwald, 1985; Greene, 1982; Auer & Bell, 1983). Potential donors should be blood typed and vaccinated for distemper, hepatitis, leptospirosis, parainfluenza, and parvovirus. A complete blood count, serum chemistry profile, urinalysis and faecal examination, need to be performed. Different diagnostic tests should be performed to screen donor dogs for endemic pathogens prevalent in the geographical region such as blood smear, PCR for haemoprotozoa, serological tests for *Brucella canis* and serum titers for infectious agents such as *Ehrlichia canis, Babesia spp.*etc. (Willer & Riedesel, 1985).

The best alternative to blood typing is crossmatching the donor and recipient blood. The use of crossmatched blood does not prevent sensitization of the recipient for future transfusions if the donor cells contained non-identical alloantigens (Turnwald & Pichler, 1985). Two types of crossmatching are performed, major and minor. The major crossmatch uses donor RBCs and recipient serum to determine whether the recipient has antibodies against the donor cells while the minor crossmatch uses recipient RBCs and donor serum to detect whether there are antibodies in donor serum against the recipient cells.

Component therapy is based on the separation of blood into multiple portions that can benefit more than one patient. The separation extends the use of a single unit of donor blood to transfuse need-based component in the recipient dog such as anaemia, hypoproteinemia and thrombocytopenia. In addition, plasma can be stored for a longer duration and can be used in emergency situations. The treatment of neoplastic diseases usually require blood components as cancer patients may suffer from either anaemia, thrombocytopenia, leukopenia, hypoalbuminemia or coagulation abnormalities due to paraneoplastic syndrome (Rudloff, 1995).

Hypoproteinemia develops in numerous disease conditions including vasculitis, protein-losing disorders, hepatic failure and peritonitis. The plasma components such as fresh frozen plasma and cryosupernatant contain albumin and are potential source of replacement therapy. When albumin levels drop below 2.0 g/ dL, plasma transfusion is recommended. (Kirby, 1992; Oster et al., 1990) Multiple plasma transfusions may be required in the larger patient.

Plasma therapy is the mainstay for the treatment of haemorrhagic disorders due haemostatic factor deficiencies, hypovolemia or hypoproteinemia. Plasma components can be transfused as a source of albumin and immunoglobulins. Fresh frozen plasma is frequently used as adjunct therapy for disseminated intravascular coagulation (DIC), thrombocytopenia and von Willebrand's disease (Stone et al., 1992).

Thrombocytopenia is the most commonly acquired haemostatic disorder in dogs and can become potentially life-threatening (Grindem et al., 1991; Bommer et al., 2008). Severe thrombocytopenia in dogs is mainly caused by immune-mediated thrombocytopenia; however, low platelet counts are also commonly associated with

inflammatory, infectious, or neoplastic diseases (Bommer et al., 2008; Botsch et al., 2009). The mechanism of thrombocytopenia depends upon the stage of the disease whether the platelets are consumed/ sequestered during the acute phase or decrease in production in chronic stage.

Platelet transfusion is highly useful in managing bleeding in patients with severe thrombocytopenia due to decreased platelet production. The main limitations of platelet-rich plasma therapy include the low therapeutic value of platelet-rich plasma (PRP) from a single donor, the short platelet lifespan requiring the earliest use of platelet-rich plasma and the risk of alloimmunization upon the first transfusion.

Until now, transfusion of whole blood is practised in clinical setup only in individual anaemic dogs. However, there is no systematic study to assess the effect of transfusion of whole blood in different canine ailments. Moreover, facilities are not available in most of the canine hospitals/institutes of India for separation of blood into its components and its storage. Therefore, the present study was planned with the following objectives

1. To compare whole blood transfusion with packed red blood cell transfusion (pRBC) in anaemic patients.
2. To assess the effect of platelet infusion in thrombocytopenic patients.
3. To evaluate the role of plasma therapy in hypoproteinemic patients.

CHAPTER II

REVIEW OF LITERATURE

2.1 Anaemia

2.1.1 Case prevalence

Oduye & Otesile (1977) conducted study on the 500 dogs of which 136 (27.2%) were found to be anaemic. Fifty-two of these affected dogs were associated with blood parasites, twenty one with hookworm infection, while ten had a mixture of blood parasites and hookworm infection. Seventeen cases were associated with various conditions such as traumatic wounds, transmissible venereal tumour, nephritis, taeniasis, anal gland adenoma, idiopathic epistaxis, lice and fleas infestation. The cause of anaemia remained undiagnosed in thirty-six cases.

Useh et al. (2003) after a study on 5278 mongrel dogs reported the prevalence of anaemia to be 40-50 percent with PCV values ranging from 7 to 36 percent. Pale mucous membranes, weakness, depression, anorexia/inappetence and reduced activity were the main clinical signs. Most of the dogs with anaemia (94.2%) had parasitic infestations. About 1580 (74%) had anaemia due to parasitic infestations and few dogs (2.6%) had anaemia due to poor nutrition, while (3.2%) had anaemia with unknown etiology.

Miller et al. (2009) conducted a study on anaemic dogs associated with lymphoma and concluded that thirty-two percent (27/84) of dogs with lymphoma had anaemia. Ninety-six percent had normocytic, normochromic anaemia, and only 4 per cent had microcytic, normochromic anaemia. The median PCV for the anaemic dogs was 35%, while the median PCV for the non-anaemic dogs was 46 percent.

Singh et al. (2012) reported anaemia in 65 dogs out of 137 (47.44%), and found prevalence of anaemia to be highest in dogs below 6 months of age (44.61%) followed by 6 months to 1 year group (15.38%), 1 to 3 year group (20.00 %) and 6 to 10 year age group (12.32 %). The lowest prevalence was found in dogs in the age group 3 to 6 years (7.69%).

Liu and Su (2015) conducted a study on 3174 anaemic dogs; the prevalence was higher in males (52.9%) as compared to females (47.1%). The commonly presented breeds were mongrel (40.0%), followed by Maltese (13.1%), Golden

Retriever (8.2%), Miniature Schnauzer (6.7%), Miniature Poodle (4.4%) and Labrador retriever (4.2%). On the basis of severity, it was categorised as mild (72.1%); moderate (20.8%); and severe (7.1%). The causes of anaemia could not be identified in 35.8 percent of cases, 45.2 percent of dogs were classified as anaemic with a single cause, and 19.0 percent of anaemic cases were associated with multiple causes.

Bhat (2016) conducted a retrospective study on the prevalence of anaemia on 1749 dogs for the period from September 2014 to August 2015 and reported that only 784 dogs were anaemic (Hb<12 g/dL). The prevalence of anaemia was 44.83 percent with a mean haemoglobin concentration of 8.36±0.09 g/dL. The highest prevalence of anaemia was recorded for Labrador (39.80%) and lowest for Bully (0.89%) and Mastiff (0.89%). The highest prevalence of anaemia in dogs was found in the month of May (14.41%) followed by July (10.97%) and August (10.08%).

Tandel (2016) conducted a prospective study on 51 anaemic dogs and a retrospective study on 352 anaemic dogs. Retrospective analysis showed that the overall prevalence of anaemia was 6.48 percent and highest prevalence was reported in the adult group (54.55%) and similarly prospective study provided the highest prevalence rate of anaemia in adult dogs (58.83%) and liver disorders (23.53%) were main contributors to the disorder.

Yu & Huang (2016) recorded that the prevalence of anaemia was highest in dogs with the modified New York Heart Association (NYHA) class IV heart failure (33.3%), followed by classes III (15.2%) and II (0%; $p < 0.001$). The presence of anaemia was associated significantly with the severity of heart failure and blood creatinine > 1.6 mg/dL.

Pallavi (2019) reported the case prevalence of severe anaemia as 2.09 percent on the basis of 17648 cases presented and 1.17 percent on the basis of 9865 CBC reports. The highest prevalence was recorded for Pitbull breed (5.26%) and lowest for Rottweiler (0.55%). Similarly, higher prevalence was found in males (1.35%) than females (1.10%). Major causes of severe anaemia were chronic kidney disease, liver disease and haemoprotozoa infections.

Meshram et al. (2019) conducted a haematological study on 108 canine blood samples of different age groups, breed and sex with a history of anorexia, loss of

appetite, diarrhoea, vomition and reported 65 (60.18%) dogs to be anaemic. The highest incidence of anaemia was observed in Mastiff and Chihuahua breed (100%) followed by Cocker spaniel (83.33%), German shepherd and Pomeranian (80.00%), Labrador (68.00 %), Bull dog and Dachshund (66.67%), Golden retriever (60.00%), Non-descript (50.00 %), Boxer and Pug (33.33%) while it was absent in Saint Bernard, Lhasa Apso and Shih Tzu breed. The observed anaemia cases were categorised as Normocytic Hypochromic (26.15%), Microcytic Hypochromic (59.09%), Microcytic Normochromic (1.515%) and Macrocytic Normochromic (12.12%).

2.1.2 Causes

King et al. (1992) conducted a study on 17 dogs to evaluate the incidence, type, and etiology associated with chronic renal failure. Nonregenerative, normochromic, normocytic anaemia was seen in 12 of 17 dogs (70.6%). There was a direct correlation between the degree of anaemia and the extent of CRF as assessed by serum creatinine concentrations. Low erythrocyte concentration depicted hypoxia and deficiency of erythropoietin in dogs in CRF (Reddan et al., 2003).

Burgess et al. (2000) reviewed 60 cases of immune-mediated haemolytic anaemia (IMHA) in the dogs. The two most commonly affected breeds were Cocker Spaniels and Labrador Retrievers. Fifty-two of the 60 dogs tested (87%) were auto agglutination positive and spherocytes were present in 45 (75%).

Nassiri et al. (2005) studied the forty cases of anaemia in dogs, out of which direct Coombs' test results were positive in 14 cases. The mean age of the dogs with was 3.7 years. The prevalence of IMHA in females was higher as compared to males and the main causes were infectious diseases and drug therapy.

Assarasakorn & Niwetpathomwat (2006) conducted a study on etiology of anaemia, the effectiveness of blood transfusion, and the incidence of transfusion reaction of 41 dogs. The mean of packed cell volume before and after treatment of all dogs was 11.3 percent and 26.5 percent, respectively. The anaemic causes of 41 dogs were categorized into three groups including blood loss (43.90%), ineffective erythropoiesis (34.15%), and haemolysis (21.95%). Acute non haemolytic transfusion reactions were observed only in 4.18 percent of dogs after transfusion, and they rapidly recovered after treatment.

Lund (2007) conducted a study on 30,946 anaemic dogs and observed various causes of anaemia as flea infestation (10.4%), hookworm disease (3.7%), heart murmur (2.2%), renal failure chronic (1.4%), haemolytic autoimmune anaemia (0.6%), nutritional anaemia (0.5%), blood loss anaemia (0.4%), anaemia due to trauma (0.3%), chronic infection (0.3%), acute renal failure (0.05%), aplastic anaemia (0.04%), babesiosis (0.02%), Heinz body anaemia (0.02%), ehrlichiosis pancytopenia (0.01%), macrocytic anaemia (0.01%), and anaemia due to endogenous estrogen (0.01%).

Fry (2010) stated that anaemia of inflammatory diseases is one of the most common causes of anaemia in dogs and cats and characterized by mild to moderate severity, non-regenerative type and it is associated with various chronic disorders such as neoplasm, inflammatory disease, infectious or immune-mediated disorders.

Polzin (2011) classified CKD into stages 3 and 4, resulting primarily from the impaired ability of kidneys to produce a sufficient quantity of erythropoietin. Chronic, low-grade gastrointestinal haemorrhage often contributes to anaemia in CKD.

Chervier et al. (2012) described the causes of anaemia (PCV<37%) in 456 dogs, excluding acute haemorrhage. Cancer-related anaemia and anaemia of inflammatory disease accounted for 33.1 and 28·5 percent cases, respectively. Most dogs with cancer-related anaemia had solid tumours (73%). AID was the second most common cause (28·5%) followed by cancer-related anaemia (33·1%). AID was diagnosed in dogs with anaemia displaying inflammation but with no signs of infectious, neoplastic or immune-mediated disease.

Dorgalaleh et al. (2013) observed the presence of anaemia and thrombocytopenia in patients with acute and chronic renal failure. Haematological changes like decreased mean corpuscular volume, RBC and impaired secretion of erythropoietin by kidneys were observed.

Ognean et al. (2015) studied that the major causes for the anaemia were coagulopathy (37.03%), haemorrhagic gastro-enteritis (29.62%), lymphoma or leukaemia (11.11%) and polytrauma (14.81%).

Bhat (2016) conducted a study on the etiology of anaemia in 1749 dogs and major causes included *Ehrlichia canis* (5.5%), *Babesia gibsoni* (3.49%), IMHA

(1.49%), Neoplasia (0.91%), *Babesia canis* (0.51), Onion/Ginger toxicity (0.29%) and non-haemolytic anaemia (32.5%).

Moraes et al. (2017) reported that IMHA is a common cause of anaemia in dogs causing phagocytosis of erythrocytes opsonized by IgG, IgM and/or complement. Tissue hypoxia and decreased haematocrit value lead to progressive kidney damage causing renal tubular necrosis.

Torres et al. (2017) compared haematologic, serum and urinary biochemistry and serum erythropoietin with the findings of bone marrow cytology obtained by aspiration of the manubrium in patients with chronic kidney diseases. Cytological findings for erythroid hypoplasia were described in 93.6 percent of dogs, and the anaemia was observed in 84.1 percent. The haematological findings were correlated with azotaemia. It was concluded that the erythroid hypoplasia has a correlation with persistent anaemia in dogs at all stages of chronic kidney disease with iron deficiency in the early stages and with peripheral destruction of erythrocytes caused by azotaemia.

Yogeshpriya et al. (2018) studied 66 cases of renal failure dogs and concluded that major haematological changes observed were increased ESR, total leukocyte count, neutrophils, blood urea nitrogen concentration and hypoalbuminemia.

Stanley et al. (2019) compared the prevalence of anaemia between hypocobalaminemic (36%) and normocobalaminemic dogs (26%) or between hypofolatemic (31%) and normofolatemic dogs (30%) but no significant differences were detected. Besides, no significant differences in prevalence of nonregenerative anaemia (69% vs 63%), macrocytosis (17% vs 0%), or anisocytosis (28% vs 0%) were detected between hypocobalaminemic and normocobalaminemic dogs. Anaemic dogs had high prevalence of vitamin B deficiencies with nonregenerative anaemia (64% hypocobalaminemic, 18% hypofolatemic) or regenerative anaemia (57% hypocobalaminemic, 21% hypofolatemic).

Parachini-Winter et al. (2019) did a retrospective cross-sectional study in 82 dogs suffering from anaemia due to lymphoma or IBD to evaluate potential relationships between the prevalence of anaemia and various RBC anomalies. The prevalence of anaemia was significantly higher in cases of lymphoma (53%) than in IBD cases (22%). The morphological RBCs anomalies were significantly higher in

dogs with lymphoma in comparison to healthy or IBD dogs. The presence of eccentrocytes was the only individual RBC anomaly significantly more common in dogs with lymphoma (29%) versus dogs with IBD (4%).

2.1.3 Clinical Findings

Dunn (1991) concluded that weakness, exercise intolerance, pallor, dyspnea, or shock were suggestive of an acute or severe anaemia.

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.

Furlanello et al. (2005) studied the clinical signs in *Babesia* and reported that dehydration (100%), apathy (74%), anorexia or decrease appetite (70%) and fever (68%) were 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.

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.

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.

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.

2.1.4 Haematological changes and biochemical changes

Cotter (1992) studied that the common findings of IMHA were polychromasia and anisocytosis in dogs that had regenerative anaemia. The positive sign of regeneration was the presence of nucleated RBC's; however, in the absence of reticulocytes, nucleated erythrocytes indicated underlying primary bone marrow disease. The significant erythrocyte morphology change noted in dogs with IMHA is spherocytosis.

Furlanello et al. (2005) conducted a study on *Babesia* in 23 dogs. Seventy percent of dogs had haemolytic anaemia and 30 percent had non-haemolytic anaemia. Sixty-nine percent of dogs showed leukopenia and 74 percent had neutropenia. Leukocytosis due to mature neutrophilia and lymphocytosis was present in one dog. Activated lymphocytes were noted in 61% percent of dogs.

Nassiri et al. (2005) studied the haematological and biochemical findings of IMHA and reported the mean PCV as 21.4± 1.4%; anisocytosis in eight dogs (61%); spherocytosis in seven dogs (54%); polychromasia in five dogs (38.5%); thrombocytopenia in seven dogs (54%); hyperbilirubinemia in eight dogs (80%) out of the ten evaluated dogs; increased activity of ALT and ALP in six (46.2%) and eight (61.5%) out of thirteen evaluated dogs, respectively.

Zygner et al. (2007) conducted a study on haematological changes in samples of blood obtained from 248 dogs naturally infected with large *Babesia*. The evaluation included red blood cell count, haemoglobin concentration, haematocrit, MCV, MCHC, leukocyte counts, thrombocyte counts, mean platelet volume (MPV), morphology of erythrocytes and leukogram. The most common disorders in affected dogs were thrombocytopenia and anisocytosis. The count of erythrocytes below reference values was detected in 26.2 percent of dogs and 31.4 percent affected animals. Haemoglobin concentration below the reference values was noted in 29 per cent of dogs, an increase of MCHC above normal values was detected in 21 percent and MCV below normal values was recognized in 2 percent of dogs.

Hodges & Christopher (2011) studied 4,387 anaemic dogs, out of which 1,426 (32.5%) had regenerative anaemia of which 168 (11.8%) had macrocytic

hypochromic anaemia. They further concluded that most regenerative anaemias in dogs were not macrocytic hypochromic.

Reddy et al. (2016) reported that dogs infected with *Babesia spp*. had reduced haemoglobin concentration, erythrocyte count, platelet count, total serum protein, serum albumin and glucose levels.

Vijayalakshmi et al. (2013) conducted a study on 12 dogs that were diagnosed with mixed infection of babesiosis and ehrlichiosis. Laboratory analysis showed marked anaemia, decreased platelets, lymphopenia, increased number of monocytes and anisocytosis.

Saraniya et al. (2015) conducted a study on 43 dogs suffering from anaemia associated with chronic renal failure. Non-regenerative anaemia was observed in 28 (65.12%) dogs and regenerative anaemia was seen in 15 (34.88%) dogs. The majority of the dogs showed normocytic normochromic anaemia as the major cellular changes, however, anisocytosis was seen in 5 (11.63%) dogs with the majority showing microcytosis**.** Echinocytes were seen in 6 (13.95%) dogs, acanthocytes in 4 (9.30%) dogs, schistocytes in 4 (9.30%) and spherocytes in 11 dogs (25.58%).

Swann & Skelly (2013) reported that the presence of spherocytes was acceptable for the diagnosis of IMHA. Spherocytes count varied with the severity of anaemia so the value of five spherocytes per high power field for the diagnosis of IMHA documented in previous publications does not hold well with every case.

Chikazawa & Dunning (2016) concluded that anaemia of inflammation is often mild to moderate, normocytic, normochromic and non-regenerative with shortened red cell span, inhibition of iron metabolism and impaired bone marrow response to erythropoietin.

2.2 Transfusion of whole blood and packed RBCs

2.2.1 Indications

Stone et al. (1992) examined the records of 405 blood transfusions to determine trends in the use of fresh whole blood and blood components. The donor dogs weighed at least 30 kg and had a packed cell volume greater than 40 percent. The commonest indications for transfusion were acute blood loss, anaemia and bleeding due to coagulopathies.

Kerl & Hohenhaus (1993) reviewed 163 pRBC transfusions and blood loss (70%), haemolysis (22%), and bone marrow hypoplasia (8%) were main contributing factors. Forty-seven percent (62 dogs) survived hospitalization. Thirteen percent (17 dogs) had acute or delayed transfusion reactions, but all of these dogs survived hospitalization. Sex and breed had no effect on anaemia. Criteria used to determine transfusion need included anaemia; history of acute blood loss; the need for anaesthesia; and evidence of weakness, tachypnea, or tachycardia. Twenty-four percent scored < 5 on the transfusion-need assessment scale.

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

Callan et al. (1996) reviewed 307 red blood cell (RBC) transfusions in dogs. A total of 658 units of RBCs including 474 (72%) units of packed red blood cells (pRBCs) and 184 (28%) units of whole blood were administered. The reasons for transfusion included haemorrhage (n=222), haemolysis (n=43), and ineffective erythropoiesis (n=42). The mean pre-transfusion packed cell volume (PCV) of dogs with haemolysis (13%) was significantly lower than the mean pre-transfusion PCVs of dogs with haemorrhage (21%) or ineffective erythropoiesis (18%).

Knottenbelt & Mackin (1998) concluded that transfusion of packed red cells is the treatment of choice for severe anaemia where the patient was normovolaemic at the time of transfusion and was particularly useful in patients with a cardiac or renal disease which would be at risk of circulatory overload from the administration of whole blood.

Hammond & Pesillo-Crosby (2008) transfused pRBCs in anaemic dogs (mean of 14.16 mL of pRBCs/ with a median of 11.44 mL/kg and a range of 5.83 to 47.80 mL/kg). Dogs with haemangiosarcoma received an average of 15.19 mL pRBCs/kg, with a median of 11.51 mL/kg and a range of 6.36 to 47.80 mL/kg. In contrast, dogs with lesions other than haemangiosarcoma received a mean of 12.59 mL pRBCs/kg with a median of 10.83 mL/kg and a range of 5.83 to 27.46 mL/kg. The volume of transfused blood did not differ significantly between the groups.

Godinho-cunha et al. (2011) concluded that the most common indications for blood transfusion included acute blood loss (47%), coagulopathy (33%) and other anaemias (20%). The mean pre-transfusion PCV with acute blood loss (18%) was

higher than in the group of coagulopathy (15%) or other anaemias (15%). The survival rates at 6 days after transfusion were greater in the coagulopathy (80.0%) and other anaemias (66.7%) than in the group of acute blood loss (42.9%).

Silvestrini et al. (2011) administered pRBCs in 56 dogs and reasons for transfusion included anaemia secondary to haemorrhage, haemolysis, and ineffective erythropoiesis.

Day (2008) concluded that common indication for blood component transfusion in dogs and cats was hypoxia resulting from a reduction in haemoglobin and subsequent tissue and or organ ischemia.

Haley et al. (2015) conducted a retrospective case study on 207 dog patients that underwent blood and component transfusion before undergoing various surgeries. Transfusion requirement (pRBC, whole blood, and bovine haemoglobin based oxygen carrier) and survival rate after 2 weeks of surgery were compared among dogs undergoing the various surgeries and it was observed that patients who underwent splenectomy and liver lobectomy were significantly more likely to receive RBC transfusion as compared to the patients for other surgeries. A significant association was found between body weight and perioperative RBC transfusion with greater odds of transfusion as body weight increased. Dogs that have received perioperative RBC transfusions were significantly less likely to survive to 2 weeks after surgery.

Lynch et al. (2015) conducted a retrospective analysis on 125 dogs admitted to the hospital with traumatic injury and recorded that most frequent signs for transfusion were perioperative haemodynamic support, shock or severe anaemia. Forty-five (36%) received transfusions out of 125 dogs, pRBC's were the most commonly administered. In dogs and cats that underwent liver lobectomy 11 out of 63 (17%) dogs and 4 of 9 cats required a blood transfusion.

Langston et al. (2017) in a retrospective study on renal patients (83 cats and 147 dogs) evaluated the use of blood products that were treated with intermittent haemodialysis to determine risk factors associated with the requirement for blood product transfusion. Blood products (whole blood, packed RBCs or stromal-free haemoglobin) were transfused in 87 percent cats and 32 percent dogs. Administration of a blood product was associated with a higher likelihood of death in dogs, but not in cats.

2.2.2 Donor selection and screening

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 (Wardrop 1997).

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 preferred than 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 smears examination results were negative. A total of 40 donor candidates (26.7%) and 108 stray dogs (32%) were seropositive to at least one vector-borne pathogen.

Yagi & Bean (2016) stated that donors should have sufficient blood volume to donate 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

2.2.3 Crossmatching

Feldman & Kristensen (1995) gave the two main reasons for the cross match that 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 the risk 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 the 3 potential donors.

Walton (2019) recommended cross match and type blood of the donor and recipient pair before blood transfusion but it is compulsory to cross match during 2nd and subsequent transfusion if it is more than 4-7 days post-transfusion.

2.2.4 Blood collection, separation 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 (Lanevschi and Wardrop 2001).

Kerl & Hohenhaus (1993) 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 triplecollection 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

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 (Hogman et al., 2002), (Wardrop et al., 1994).

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 to 3 to 5 week, depending on the preservative (Wardrop, 1995)

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 (Wardrop et al., 1994).

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.

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

Lanevschi & Wardrop (2001) stated that 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.

2.2.5 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) 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 acute kidney 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.

2.2.6 Adverse reaction of blood transfusion

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 (Turnwald and Pichler, 1985).

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 by emesis, 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 2007 consensus 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 significantly less 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.

2.2.7 Life span of transfused RBCs

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

Giger (2010) 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.

The average life span of canine RBCs is almost 120 days but may be reduced due to the collection and storage process. Response to transfusion is also associated with the host response and ongoing disease process (Walton, 2019).

2.2.8 Post transfusion follow up and survival of patients

Saini (2001) conducted a study on blood transfusion in 6 anaemic dogs and transfused whole blood into 6 animals @ 15mL/kg Bwt. Blood transfusion resulted in improvement of erythrocytic indices (MCV, MCH, MCHC) along with the increase in haemoglobin, (PCV) packed cell volume and TEC (Total erythrocytic count). and concluded that mean haemoglobin and PCV before transfusion were 4.74± 0.70 g/dL and 14.25 ± 2.51% percent respectively, which were increased to ($p<0.01$) 5.83 ± 0.65 g/dL and 56 percent increase respectively after 24 hours which further hiked up to 6.79 ± 0.44 g/dL and 82 percent increase respectively after 48 hours of transfusion.

Weingart et al. (2004) conducted fresh blood transfusion in 91 cats. Transfusions were required because of blood loss anaemia (n=40), haemolytic anaemia (n=13), ineffective erythropoiesis (n=35), hypoproteinaemia (n=2) or coagulopathy (n=2). The anaemic cats had a pre-transfusion, haematocrit of 5–20 per

cent (*m* [median] =13), and received one to six transfusions (*m*=1). The survival rates of the anaemic cats at 1 and 10 days after transfusion were 84 and 64 percent, respectively. No death was ascribed to transfusion reactions.

Rutan (2007) stated that post-transfusion evaluation of PCV, total protein should be done at 1, 24 and 72 hours after transfusion. Haemoglobin increases after transfusion however, the intravascular volume will take 12 to 24 hour to stabilize after transfusion due to fluid shift to and from the extra vascular space. If the PCV of patient is less than expected post transfusion, evidence of continued haemorrhage or haemolysis should be checked.

Godinho-Cunha et al. (2011) studied a total of 19 transfusions in a group of 15 animals (9 dogs, 6 cats) in which three animals received more than one transfusion. The mean pre-transfusion packed cell volume (PCV) of animals with acute blood loss (18%) was higher than in a group of coagulopathy (15%) or other anaemias (15%). Overall survival was dependent upon post-transfusion rectal temperature, observed PCV change, the difference between the obtained and the calculated PCV, and administered transfusion volume ($p< 0.05$).

Ognean et al. (2015) compared the statistical analysis in transfused patients the post-transfusion of the red blood cells count and haemoglobin concentration showed significant post-transfusion increase and an extremely significant increase of the haematocrit value. The transfusion was well tolerated, except for four patients who presented immediate transfusion reactions with mild consequences (tachycardia, myoclonus and hyperthermia). After the blood transfusion therapy, 18 (66.6%) patients clinically recovered and 9 (33.3%) died from severe clinical complications without presenting any adverse transfusion reactions.

Kumar & Kumar (2016) conducted a study to compare the efficacy of fresh and preserved blood transfusion in 25 anaemic pups aged between 3-5 months and divided them into five groups. Group T1 pups were transfused with fresh whole blood and Group T2, T3, and T4 received stored blood of 7, 14 and 21 days, respectively @ 20 mL/kg body weight as a single therapy. The clinico-biochemical parameters before blood transfusion showed a significant increase in pulse and respiration rates and a significant decrease in total protein, albumin and blood glucose concentration. After 24, 72 and 120 hrs of post-transfusion, a significant improvement

in the clinic-biochemical parameters was recorded, whereas no significant change was noticed in non transfusion group. They concluded that blood transfusion, wheter the blood is fresh or stored, it can improve clinical as well as haematobiochemical status so, served as an important life saving measure in severely anaemic dogs.

Sultana (2018) conducted a study of whole blood transfusion on ten stray dogs (5 recipients and 5 donors) in Teaching Veterinary Hospital, Chittagong Veterinary and Animal Sciences University, Chittagong, Bangladesh. The blood parameters were increased slightly in one of the recipients. The highest PCV (2.2%), Hb (0.44%) and neutrophil (1.1%) were observed on 3rd day after transfusion. The maximum heart rate was 90.2 ±1.7/min recorded after 10 minutes of transfusion. The mean control value of rectal temperature was 100.88 ± 0.23/min ºF and the maximum value was 102± 0.31/min º F after 20 minutes of blood transfusion. It was concluded that blood transfusion is an important tool in saving critical native dogs.

Medina Valentin et al. (2018) conducted a retrospective study on 86 anaemic dogs who received about 10-20 mL/kg of packed RBC or fresh whole blood. Pre-transfusion PCV of the recipient influenced the degree of the increase of post-transfusion PCV only in chronic anaemia. The type of blood product did not affect post-transfusion PCV. There were no differences between the increases of PCV in recipient affected by acute or chronic anaemia receiving blood products with different PCV values. The increase in PCV post-transfusion was greater when less than 10-days' old stored blood product was used. In chronic anaemia, lower pre-transfusion PCV was observed which was related to a greater increase of post-transfusion PCV. In both chronic and acute anaemias, both pRBC and stored whole blood were important for improving the post-transfusion PCV.

2.3 Thrombocytopenia

2.3.1 Case prevalence and causes

Thrombocytopenia is caused by canine ehrlichiosis that leads to decrease in platelet count, consumption and sequestration of platelets leading to prolonged bleeding time and dysfunction of platelets. Administration of tetracyclines as well as supportive therapy including intravenous poly ionic fluids and transfusion of blood products has positive results (Woody and Hoskins 1991; Van Heerden 1992).

Grindem et al. (1991) studied and classified thrombocytopenia in 987 of 18,910 (5.2%) dogs based on etiology which revealed 5 percent in immune-mediated thrombocytopenia 13 percent as neoplasia-associated thrombocytopenia 23 percent in inflammatory/infectious thrombocytopenia; and (59%) as miscellaneous thrombocytopenia. Dogs with immune-mediated thrombocytopenia had significantly lower platelet counts (mean 36,760 +/- 50,288 microliter) than dogs in the other three groups.

Matthewman et al. (1993) examined 105 dogs and found that 52 percent had antibodies reactive with *E.canis* in indirect fluorescent antibody studies, 26 percent had *B. canis* parasite in peripheral blood smears, and 17 percent had both infections. Anaemia and thrombocytopenia were typical laboratory findings, with a substantially higher prevalence of non-regenerative anaemia in dogs with antibodies to *E.canis* compared to dogs with both infections. Thrombocytopenia was found to be more common in dogs with babesiosis than in dogs with antibodies to *E.canis*.

Harrus et al. (1997) described the principal findings of erlichiosis dogs included lymphadenomegaly, pale mucous membranes, fever and the presence of ticks on physical examination while as the main abnormal haematological and biochemical findings included thrombocytopenia, the presence of giant platelets, low haematocrit, monocytosis and low albumin concentrations.

Bulla et al. (2004) used platelet counts as a screening test for *E. canis* in an endemic area, 217 whole blood samples from dogs were divided into three groups on the basis of platelet counts; 71 dogs were non-thrombocytopenic. Sixty-seven of the 217 samples (30.9%) were positive for the presence of the *E. canis* 16S rRNA gene. They concluded that platelet counts might be a good screening test for canine monocytic ehrlichiosis, and the degree of thrombocytopenia may increase extent of diagnosis.

Furlanello et al. (2005) conducted a study on *Babesia* in dogs, significant prolonged APTT) was only recorded in one case. In four dogs, both plasma fibrinogen/fibrin degradation products (FDPs) and D-Dimer were increased. Antithrombin (AT) was slightly decreased in 11 of the 23 dogs. Mild elevation of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine kinase (CK), total bilirubin and lactic acid and

decrease of total iron and total iron binding capacity (TIBC) were recorded in the majority of cases.

Macieira et al. (2005) studied to determine the prevalence of *E. canis* in thrombocytopenic dogs in thirty-six (32.1%) of the thrombocytopenic dogs and 4 (3.5%) of the non-thrombocytopenic both the groups were positive for rickettsial gene sequences (P <.0001). The author concluded that the prevalence of *E. canis* infection was higher in thrombocytopenic dogs, less than one-third of these dogs had demonstrable *E .canis* infection and thrombocytopenia is not specific for the detection of *E. canis* even in endemic area.

Neumann (2006) compared the platelet counts of 52 dogs with different liver diseases and compared them with 52 healthy dogs to investigate the influence of different liver diseases including degeneration, hepatitis and liver tumours, on the platelet counts. Dogs with liver degeneration have thrombocytosis in 41 percent of the cases and a group of dogs with liver tumours (malignant histiocytosis, hepatoma, malignant lymphoma anaplastic sarcoma, cholangiocarcinoma, hepatocellular carcinoma) had thrombocytopenia in 50 percent of the cases. The patients with liver disease and a control group of healthy dogs showed significantly higher platelet counts in cases of liver degeneration and significantly lower platelet counts in cases of liver tumour. The comparison between the dogs with different liver diseases showed significantly lower platelet counts in dogs with liver tumours when compared to dogs with liver degeneration.

Zygner et al. (2007) studied canine babesiosis in 248 dogs. Thrombocytopenia was detected in (99.5%) of infected animals, but only (15.3%) showed increased Mean Platelet Volume (MPV). The most common disorder in affected dogs were thrombocytopenia and anisocytosis.

Tick infestation and cancer were the main findings reported in dogs with thrombocytopenia in a clinical data. Low platelet counts are believed to be commonly associated with inflammatory, infectious or neoplastic diseases (Bommer et al., 2008; Botsch et al., 2009).

Smith et al. (2014) observed that (PDW) increased in dogs with haemangiosarcoma compared to control groups Carcinoma, lymphoma and

haemangiosarcoma were the most commonly reported cancer in thrombocytopenic dogs.

Schwartz et al. (2014) suggested that presumptive primary immune-mediated thrombocytopenia in dogs is characterized by increased MPV levels due to large immature platelets` production during a regenerative process.

Sharma et al. (2015 a) conducted a study on cases of thrombocytopenic and anaemia cases of ehrlichiosis in canines. Animals were treated with doxycycline @10 mg/kg body weight twice a day and whole blood transfusion was done as supportive treatment for low RBC and platelet counts. The complete recovery and improvement in haemorrhagic observed was observed after 20 days.

Souza et al. (2016) recorded the clinical data of thrombocytopenic animals (n = 45) which revealed that (31.11%) of the dogs were cancer patients, (24.44%) had a tick infestation and suspected haemoparasitosis, (11.11%) only showed prostration and loss of appetite with no conclusive diagnosis, (6.67 %) had a respiratory disease, (4.44 %) had renal failure and (22.22%) consisted of dogs with other clinical findings. Microfilariae were found in blood smears in two thrombocytopenic dogs.

2.3.2 Haematological characteristics

A decreased MPV may be associated with early stages of immune-mediated thrombocytopenia and it is believed that this reduction could be caused by the presence of small platelets (Northern and Tvedten 1992, Topper and Welles 2003).

Thrombocytopenic animals on morphological analysis revealed macroplatelets and platelet anisocytosis in morphological studies. Large platelets can be observed in peripheral blood when bone marrow is intact and overstimulated to produce platelets (Kaito et al., 2005, Bommer et al., 2008).

Severe thrombocytopenia in dogs is mostly caused by immune-mediated thrombocytopenia; however, low platelet counts are also commonly associated with inflammatory, infectious or neoplastic diseases (Bommer et al., 2008, Botsch et al., 2009).

Dircks et al. (2009) conducted a study on 83 thrombocytopenic dogs and PBAs were detected in 37 of 83 (45%) dogs .Spontaneous bleeding was observed in 18 dogs (86%) mainly in the form of surface bleeding. Nineteen dogs (91%) with

pIMT had platelet counts below 20,000/µl. In 26 of 83 (31%) dogs, an associated infectious disease was detected; PBAs were detected in 13 of these 26 dogs. A positive PBA test result was an inconsistent finding in dogs with anaplasmosis, ehrlichiosis, leptospirosis, and sepsis, whereas all 5 dogs with babesiosis had positive results for PBAs.

Souza et al. (2016) divided the thrombocytopenic dogs into two groups with less than 150,000 platelets/ µL of blood (19/42.2%) and dogs with over 150,000 platelets /µL of blood (26/ 57,8%). Mean values of Platelet Distribution Width (PDW) and Platelet large cell ratio (P-LCR) indices in thrombocytopenic animals were significantly higher than the mean values of animals with normal platelet counts.

2.4 Coagulation time

Prothrombin time (PT) is a screening test for factors II, V, VII and X of the extrinsic and common pathways, while as activated partial thromboplastin (APTT) is a screening test for factors II, V, VIII, IX, X, XI and XII of the intrinsic and common pathways (Proctor and Rapaport, 1961).

Measurement of prothrombin time (PT) and activated partial thromboplastin time (APTT) are done in citrated plasma and are the most commonly employed laboratory tests in patients suspected of having a coagulopathy (Kazakos et al.,2005; Bruchim et al., 2006; Kummeling et al., 2006).

Giurgiu et al. (2009) studied the reference values for PT and APTT) in 40 healthy male and female dogs. Various breeds were presented, which were divided into two groups based on their age: under 1.5 years old (n=15) and over 1.5 years old (n=25). The value for APTT in 1.5 years old dogs was 16.74± 0.35 sec, while for the adult dogs, it was 12.36 ± 0.68 sec. Also, PT rage value was 5.3±0.63 sec in adult dogs and 5.7±0.4 sec in young dogs. In the experimental conditions mentioned previously, the human commercial kits proved their utility in establishing reference values of two coagulation parameters in the dog.

Geffre et al. (2010) conducted a study in 139 healthy fasting purebred dogs to determine reference intervals for prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen, and anti thrombin (AT) in citrated plasma The levels were 6.9–8.8 sec, 13.1–17.2 sec, 1.24–4.30 g/l, and 104–188% for PT, APTT, fibrinogen, and AT, respectively.

Sumathi et al. (2012) established a reference range values of APTT in dogs as 48.43± 4.01 seconds and for PT, it was 12.12±0.57 seconds. The range value for APTT was 43.17± 2.39 seconds with a minimum and maximum of 22.6 and 56.6 seconds respectively.

2.4.1 Haemostasis and platelet relationship

Acquired thrombocytopathia can occur due to numerous conditions of conditions including *Ehrlichia spp*. infection, snakebite envenomation, neoplasia, uremia, DIC, anaemia, endotoxemia, and pharmacologic manipulation with antiplatelet drugs (Italiano et al., 1999; Olsen 2000; McMichael 2005).

Platelets contain two types of granules within called alpha and delta (dense) granules. Substances stored within alpha granules include platelet-derived growth factor, fibronectin, transforming growth factor β, β-thromboglobulin, platelet factor 4, fibrinogen, clotting factors V and VIII, and vonWillebrand factor (vWF). Delta (dense) granules contain ADP, ATP, epinephrine, serotonin, calcium ions, and histamine (McMichael 2005).

Radia (2013) categorized immune-mediated thrombocytopenia as primary, where an underlying trigger for the immune system deregulation cannot be identified, or secondary to neoplasia, drug reaction, or infection Platelet lifespan can be reduced from days to a few hours. Increased platelet consumption is often a result of haemorrhage or disseminated intravascular coagulation.

2.4.2 Liver disease relationship with thrombocytopenia and coagulation factors

Badylak et al. (1983) conducted a study in which 93 percent of dogs with a variety of liver diseases, including degeneration, inflammation, cirrhosis, or neoplasia, had at least one abnormal coagulation test value. The PT and PTT were abnormal in (50%) and (75%) of the dogs.

Witters et al. (2008) stated that the liver, in conjunction with the kidneys, is responsible for producing thrombopoietin (TPO), a cytokine that regulates megakaryocyte formation and platelet production.

Prins et al. (2010) compared that platelet count in dogs with normal livers and dogs with a variety of liver diseases, including chronic hepatitis, chronic hepatitis

with cirrhosis, non-specific reactive hepatitis, congenital portosystemic shunt, and steroid-induced hepatopathy. The mean platelet concentration was significantly lower in dogs with chronic hepatitis and cirrhosis compared to other dogs in the study

Prins et al. (2010) conducted the study in dogs with liver diseases, coagulation abnormalities were present in (57%) of dogs with hepatic disease. Haemostatic abnormalities were primarily seen in dogs with cirrhosis and was attributed to reduced synthesis rather than increased consumption of coagulation factors. Mean platelet numbers, AT and factor IX activity were significantly lower in dogs with chronic hepatitis with cirrhosis, compared to dogs with other hepatopathies and dogs with chronic hepatitis with cirrhosis showed lower activities for factors II, V, VII, VIII, IX, X, XI, and XII but only factor IX activity was significantly lower when compared to control dogs

Fleming et al. (2011) stated that liver disease is common in middle age and older dogs, and is reported to be the cause of death in approximately 5 percent of adult dogs

Kavanagh et al. (2011) concluded that chronic liver disease could result in secondary haemostasis defects through decreased coagulation factor production (II, V, VII, IX, X, XI, and XIII), decreased fibrinogen production, decreased vitamin K mediated carboxylation of coagulation factors (II, VII, IX, and X), and changes in production of anticoagulants

Kelley et al. (2015) conducted a study evaluating thrombo elastography in dogs with acute liver disease which revealed 52 percent of dogs to be hypocoagulable, 38 percent to be normocoagulable, and 10 percent to be hypercoagulable.

Lester et al. (2016) studied that thrombocytopenia was documented in dogs with acute liver failure 51 percent of dogs and 29 percent of dogs with acute liver failure had an evidence of haemorrhage.

Fry et al. (2017) conducted a study in dogs with chronic hepatopathies 10 out of 21 had significantly lower platelet counts compared to reference intervals and the platelet count of dogs with congenital portosystemic shunts were lower as compared to healthy dogs.

2.5 Platelet therapy in thrombocytopenic patients

Crystal & Cotter (1992) reported that an increase of 13,000 platelets/µL might be obtained by transfusing 12.5mL/kg body weight of fresh whole blood from a donor with a platelet count of 200,000/ µL. The presence of concurrent fever, sepsis, DIC, bleeding, splenomegaly or immune-mediated thrombocytopenia exist the results will be mitigated.

Slitcher et al. (2010) concluded that when prophylactic platelet transfusions are administered at a trigger threshold platelet count of < 10×10 9 (≤10,000/µL), the platelet dose had no significant effect on the incidence of bleeding in patients with hypo proliferative thrombocytopenia.

Jandrey et al. (2012) administered platelet-rich plasma in German shepherd dogs with platelet procoagulant deficiency (Scott syndrome) prophylactically prior to elective neutering at a median dose of 5.7×10^9 platelets/kg Bwt. and in management of nonsurgical haemorrhage, mainly epistaxis, and it proved to have good efficacy

2.6 Plasma therapy in hypoproteinemia and hypovolemia patients

Williams (1994) suggested that in cases of acute pancreatitis, plasma transfusion may be indicated to replace plasma protease inhibitors and albumin.

De Gopegui & Feldman (1997) concluded that the liver is the most important site for the synthesis of noncellular components of haemostasis which included coagulation factors and coagulation inhibitors. In patients with compromised liver status, the levels of haemostatic proteins are altered so, in general, it is associated with increased haemorrhagic tendencies. Fresh frozen plasma or fresh whole blood may be administered in patients with severe liver damage.

Moore (1998) suggested that the doses of fresh frozen plasma for albumin replacement range from 6 to 10 mL/kg every 8 hours to increase albumin concentration by 0.5 g/dL.

Stokol & Parray (1998) conducted a study on the efficacy of fresh frozen plasma over cryoprecipitate. Six of the nine dogs treated with FFP experienced adverse effects ranging from mild pruritus to pallor and weakness, whereas none of the dogs treated with Cryoprecipitate had any observable adverse reactions (P= .009)

and recommends Cryoprecipitate over FFP for treatment or prophylaxis of haemorrhagic episodes in dogs with vWD (vonWillebrand disease) or haemophilia A.

Lanevschi & Wardrop (2001) concluded that transfusion medicine has become more feasible in small animal practice due to the factors which included improved access to blood products through either site-donors, external donor programs, or the availability of blood components substitutes.

Logan et al. (2001) administered fresh frozen plasma in 74 dogs and could save 50 (68%) dogs until discharge from the hospital.

Rozanski & Laforcade (2004), after a study in 24 critically ill dogs, concluded that fresh frozen plasma transfusion is not generally effective at increasing serum anti thrombin activity.

Snow et al. (2010) reviewed the data of plasma transfusion in which plasma was administered to 112 dogs and 23 cats from 2006 to 2008 and 171 dogs and two cats from 1996 to 1998. Very few patients received plasma for the treatment of hypoalbuminemia in (2006-2008: (15%) compared to 1996-1998: (53%) or while significantly more patients received plasma for coagulopathy (2006-2008: (80%) as compared versus 1996-1998: (31%). There was no difference in serum albumin concentration following plasma transfusion while median PT and APTT were significantly decreased post plasma administration. No association was found between the volume of plasma administered and outcome.

Desborough & Stanworth (2012) noticed that use of frozen plasma had an effect on correcting abnormal coagulation tests when mild and moderate results are recorded.

CHAPTER III

MATERIALS AND METHODS

The present study was conducted on dogs presented to Medicine OPD of Small Animal Clinics, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana from April 2019-Feb 2021. The criteria of selection of cases for transfusion therapy was as under:

a) Severely anaemic (Hb <5 g/dL and <15% PCV)

b) Thrombocytopenic dogs (Platelet count < 50×10^3)

c) Hypoproteinemia (Total protein and Albumin <4.5g/dL and 2.0g/dL)

3.1 Signalment, history and physical examination

Case number, date of presentation, name, address, and phone number of the dog owner was recorded. A detailed history regarding the prior treatment, any previous major illness, prolonged use of any drug, vaccination status, deworming status, tick infestation, and dietary intake was recorded. Information regarding color and frequency of vomitus, faeces, and urine were also recorded. A complete physical examination of the animal was carried out and parameters like rectal temperature, respiration rate, heart rate, general attitude, posture, body condition, and color of the mucus membrane were recorded. A detailed percussion and palpation of the abdomen, especially of the lumbar region was done to check for pain, fluid or abnormality. Auscultation of the thorax was done to note cardiac rhythm. Clinical assessment of anaemic dog was done as per Anaemic Dog Clinical Assessment Score **(**ADCAS score) Kisielewicz (2016).

Table 1: Anaemic dog clinical assessment score (ADCAS)

	Normal (0)	**Mild (1)**	**Moderate (2)**	**Severe (3)**
Mucous membrane color	Salmon pink	Slightly pale	Moderately pale	
Pulse quality	Normal	Bounding	Weak	-
Heart rate(bpm)	65-109	110-140	>140	-
Respiratory rate	15-24	25-40	>40	-
Mentation	Bright walking	Quiet, able to walk	Lethargic, able to stand	Lethargic, Unable to stand

3.2 Diagnostic tests

3.2.1 Blood collection

The dogs were properly restrained in the lateral recumbency; blood was aseptically withdrawn from the cephalic or the saphenous vein. Two mL of blood was collected in sodium EDTA vacutainer vials for the haematological analysis and/ or examination of haemoprotozoa and rickettsia by blood smear examination or by PCR analysis. Five mL of blood was collected in serum collection tubes, which was then centrifuged to extract the serum for biochemical analysis

3.2.2 Haematology

Fully Automatic Laser Based Haematology Analyser (ADVIA ® 2120 Haematology system, Siemens Healthcare diagnostics Inc., USA) was used for the analysis of haematological parameters such as Haemoglobin (Hb, g/dL) , Packed cell volume (PCV%), Total leukocyte count (TLC, 10^3 /mm^3), Platelet count (10^3 /mm^3) , Differential leukocyte count (DLC) on Leishman stained blood films.

3.2.3 Blood biochemical analysis

Virtos DT 350 Chemistry system (Ortho Clinical Diagnostics, Johnson & Johnson Company) employing Virtos DT slides was used for estimation of renal function test i.e. Blood urea nitrogen (BUN, mg/dL), creatinine (mg/dL) ;liver function test i.e. Alanine aminotransferase (ALT, U/L), Aspartate Aminotransferase (AST, U/L), Alkaline phosphatase (ALKP, U/L), Gamma Glutamyl Transferase (GGT, U/L), Total bilirubin (mg/dL), Total protein (TP, g/dL) and Albumin (g/dL) etc.

3.2.4 Blood smear examination

Blood samples from cases with anaemia and thrombocytopenia were stained with leishman stain and subjected to microscopic examination to detect the different haemoprotozoan and rickettsial infections viz., *Babesia gibsoni*, *Babesia canis vogeli*, *Hepatozoon canis* and *Ehrlichia canis* etc.

3.2.5 Faecal examination

The faecal samples were taken per-rectum directly from the dogs using a gloved finger. The samples were subjected to floatation technique using saturated salt solution (specific gravity 1.20). Identification of eggs was made by observing their size and morphological characters under a microscope.

3.2.6 Radiography and ultrasonography

Radiography and ultrasonography were performed to identify the tumorous growth, tumour metastasis, hepatomegaly, chronic kidney disease, ascites and splenomegaly.

3.3 Immunological tests

3.3.1 In-saline agglutination test

The in-saline auto-agglutination test was performed by mixing one drop of blood with 1-4 drops of normal saline (0.9%) at room temperature on a glass slide Day (2008). RBC agglutination was immediately evaluated macroscopically and microscopically. 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 (Plate 1c and 1d).

3.3.2 Coomb's test

Coomb's test was done in cases suspected for immune-mediated haemolytic anaemia. The criteria for case selection were PCV <15 percent, presence of spherocytes (Plate 1a) in blood smear, regenerative anaemia (Plate 1b), presence of microscopic agglutination and exclusion of other cases of anaemia (Piek et al., 2008).

Coomb's test was performed within 24 hr. on blood samples collected in EDTA from the affected dogs. Two mL blood was centrifuged for 5 min at room temperature and 0.1 mL of packed erythrocytes were added to 4.9 mL of phosphate buffered saline (PBS). Blood cells were washed four times in PBS, and finally suspended in 4.9 mL of PBS to prepare 2 percent suspension.

3.3.2.1 Test procedure

100 µl of PBS was added to 4 test tubes (12 x 75mm). Two-fold serial dilutions of the Coomb's reagent was made by adding 100 µl of Canine Coomb's reagent (VMRD, Pullman, WA, USA) in the first tube, mixed and transferred 100 µl to the 2nd tube then similarly from the second tube transferred 100µl to the 3rd tube and after mixing 100 µl was discarded from 3rd tube. The control 4th tube contained only PBS. 100µl of washed (2%) suspended erythrocytes in all the tubes was added, mixed and incubated at 37ºC for 30 min. After incubation, all the tubes were centrifuged for 1 min.

3.3.2.2 Interpretation

The contents of each tube were evaluated by placing a small amount of solution on a clean glass slide, covered with glass coverslip and observed under 100X. The presence of clumps and large aggregates of erythrocytes was taken as positive for Coomb's test and absence of clumping was considered negative (Plate 1e and 1f). The control tube cells should not contain any clumps.

3.4 Tests of secondary haemostasis

The dogs were properly restrained in the lateral recumbency and 5 mL of blood was collected aseptically from the cephalic or saphenous vein in 3.2 percent sodium citrate vacutainer vials for coagulation time. Prothrombin time (PT) and Activated partial thromboplastin time (APTT) were estimated by an automated coagulation analyser.

3.5 Blood transfusion

3.5.1 Selection of clinical cases

The dogs having Hb level below 5g/dL and PCV level below 15 percent were selected for blood transfusion irrespective of etiology. These dogs mostly exhibited clinical signs of anaemia, manifested as tachycardia, dyspnea, weakness, lethargy, recumbency, cold extremities and low body temperature.

3.5.2 Selection and screening of donor animals

Donor dogs were mostly arranged by the owner of the recipient dog and in some cases, by India For Animals on personal request basis. Donors selected for the blood donation were 1 to 8 years old, with minimum body weight of 20 kg, clinically healthy with normal feed and water intake. The donors were screened against common endemic pathogens before using them for blood collection. Minimum Hb and PCV level for considering a dog as a donor were 12g/dL and 35 percent respectively. Thin blood smears were used for the screening of babesiosis, ehrlichiosis and hepatozoonosis, while Rose Bengal plate agglutination test was used for diagnosis of brucellosis.

3.5.3 Cross matching

Cross-matching of donor and recipient blood was done by major and minor cross-matching. Blood from the donor as well as recipient was collected in EDTA

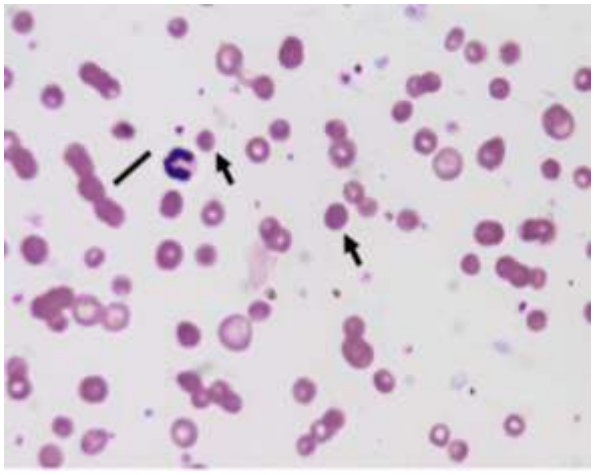


Plate 1a: Spherocytosis (arrow) along with agglutination (line) 100X

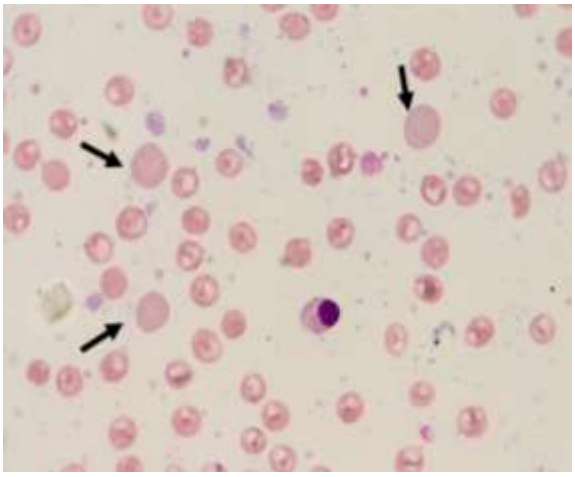


Plate 1b: Blood smear showing polychromatophilic RBCs 100 X

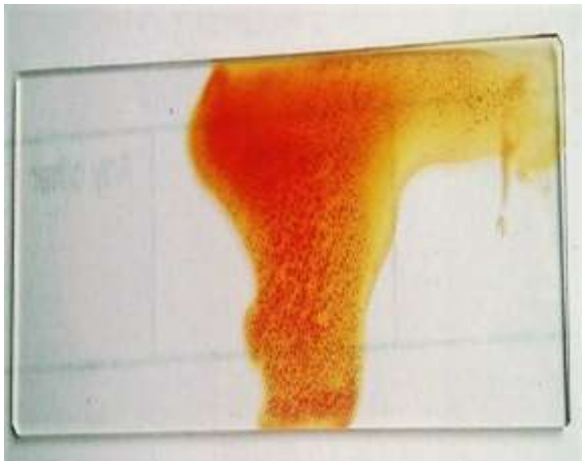


Plate 1c: Positive slide agglutination test indicating IMHA

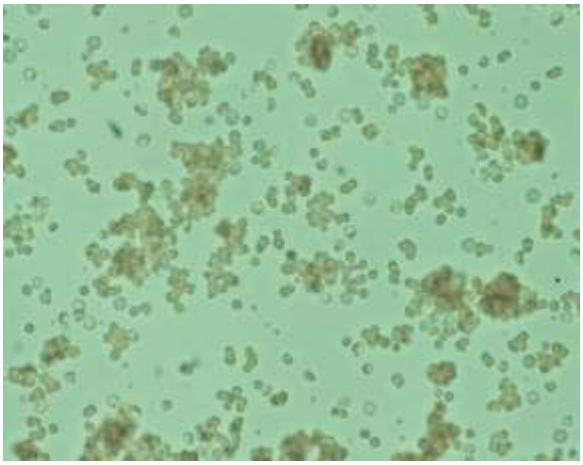


Plate 1d: Positive saline agglutination test indicating IMHA 40 X

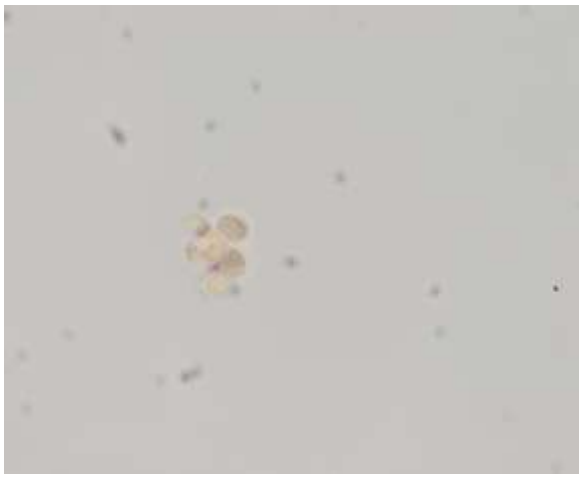


Plate 1e: Positive Coombs test showing a clump of RBCs confirming IMHA 100X

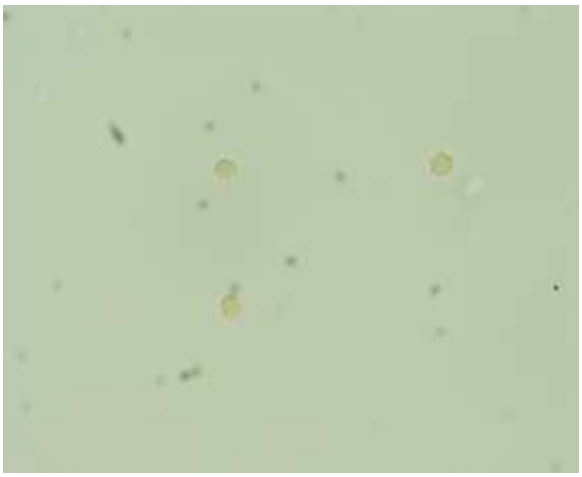


Plate 1f: Negative Coombs test showing a separate RBCs (100X)

Plate 1: Diagnosis of IMHA in dogs

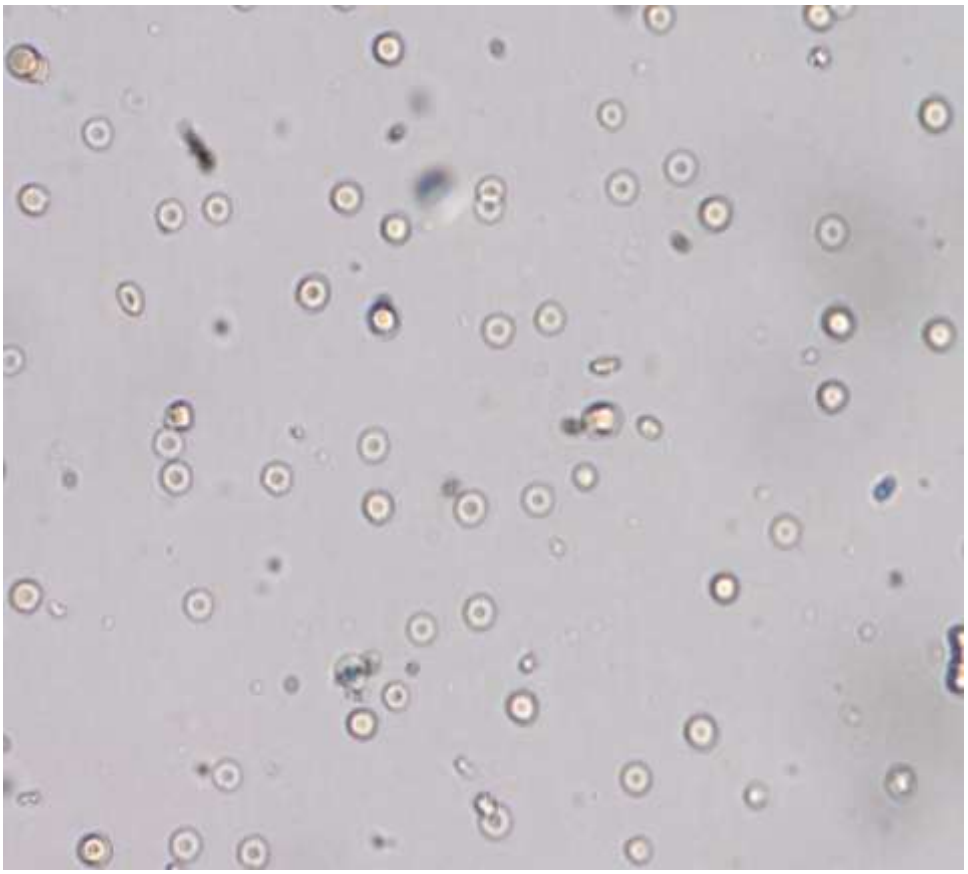


Plate 2: Compatible cross match in a dog (40X)

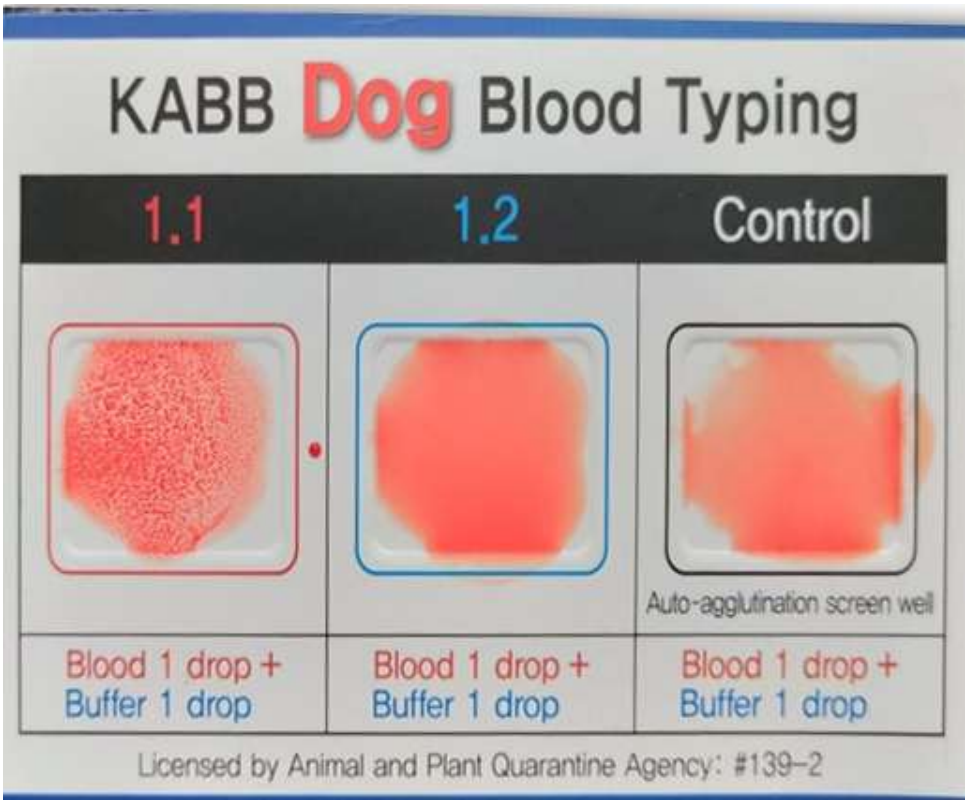


Plate 3: Blood typing card showing DEA 1.1 positive blood type

vials. Blood was centrifuged and separation of plasma from the RBCs was done in Eppendorf tubes. 200 µl of RBCs were taken in a centrifuge tube and 4.8 mL of 0.9 percent NSS was added. After proper mixing of the tube, it was centrifuged for one minute. The process was repeated three times, removing the supernatant every time. The final resuspension of 2% to 4% RBC solution was made in NSS (200 µl of blood in 4.8 mL of saline gives a 4% solution). Labelling of the tubes was done to make the following mixtures:

- Major cross-match (2 drops of recipient serum with one drop of donor RBC suspension).
- Minor cross-match (1 drop of recipient RBC suspension with 2 drops of donor serum).
- Control (1 drop of recipient RBC suspension with 1 drop of recipient serum).

All three tubes were incubated for 15 to 30 minutes at 37°C and then centrifuged for 15 seconds. The supernatant was examined for haemolysis or haemagglutination grossly as well as microscopically. Clumping of RBCs was taken as an incompatible match whereas individual RBCs on the slide were considered as compatible (Plate 2). Blood typing card is shown in Plate 3.

3.5.4 Blood collection from donor dogs

Citrate-phosphate-dextrose solution (CPDA) bag was used for whole blood collection (Plate 4a); Double bag with one containing CPDA and another empty satellite bag allowed separation of pRBCs and PRP (Plate 4b). Triple bag with one containing CPDA; second bag with Saline, Adenine, Glucose and Manitol (SAGM) as an additive solution and a third empty satellite bag for separation into pRBCs, platelets and plasma (Plate 4c). The bag was placed on blood collection system on the Blood Collection Monitor Terumo Penpol (Plate 5). The canine donor was positioned in lateral recumbency and the venipuncture site (jugular vein) was clipped and prepared with surgical scrub to minimize the chances of bacterial contamination. The jugular vein was punctured with a needle provided with bag. The progress of blood collection was monitored via the blood collection monitor. Once the desired amount was collected, the blood line was re-clamped close to the needle and the needle was removed from the donor. Pressure was applied digitally or with a pressure wrap on the

venipuncture site until haemostasis was achieved. The donors were monitored further for any signs of hypotension or hypovolemia such as weakness, pale mucous membranes, prolonged capillary refill time, weak pulses, or altered mentation etc.

3.5.5 Blood component separation

The process of converting a whole blood collection into high-quality blood products is influenced by several factors including centrifuge settings and pre-storage conditions. Whole blood (WB) was centrifuged for 10 minutes (22°C) at 2000x g to prepare platelet-rich plasma (PRP). The PRP was expressed into a satellite bag by plasma extractor. Feldman & Sink (2008) analysed centrifugation techniques and concluded that 2000g for 3 min at 22-25 °C was the correct way for PRP separation.

3.5.5.1 Whole blood (WB) processing:

The centrifuge (plate 6a) was turned on it was allowed to cool to reach the target temperature (22-25°C). The collected WB was weighed along with satellite bags (Plate 6b). The bag with the blood was put in the centrifuge bucket and the attached bags were folded in the same bucket. The centrifuge buckets were accurately balanced by using suitable balances including pieces of tubing, unused blood collection bags, or other soft plastics and rubbers (Plate 6c). It was ensured that buckets swung freely in their axis. The bags were centrifuged as per appropriate protocol. In the meantime, equipments were prepared such as plasma expressor, tube sealer, tube stripper, and scissors. After the centrifugation, it was carefully inspected and ensured that the plasma was clear. The bucket was carefully removed from the centrifuge and the bag was placed on the plasma expressor and it was gently released by pressing the plate (Plate 6d). The seal at the top of blood collection bag was snapped and the PRP was allowed to run into the satellite bag. The line was sealed with tube sealer and ensured that the seal allows both the plasma and red cells to remain sterile once physically separated from each other (Plate 6e). The platelets were put on a platelet agitator maintained at room temperature (Plate 6f).

3.5.5.2 Plasma

The plasma was prepared by the same process as the blood as for WB processing, followed by hard spin 3500 rpm for 10 minutes. The plasma bag was removed from the centrifuged red cells or platelets (PRP method). The bag was labelled according to local convention and weighed. It was placed in a freezer at -40°C (Plate 6g).

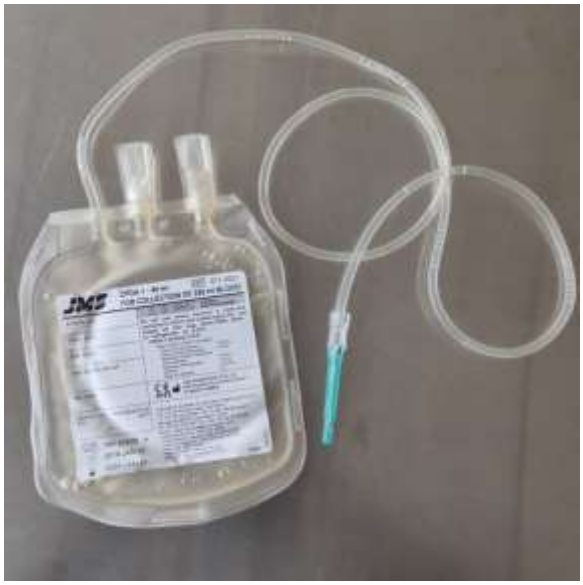


Plate 4a: Single bag

Plate 4b: Double bag

Plate 4c: Triple bag

Plate 4: Different types of blood bags

Plate 5: Collection of blood from donor dog

Plate 6a: Temperature controlled centrifuge (Cryofuse) used to process whole- blood derived products

Plate 6b: Blood weighing balance

Plate 6c: Centrifugation of whole blood for separation into its components

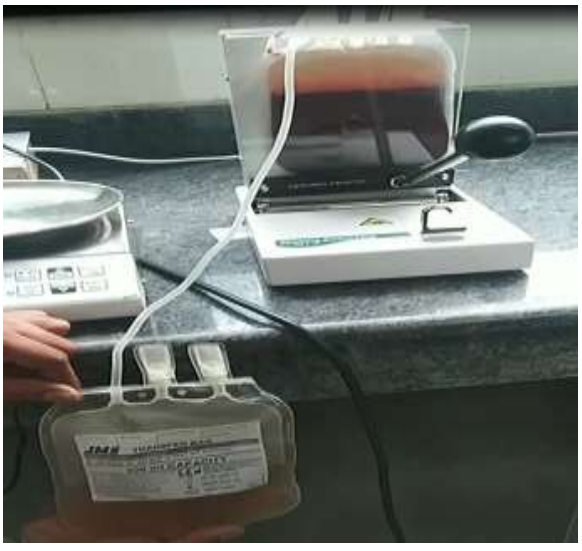


Plate 6d: Plasma extractor is slowly pressed against the bag to express the plasma into an attached bag

Plate 6e: Tube sealer used to permanently close a fluid path and creation of pig tails for compatibility testing

Plate 6f: Platelet agitator cum incubator for storage of platelets at 22°C

Plate 6g: Deep freezer -40 °C used for the storage of fresh frozen plasma

Plate 6: Different types of instruments used in canine blood banking

3.5.6 Storage of fresh platelets and fresh frozen plasma

The platelets have a short shelf life and PRP was stored at 22°C with continuous gentle agitation for up to 5 days (Fung 2014) while frozen plasma products were stored at temperature of -40 °C. Plasma bags were frozen in a horizontal position and then stored upright.

3.5.7 Thawing, administration, and dosage of plasma

Plasma was thawed at temperatures between 30 and 37°C in a water bath. The bag was immediately transfused to the recipient dog. Plasma was infused slowly at initial rate of <5mL/kg/hour for 15–30 minutes, with the recipient closely monitored for signs of transfusion reaction. After the initial period, an administration rate of 5–10mL/kg/hour was maintained. Total FFP was transfused at a dose rate of 10–12mL/kg Bwt.

3.5.8 Administration of blood and packed RBCs

Before transfusing blood into the recipient dog, a risk note for performing the transfusion was under taken from the owner (Appendix 1). The injections of Dexamethasone @ 0.25mg/kg and Pheniramine maleate @ 0.5mg/kg were administered intramuscularly 15-20 minutes before initiating transfusion. Blood was given IV through a cephalic or saphenous vein via a blood administration set with a micropore filter (170 µm) pores to reduce the risk of entry of microthrombi into the general circulation (Plate 7a). No other drug or solution containing calcium or glucose was given through the same IV line except 0.9% normal saline solution. The amount of blood required in recipient dogs was calculated by either of two methods. In large recipient dogs, blood was given @ 10-20 mL/kg Bwt. as sometimes due to low blood collection volume from a donor. However, for small breed recipients, it was calculated as:

$$\text{Volume of the blood (mL)} = 90 \times \text{body weight (kg)} \times \frac{\text{Desired PCV- Actual PCV}}{\text{Donor PCV}}$$

The transfusion rate was 0.25mL/kg Bwt. for the first 15 minutes to check any adverse reactions and then gradually increased to 2-10 mL/kg/hr as per Harrell and Kristensen (1995). The transfusions were usually completed within 4 hours of blood collection to minimize the risk of bacterial contamination.

3.5.9 Administration of Platelet rich plasma (PRP) and Fresh Frozen plasma (FFP)

Before transfusing platelet and plasma into the recipient dog, a risk note for performing the transfusion got signed by the owner (Appendix1). The cross-matching for the plasma and platelet can be optional. However, the major cross - match was performed in every patient. Both FFP and PRP were given IV through cephalic or saphenous vein via a blood transfusion set. No other drug or solution containing calcium or glucose was given through the same IV line except 0.9% normal saline solution (Plate 7b).

3.5.10 Monitoring of patient

Before transfusion, a baseline evaluation of recipient viz temperature, heart rate, respiration rate, mucus membrane was made. Constant monitoring of the patient was done during transfusion for the first 20 minutes and after that at an interval of every 15 – 20 minutes. For monitoring of the patients, a monitoring chart was prepared. (Appendix 2). Emergency drugs such as calcium gluconate, adrenaline, saline, pheniramine maleate, steroids, and oxygen supply were arranged all the time for any emergency. For whole blood, pRBCs and plasma transfusion, follow up haematology was performed on 3rd day and 7th day. Platelet count was performed one hour after administration of platelet-rich plasma and then after 3rd days of transfusion.

3.6 Canine coagulation factor studies

Estimation of coagulation factors (II, V, VII, VIII IX X and vWF) was done by ELISA kits (Biotech Bioassay) as per the manufacturer's recommendation (Plate 8). The studies were performed in healthy serum and plasma as well as in patients suffering from trauma, diabetes mellitus, bleeding dogs with or without thrombocytopenia, acute hepatitis and chronic liver disease.

3.6.1 Reagent preparation

All reagents were allowed to reach room temperature (37ºC) before use. Each standard was reconstituted with standard diluent. Reconstituted material was allowed to stand for at least 20 minutes and mixed gently. Wash buffer1X was prepared by adding the contents of the bottle to an appropriate amount of distilled water.

3.6.1.1Assay Procedure

All the reagents, standard solutions and samples were prepared as instructed. The number of strips required for the assay were determined and inserted in the

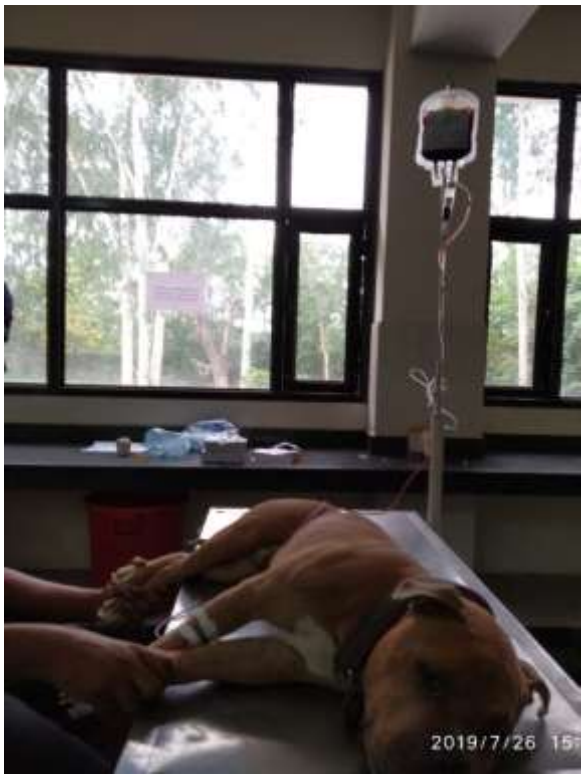


Plate 7a: Administration of pRBCs in anaemic dog

Plate 7b: Administration of platelet rich plasma in thrombocytopenic dog

Plate 7: Administration of different types of blood products

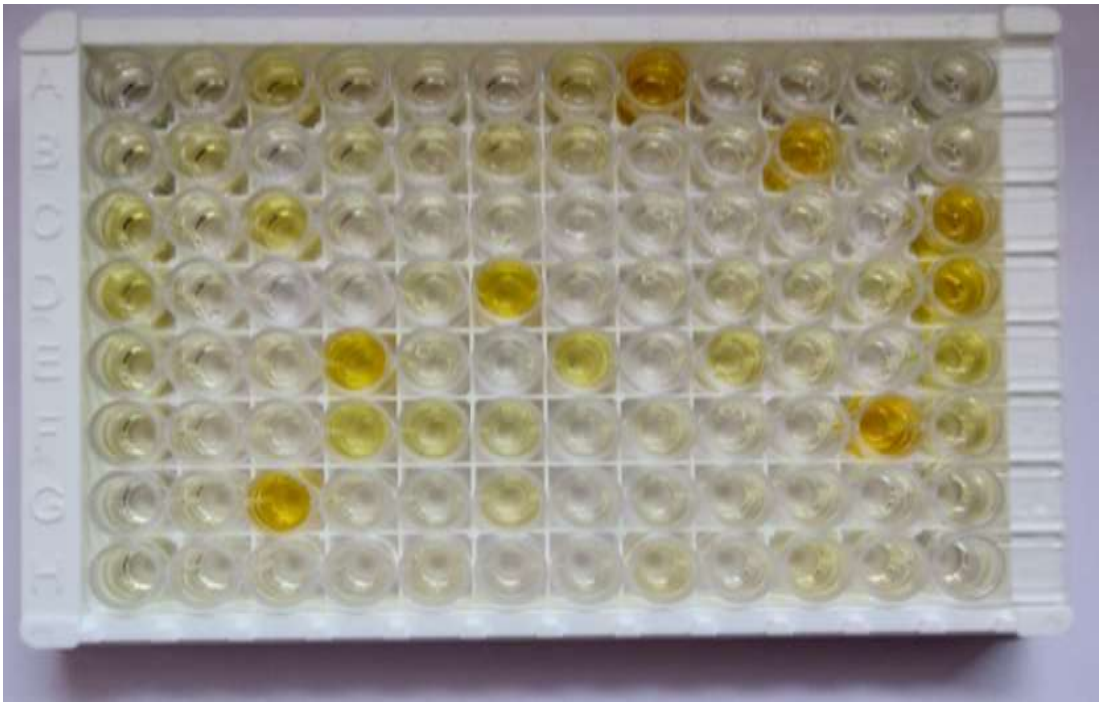


Plate 8: Estimation of coagulation factors (II, V, VII, VIII, IX, X and vWF)

frames. 50 µl standard was added to standard well and 40 µl sample was added only to sample wells then 10 µl anti F-9 antibody was added to sample wells and then 50 µl streptavidin-HRP was added to sample wells as well as standard wells. The sealer was removed and plate was washed 5 times with wash buffer. The wells were soaked with at least 0.35 mL wash buffer for at least 30 seconds for each wash. The plate was blotted onto paper towels and 50 µl substrate solution A was added to each well and then 50 µl substrate solution B was added to each well. The plate was incubated in dark and covered with a sealer for 10 minutes at 37 minute. 50 µl Stop solution was added to each well, the blue color changed into yellow immediately. Each well's optical density (OD value) was determined immediately using a microplate reader set to 450nm within 10 minutes after adding the stop solution.

3.6.1.2 Calculation of results

The mean absorbance value (OD 450) for each set of reference standards, controls and samples was calculated. The standard curve was made by plotting the mean absorbance of each reference standard against its concentration on graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis. On the basis of mean absorbance value of each sample, the concentration of the sample was determined from the standard curve.

3.7 Molecular diagnosis

The blood samples from the cases of anaemia and thrombocytopenia (n=80) as well as healthy donor dogs (n=20) were subjected to DNA extraction. Upon standardization of the respective parasite-specific PCR assays, the amplicons for the positive control and samples were observed in (1.5%) electrophoresis at 672 bp (*Babesia gibsoni*), 601 bp (*B. canis vogeli*) 380 bp (*Erlichia canis*) and at 666 bp (*Heptazoon canis*).

3.7.1 Genomic DNA extraction for analysis of blood samples by Polymerase Chain Reaction (PCR)

For conducting the PCR assay, whole genomic DNA was extracted from 300 µl of whole blood using Geneaid® DNA Isolation kit (Blood) Quick protocol according to the manufacturers' recommendations.

3.7.2 Standardization of PCR assays

3.7.2.1 Standardization of *Babesia gibsoni* PCR assay (Bg-PCR)

The Bg-PCR was standardized by using primers targeting a partial 18S rRNA in the gene sequence of *B. gibsoni* Singh et al. (2014). The sequences of primers employed in PCR assay are as follows:

Forward primer (GIB599): 5' CTC GGC TAC TTG CCT TGT C 3'

Reverse primer (GIB1270): 5' GCC GAA ACT GAA ATA ACG GC 3'

3.7.2.2 Standardization of *Babesia canis vogeli* PCR assay (Bc-PCR)

The Bc-PCR was standardized using primers targeting a 18S rRNA in the gene sequence of *Babesia canis vogeli* as described by Duarte et al. (2008). The sequences of primers employed in PCR assay are as follows:

Forward primer (BAB1): 5' GTG AAC CTT ATC ACT TAA AGG 3'

Reverse primer (BAB4): 5' CAA CTC CTC CAC GCA ATC G 3'

3.7.2.3 Standardization of *Hepatozoon canis* PCR assay (Hc-PCR)

The Hc-PCR was standardized by using primers targeting a partial 18S rRNA gene sequence of Hepatozoon species Inokuma et al. (2002). The sequences of primers employed in PCR assay are as follows:

Forward primer (HEP-F): 5' ATA CAT GAG CAA AAT CTC AAC 3'

Reverse primer (HEP-R): 5' CTT ATT ATT CCA TGC TGC AG 3'

3.7.2.4 Standardization of *Ehrlichia canis* PCR assay (Ec-PCR)

The Ec-PCR was standardized using primers targeting a partial Vir B9 gene sequence of *Ehrlichia canis* as described by Kledmanee et al. (2009). The sequences of primers employed in PCR assay are as follows:

Forward primer (Ehr1401F): 5' CCA TAA GCA TAG CTG ATA ACC CTG TTA CAA 3'

Reverse primer (Ehr1780R): 5' TGG ATA ATA AAA CCG TAC TAT GTA TGC TAG 3'

3.7.3 Thermocycler conditions

The PCR was set up in 25 µL reaction consisting of 10X Dream Taq buffer (Thermo scientific), 200 µM of 10 mM dNTP mix (Thermo scientific), 1.0 mM of 25

mM $MgCl_2$ (Biolabs), 1.0 U of Taq DNA polymerase (recombinant) (Thermo scientific), 15 pmol each of the respective primers and 3.0 µl of template DNA source. The final volume was made up to 25 µL by adding the requisite amount of nuclease free water (Thermo scientific). The composition of the master mix is given in (Table 2) and cycling conditions PCR in (Table 3)

Table 2: Composition of master mix used for PCR assay

	PCR component	**Volume (µL)**
1	10X PCR buffer+20 mM $MgCl_2$	2.5
2	10 mM dNTP mix	0.5
3	Forward primer	1.5
4.	Reverse primer	1.5
5	Dream *Taq* Hotstart DNA Polymerase (recombinant)	0.25
6	DNA sample	3.0
7.	Nuclease free water	15.75
Total Volume		25

Table 3: Cycling conditions for performing the PCR assay

Cycling conditions PCR					
	B.gibsoni	***B canis vogeli***	***H.canis***	***E.canis***	
Initial denaturation	95°C for 5 min				1 cycle
Denaturation	95°C for 30 sec	95°C for 30 sec	95°C for 30 sec	95°C for 30 sec	40 cycles
Annealing	63 °C for 30 sec	62 °C for 30 sec	58°C for 30 sec	62 °C for 30 sec	
Extension	72°C for 45 sec	72°C for 1 min	72°C for 45 sec	72°C for 45 sec	
Final extension	72°C for 7 min	72°C for 7 min	72°C for 7 min	72°C for 7 min	1cycle
4 °C for ∞					

3.7.4 Agarose gel electrophoresis for detection of the PCR amplified products

PCR products were analysed using conventional agarose gel electrophoresis in 1.5 % w/v agarose containing Good view nucleic acid stain (Helix Biosciences) at 100 V for 30-45 min. Briefly, agarose (1.5%) (w/v) suspension was made in 1X TAE buffer and heated until the agarose was completely dissolved in microwave oven to have a clear transparent solution. After that, 5 µL of nucleic acid stain (10 mg/mL) was added to make the final concentration of 0.5 µg/mL and the solution was cooled. Comb was fitted in a balanced and sealed tray and the gel was casted. Then the solidified gel was placed in horizontal gel electrophoresis apparatus containing sufficient amount of 1X TAE buffer. Comb was removed and DNA sample was laid in wells. The respective PCR amplified products (15 µL) were mixed with 3 µL of 6X Tri Track DNA loading dye (Thermo scientific), loaded in the wells of stained gel and run for 30-45 min at 100 V along with suitable DNA Ladder. The amplicons were visualized under UV light and gel documentation system (Bio-Rad) and the size of PCR amplicon was estimated. Suitable positive and negative controls were also run alongside.

3.8 Retrospective studies

3.8.1 Thrombocytopenia

Hospital prevalence of thrombocytopenia was determined on dogs presented to small animal Clinics, GADVASU, Ludhiana for two years Dogs with thrombocytopenia were graded as per (Mc Conell, 2000).

a. Normal $\geq 165 \times 10^3$.

b. Mild thrombocytopenia: $90\text{-}150 \times 10^3$.

c. Moderate thrombocytopenia: $50\text{-}90 \times 10^3$.

d. Severe thrombocytopenia: $<50 \times 10^3$.

Epidemiological analysis on data relating to thrombocytopenia was done with respect to breed, season, sex, age and etiology.

- **Season**: Winter (November, December, January and February), summer (March, April, May and June), monsoon (July, August, September and October).
- **Sex**: Male and female dogs.
- **Age**: ≤6 months, >6 months to 1y, >1 y to 5 y, >5 y to 10 y and >10 y.

3.8.2 Abnormal protein concentrations

Hospital prevalence of abnormal protein concentrations was determined in dogs presented to small animal Clinics, GADVASU, Ludhiana for two years and were divided into three groups (Porter and Kaplan 2011) as below:

- Hypoproteinemia+hypoalbuminemia (Total protein>5.4g/dL and albumin>2.3g/dL).
- Hypoproteinemia+normoalbuminemia (Total protein >5.4g/dL and albumin 2.3-3.1 g/dL)/
- Normoproteinemia+hypoalbuminemia (Total protein 5.4-7.5 g/dL and albumin >2.3 g/dL).

Epidemiological analysis on data relating to abnormal protein concentrations was done with respect to etiology, breed, sex, and age (0-1yr, 1- 5 yr, 5-10 yr and >10 yr).

3.9 Statistical analysis

The statistical analysis was done for each response variable with the help of one way ANOVA and t-test

CHAPTER IV

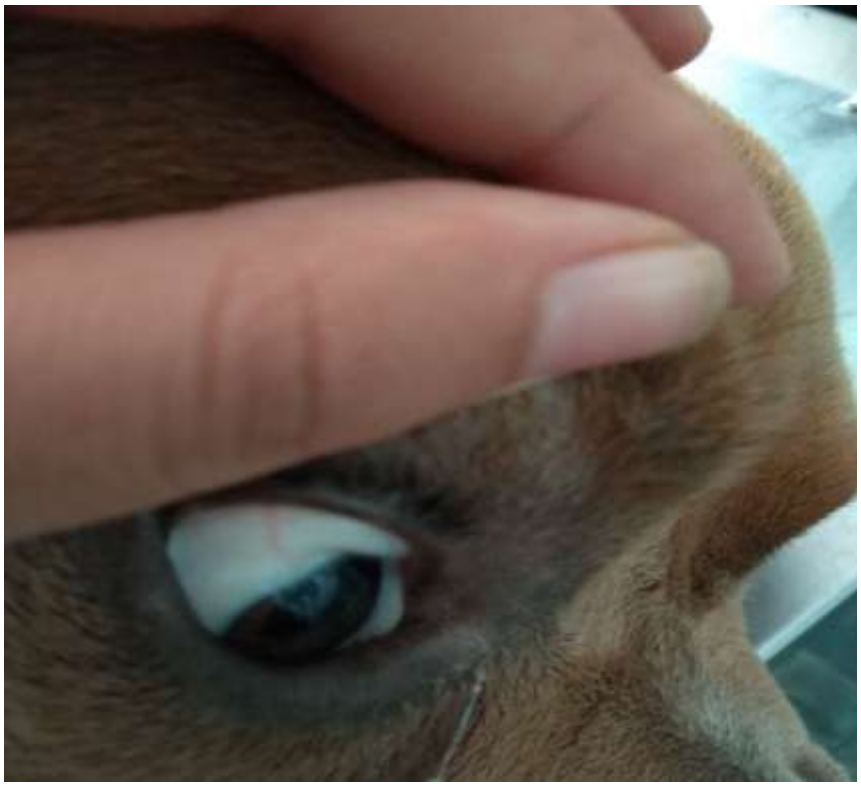
RESULTS AND DISCUSSION

4.1 Observations on severely anaemic dogs

A detailed study was carried out on clinical signs and physical examination of 91 severely anaemic dogs subjected to transfusion (Hb<5 g/dL and PCV 15%). The detailed study on clinical parameters, signs, haematology, biochemistry and survival was done on initial presentation and followed up.

4.1.1 Clinical signs

As shown in Table 4, (Fig 1) and as per ADCAS score (Fig.2) severely pale mucous membrane was be most significant sign of severe anaemia (78.02%) (Plate 9) followed by tachypnea (72.52%), lethargic but able were to stand (68.10%), in appetence/anorexia (61.53%), tachycardia (57.14%), vomition (54.94%), weak pulse (51.64%) and melena (30.76%). Similarly, Pallavi (2019) recorded pale mucous membrane to be the most significant sign of severe anaemia with (91.43%) followed by inappetence/anorexia (81.44%), lethargy (78.57%), tachycardia (71.43%), tachypnea (70%), vomition (52.86%) and melena (48.57%). Similarly, Tandel et al. (2019) reported pale mucous membrane (86.27%), hypothermia (68.63%), tachycardia (70.59%), tachypnea (74.51%) and increased capillary refilling time (64.71%) as signs of anaemia. Bhat (2016) also observed pale mucous membrane (95.16%), lethargy (75.81%), tachycardia (69.35%), tachypnea (51.61%), vomition (46.77%) and melena (46.77%) as important clinical signs of anaemia. Clinical examination of the anaemic patients revealed pale mucous membranes, tachypnea, tachycardia, systolic murmur, hepatomegaly and/or splenomegaly, lymphadenomegaly, fever in another study (Mackin, 2014). Similar signs were also recorded by many previous studies, (Nassiri et al., 2005, Piek et al., 2011).



(a)

(b)

Plate 9: (a) Pale conjunctival mucous membrane (b) Lethargic but able to stand anaemic dog

Table 4: Clinical signs and physical examination of anaemic dogs

Parameters	Clinical Signs	Pre transfusion (n=91)
Mucous membrane	Severely pale	71(78.02)
	Pale	8(8.79)
	Icteric	8(8.79)
	Salmon Pink	4(4.39)
General state	Lethargic but able to stand	62(68.10)
	Recumbent	18(19.70)
	Active	11(12.00)
Pulse quality	Weak	47(51.64)
	Bounding	38(42.76)
	Normal	6(6.59)
Tachypnea (>30/min)		66(72.52)
Anorexia/Inappetance		56(61.53)
Tachycardia(>140/min)		52(57.14)
Vomition		50(54.94)
Melena		28(30.76)
Fever		13(14.28)
Loose feces		9(9.89)
Ticks		8(8.79)
Epistaxis		7(7.69)
Weight loss		6(6.59)
Abdominal distention		6(6.59)
Haematuria		6(6.59)
Haematemesis		6(6.59)
Dark yellow urine		5(5.49)
Blindness		2(2.19)
Nervous Signs		2(2.19)
Constipation		2(2.19)
Haematochezia		2(2.19

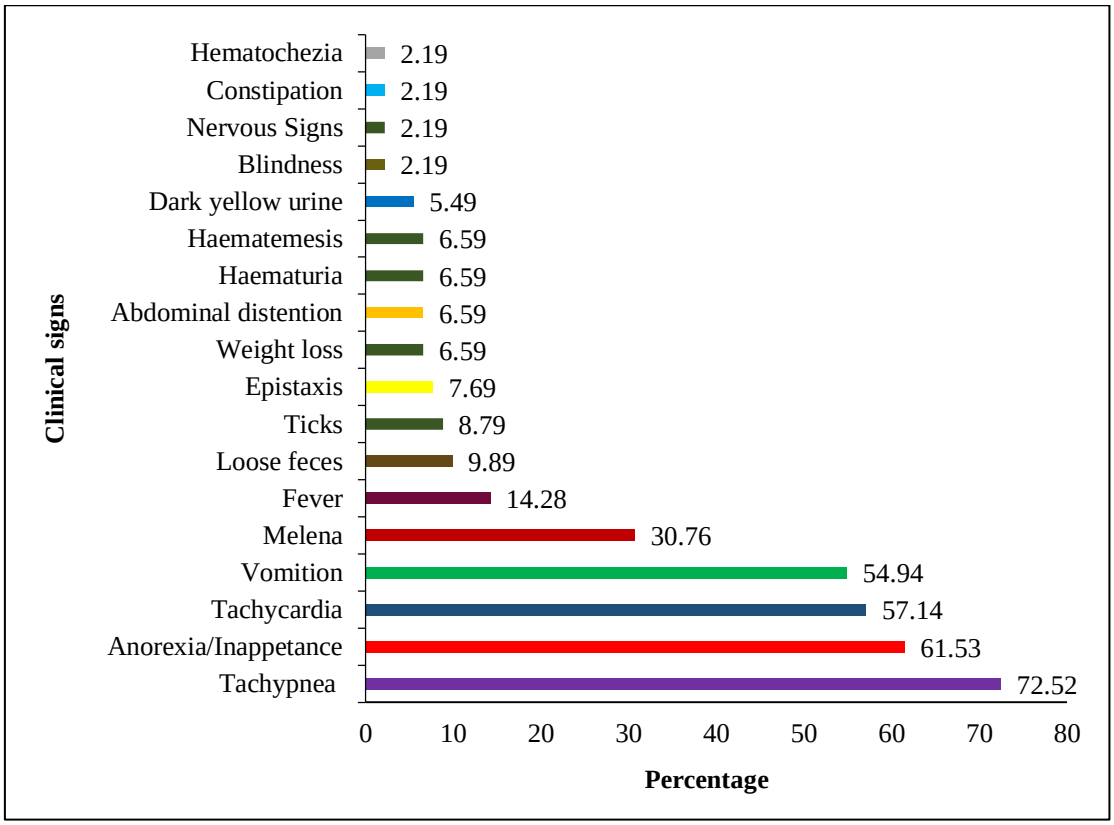


Fig. 1: Bar diagram demonstrating clinical signs in anaemic dogs

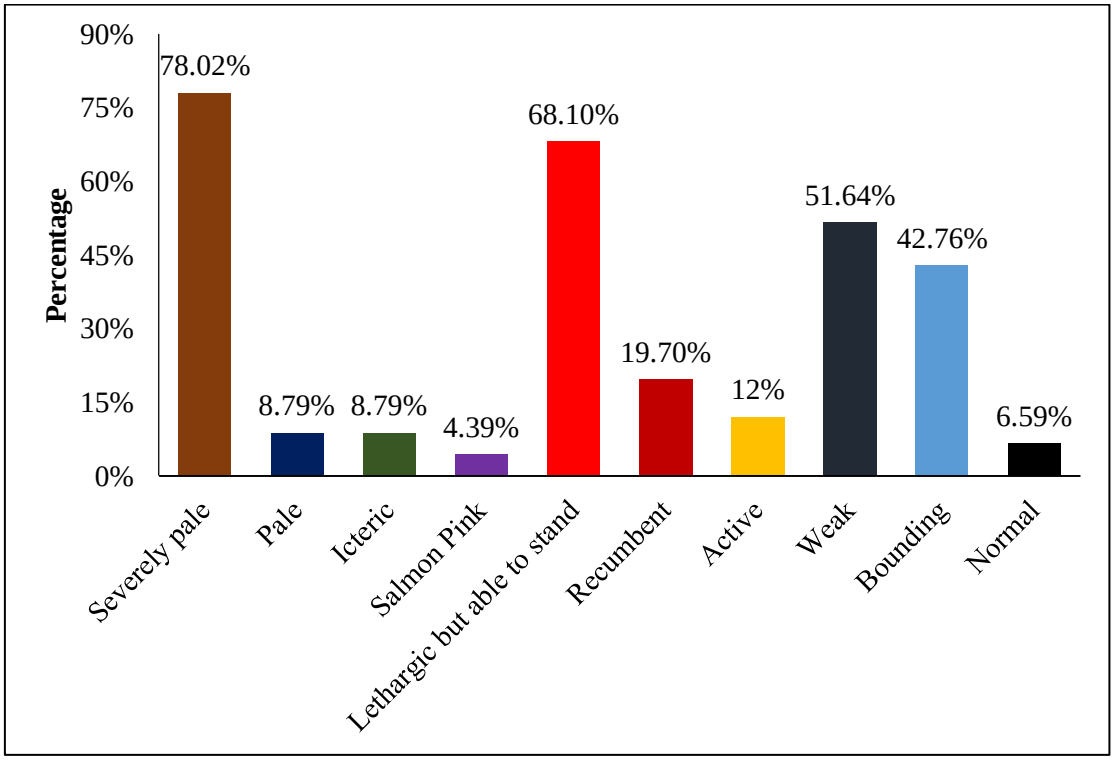


Fig. 2: Bar diagram showing clinical signs in anaemic dogs based on ADCAS score

4.1.2 Epidemiological studies on severe anaemia

The highest prevalence of anaemia in our study was recorded for Labrador retriever (29.67%) followed by mixed breeds (17.58%) and German shepherd (12.08%) (Table 5 and Fig. 3). Our study was in accordance with Tandel et al. (2019), who revealed a high prevalence of anaemia in Labrador (35.29%) followed by non-descript (ND) (19.60%), German shepherd and Doberman (13.73% each). Lynch et al. (2016) also recorded highest distribution of anaemia in mixed breeds (n = 132) followed by Labrador retrievers (n = 74), Golden retrievers (n = 39), and German shepherd dogs (n = 29). The highest prevalence of anaemia was recorded in males (74.73%) as compared to females (25.27%) in our study. Similarly, Tandel et al. (2019) recorded that prevalence of anaemia was higher in males (52.94%) than in female dogs (47.60%). Similarly, Liu and Su (2015) examined 3174 dogs with anaemia out of which 1680(52.9%) were males and 1494(47.1%) were females.

Table 5: Epidemiological data analysis in severely anaemic dogs

Breed	**No. (%)**
Labrador Retriever	27(29.67)
Mixed breeds	16(17.58)
German Shepherd	11(12.08)
Pug	8(8.79)
Pitbull	6 (6.59)
American Bully	6(6.59)
Pomeranian	4(4.40)
Daschund	4(4.40)
Beagle	3(3.30)
Cocker Spaniel	3(3.30)
Rottweiler	3 (3.30)
Sex	
Male	68(74.73)
Female	23(25.27)
Age (years)	
0-3 yr	41(45.05)
3-6 yr	16 (17.58)
6-9 yr	24 (26.38)
9-12 yr	10(10.99)

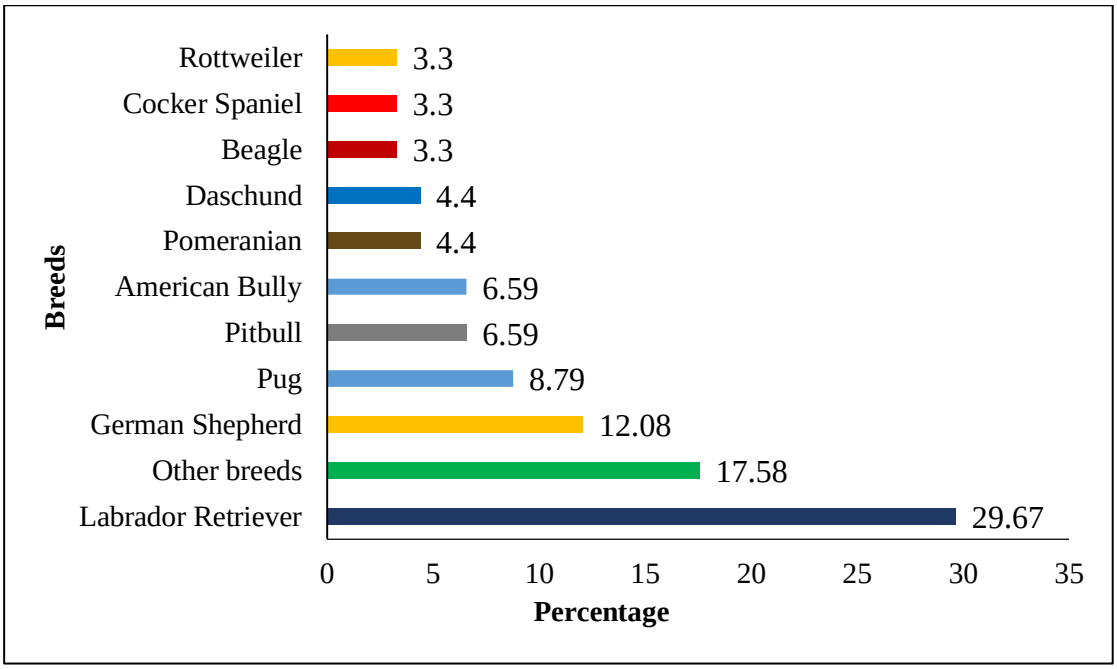


Fig. 3: Bar diagram demonstrating breed-wise proportion of severe anaemia in dogs

In our study the highest prevalence of anaemia was observed in younger age group of 0-3 yr (45.05%) (Fig. 4). This finding can be due to higher presentation of younger dogs in our clinics. Similarly, Meshram et al. (2019) reported the highest incidence in younger group (38.46 %) followed by senile group (37.87 %) and adult group (22.72 %).

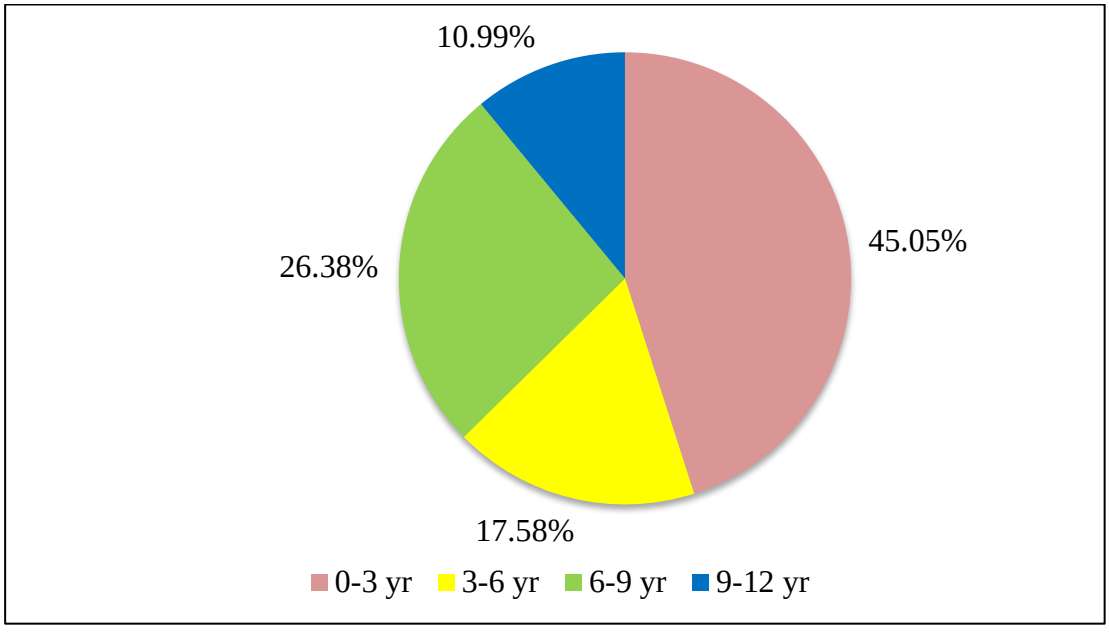


Fig. 4: Pie diagram representing age-wise proportion of severe anaemia in dogs

Bhat (2016) reported 1-5 years age group as most susceptible (37.88%) and dogs more than ten years with minimum prevalence (6.63%) of anaemia. However, our study was in contrast to Tandel et al. (2019), as they reported a higher rate of anaemia (58.83%) in the adult age group as compared to the younger (21.57%) and senile group (19.60%).

4.1.3 Biochemical alterations

Biochemical alterations recorded in anaemic cases mainly corresponded to primary etiology. The values of biochemical alterations are given in Table 6. In liver diseases, AST and ALT levels were higher in dogs while the levels of total protein and albumin in dogs were low in dogs with anaemia. Increased ALT and AST may be attributed to the haemolysis and cellular damage to the hepatic cells (Shrivastava et al., 2014). It may be attributed to hepatocellular degeneration or hepatic dysfunction (Niwetpathomwat et al., 2005; Guadarrama-Olhovich et al., 2013). The current study's lower TPP level was consistent with previous studies (Elhamiani -Khatat et al., 2015; Bilwal et al., 2017). Hepatic dysfunction leads to hypoproteinemia in dogs with anaemia (Agnihotri et al., 2012). In chronic kidney diseases, creatinine level was increased in accordance with (Elhamiani -Khatat et al., 2015). Similarly, Mann, (2013) also found the overall mean creatinine levels in renal failure dogs to be 9.01±1.09 mg/dL and mean BUN as 134.65±14.27 mg/dL. Waldrop et al. (2003) recorded the biochemical profile of 47 severely anaemic dogs median BUN was 23 mg/ dL (range, 6 to 226 mg/ dL), median creatinine concentration was 0.6 mg/ dL (range, 0.2 to 4.7 mg/dL), seven percent of dogs were panhypoproteinemic, with a TP - 4.2 g/ dL (range, 3.3 to 6.6 g/ dL).

4.1.4 Haematological alterations

A total of 93 transfusions were performed in 91 dogs (one dog received transfusion thrice) and were classified into 13 groups based on etiology as shown in Table 7 and Fig. 5. The number of anaemic cases were highest in kidney disease (23.08%), followed by liver disease (15.38%), haemoprotozoan infection (15.38%) and lowest in haematuria (1.09%). Similarly, Tandel et al. (2019) reported that liver (33.30%) and renal (29.16%) disorders were major etiological factors contributing to the etiology of anaemia.

Table 6: Biochemical alterations in severely anaemic dogs based on etiology

Etiology (n=91)	ALT (IU/L)	ALKP (IU/L)	Total Bilirubin (mg/ dL)	Total Protein (g/ dL)	Albumin (g/ dL)	Blood urea nitrogen (mg/ dL)	Creatinine (mg/ dL)	Phosphorus (mg/ dL)	AST (IU/L)
CKD	77.27±13.57 (19-154)	71.50±14.72 (19-154)	0.20±0.08 (0.1-0.6)	5.36±0.35 (4.6-8)	2.48±0.15 (2.1-3.7)	132.28±11.33 (31-200)	7.67±0.66 (2.80-13.2	9.95±0.99 (2.90-20.0)	47.00±5.84 (31-63)
Liver disease	187.46±34.98 (36-450)	581.66±178.43 (75-1525)	1.61±0.44 (0.2-4.1)	4.82±0.25 (3.2-6.3)	1.97±0.15 (0.2-1.6)	26.00±5.07 (10-73)	0.92±0.12 (0.2-1.6)	4.11±0.33 (2.1-6)	266.66±34.98 (75-858)
Pancytopenia	242.11±99.97 (20-812)	43.00±9.19 (30-56)	0.70±0.08 (0.1-0.7)	5.50±0.92 (3.4-10.5)	2.20±0.33 (1.6-3.7)	25.50±3.24 (12-40)	1.80±0.26 (0.6-1.8)	4.75±0.01 (4.7-4.8)	86.00±41.64 30-230
Haemoprotozoa	112.16±60.35 (27-465)	-	0.55±0.20 (0.1-1.5)	6.07±0.11 (5.7-6.5)	2.20±0.08 (1.9-2.5)	34.60±10.25 (10-84)	0.91±0.18 (0.3-1.9)	3.80±0.16 (3.4-1.9)	35.75±4.95 (19-54)
AID / Septicemia	47.86±7.48 (20-78)	-	0.17±0.02 (0.1-0.2)	6.38±0.32 (4.8-7.6)	2.60±0.29 (1.4-3.8)	18.86±0.09 (12-31)	0.90±0.09 0.3-1.1	-	82.42±15.48 (34-170)
GI diseases	52.67±8.99 (31-67)	-	-	4.66±0.13 (4.2-5.2)	2.02±0.20 (1.5-2.6)	21.33±4.72 (10-29)	0.90±0.28 (0.2-1.3)	4.20±0.37 (3.4-5.0)	53.33±10.37 (31-75)
Neoplasia	196±79.42 (41-437)	385.00±29.34 (325-450)	1.27±0.50 (0.2-2.8)	5.52±0.56 (4.2-6.8)	2.37±0.18 (1.8-2.8)	23.25±7.51 (8-47)	1.05±0.11 (0.7-1.3)	4.90±0.75 (3.6-6.2)	58.00±0.00 (325-450)
IMHA	321.00±23.52 (265-358)	-	9.50±5.47 (2.2-22.9)	7.30±0.29 (6.8-8.0)	2.90±0.08 (2.8-3.1)	16.66±3.60 (10-25)	0.73±0.14 (0.4-1)		114.00±0.00
MODS	488.25±159.83 (154-900)	-	1.70±0.35 (1.2-2.2)	4.35±0.33 (3.2-4.8)	1.62±0.13 (1.2-1.9)	103.50±25.90 (15-140)	4.25±1.22 (3.2-4.8)	13.70±0.46 (12.9-14.5)	561.00±26.55 (515-606)
Surgery & Trauma	44.66±6.41 (34-60)	65.00±0.00 (0-65)	0.13±0.02 (0.1-0.2)	3.80±0.18 (3.4-4.2)	1.83±0.02 (1.8-1.9)	19.00±1.70 (15-22)	0.80±0.04 (0.8-0.9)	3.60±0.24 (3.4-4.2)	39.66±4.38 (32-37)
Idiopathic	50.00±6.50 (41-66)	78.00±4.90 (70-90)	0.20±0.04 (0.1-0.3)	5.20±0.53 (4.5-6.5	2.20±0.17 (1.8-2.5)	16.33±4.90 (8-25)	0.86±0.10 (0.7-1.2)	3.80±0.20 (3.5-4.5)	45.00±5.70 (34-58)
Pyometra	76.00±1.41 (74-78)	30.00±1.41 (28-32)	-	5.95±0.14 (5.4-6.5)	2.00±0.14 (1.8-2.2)	43.50±4.59 (37-50)	1.45±0.24 (1.1-1.8)	5.35±0.60 (4.5-6.2)	-

Table 7: Change in haematological values upon transfusion of whole blood or pRBCs in different disorders

Etiology (n=91)	Day	Hb (g/dL)	TEC ($\times 10^6$/ µl)	PCV (%)	Platelets ($x10^3$/µl)
CKD	0(21)	3.48±0.22 (1.50-5.10)	1.66±0.10 (0.83-2.50)	10.31±0.60 (5.20-15.00)	251.47±31.73 (34-478)
	3(20)	6.00±0.39 (2.30-7.60)	3.21±0.19 (1.12-4.40)	20.00±1.15(9.30-30.40)	228.45±29.27(20-460)
	7(13)	6.45±0.45 (2.90-8.10)	3.43±0.36 (1.32-6.20)	20.85±1.65(10.00-28.00)	
Liver Disease	0(14)	2.76±0.14 (2.00-3.70)	1.50±0.09 (1.00-2.32)	9.54±0.60 (6.00-14.00)	170.64±42.62 (25-539)
	3(14)	5.60±0.20(4.20-7.30)	2.91±0.13 (1.85-3.70)	18.38±1.03 (12.00-26.00)	174.00±37.52(35-450)
	7(12)	7.37±0.17(6.20-8.20)	3.62±0.29 (1.20-5.10)	24.13±1.17 (20.00-32.00)	
Pancytopenia	0(6)	3.00±0.27 (2.1-4.10)	1.70±0.10(1.25-2.10)	9.83±1.07(5.00-13.80)	21.00±5.16 (8-44)
	3(4)	4.66±0.60(3.00-6.50)	2.31±0.29(1.52-3.23)	16.88±1.56(10.0-19.40)	47.40±18.34 (19-129)
	7(2)	5.30±0.36(4.40-6.00)	2.75±0.32(2.10-3.23)	16.88±1.93(12.0-22.0)	-
Haemoprotozoa	0(14)	2.95±0.28 (0.90-4.40)	1.51±0.12(0.50-2.3)	9.73±0.86 (3.10-14.0)	137.64±39.93 (3-461)
	3(12)	5.05±0.50(1.50-7.50)	2.76± 0.35(1.20-5.29)	18.25±1.93 (5.00-30.0)	193.91±44.75 (20-460)
	7(8)	8.13±0.45(7.00-11.30)	4.19±0.36 (3.00-5.9)	26.88±1.23(21.00-32.00)	
AID/ Septicaemia	0(9)	3.44±0.34(2.10-5.00)	1.49±0.14 (1.04-2.33)	11.98±0.92 (7.20-15.00)	198.88±45.03 (20-450)
	3(9)	6.08±0.40(3.80-8.00)	3.03±0.35(1.40-2.33)	19.98±1.10(14.0-25.0)	220.00±38.88 (45-460)
	7(7)	8.14±0.22(7.00-8.80)	4.60±0.14(4.20-5.20)	25.95±0.86 (21.00-28.70)	
GI diseases	0(5)	4.00±0.42(2.80-5.00)	2.24±0.21(1.46-2.78)	12.88±1.20 (8.00-15.00)	190.00±39.78 (27-300)
	3(5)	7.18±0.95(5.60-11.4)	3.56±0.49 (2.00-5.20)	20.16±1.36 (17.00-25.80)	252.40±51.92 (68-404)
	7(5)	8.28±1.05 (7.0-13.0)	3.67±0.50(2.1-5.5)	25.60±2.16 (21.0-35.00)	

Etiology (n=91)	Day	Hb (g/dL)	TEC (×10^6/ µl)	PCV (%)	Platelets (x10^3/µl)
Neoplasia	0(4)	3.50±0.44 (2.1-4.4)	2.11±0.25(1.6-2.76)	12.62±1.61 (8-15.90)	227.25±87.51 (10-499)
	3(4)	6.00±0.35(5.0-6.8)	3.22±0.23(2.53-3.8)	19.9±1.49 (15.0-22.6)	200.00±52.32 (30-300)
	7(4)	6.55±0.46(5.2-7.6)	3.35±0.44 (2.01-4.40)	20.6±1.72 (16.4-24.0)	
IMHA	0(4)	2.70±0.60 (1.70-4.7)	1.22±0.29 (0.70-2.12)	8.95±1.86(4.80-14.70)	100.00±47.26 (9-238)
	3(4)	4.22±0.80 (2.40-6.40)	1.61±0.37 (0.81-2.48)	13.75±2.13 (9.00-18.00)	102.75±39.55(12-200)
	7(4)	6.65±1.41 (1.80-9.00)	3.29±0.79 (0.66-4.60)	20.75±4.86(4.00-28.00)	
Multiple Organ Dysfunction Syndrome	0(4)	3.46±0.61 (1.40-4.60)	1.84±0.32 (0.72-2.26)	11.85±1.92(5.5-15)	223.75±97.43 (14-537)
	3(3)	6.70±0.11 (6.4-6.9)	3.72±0.32(3.16-4.47)	22.00±0.40 (21.00-23.00)	382.00±128.78 (154-742)
	7(3)	7.43±0.23 (7.00-8.1)	4.00±0.41 (3.50-5.02)	26.00±0.82(24.0-28.0)	
Surgery & Trauma	0(3)	3.90±0.47 (2.8-4.8)	1.91±0.50(0.73-2.8)	10.13±0.48(9-11)	226.67±81.15 (30-350)
	3(2)	7.50±0.87(6.00-9.00)	3.85±0.46 (3.2-4.5)	23±3.53(18-28)	260.00±17.32 (230-290)
	7(2)	9.75±1.30(7.5-12.0)	5.15±0.25(4.8-5.5)	27±0.71 (26-28)	
Idiopathic	0(4)	3.75±0.5(2.5-5.0)	1.56±0.21(1.06-2.1)	12.55±1.23 (9-14.7)	50.25±29.15 (20-152)
	3(1)	4.50±0.00 (4.5)	2.10±0.00 (2.1)	18.00±0.00 (18.0)	180.00±00.00 (0-180)
	7(1)	6.00±0.00 (6.0)	3.10±0.00 (3.1)	20.00±0.00 (20.0)	
Pyometra	0(2)	4.10±0.07 (4.0-4.20)	1.90±0.21 (1.60-2.20)	12.00±0.00 (12-12)	255.50±14.53 (50-461)
	3(2)	5.85±0.25 (5.5-6.2)	3.35±0.39 (2.8-3.35)	18.00±0.00 (18-18)	260.00±13.43(70-450)
	7(2)	7.50±0.35 (7.0-8.0)	4.15±0.74 (3.1-4.15)	25.00±0.70 (24-26)	
Haematuria	0(1)	3.00±0.00	1.20±0.00	7.00±0.00	250.00±0.00
	3(1)	5.00±0.00	2.80±0.00	12.00±0.00	220.00±0.00

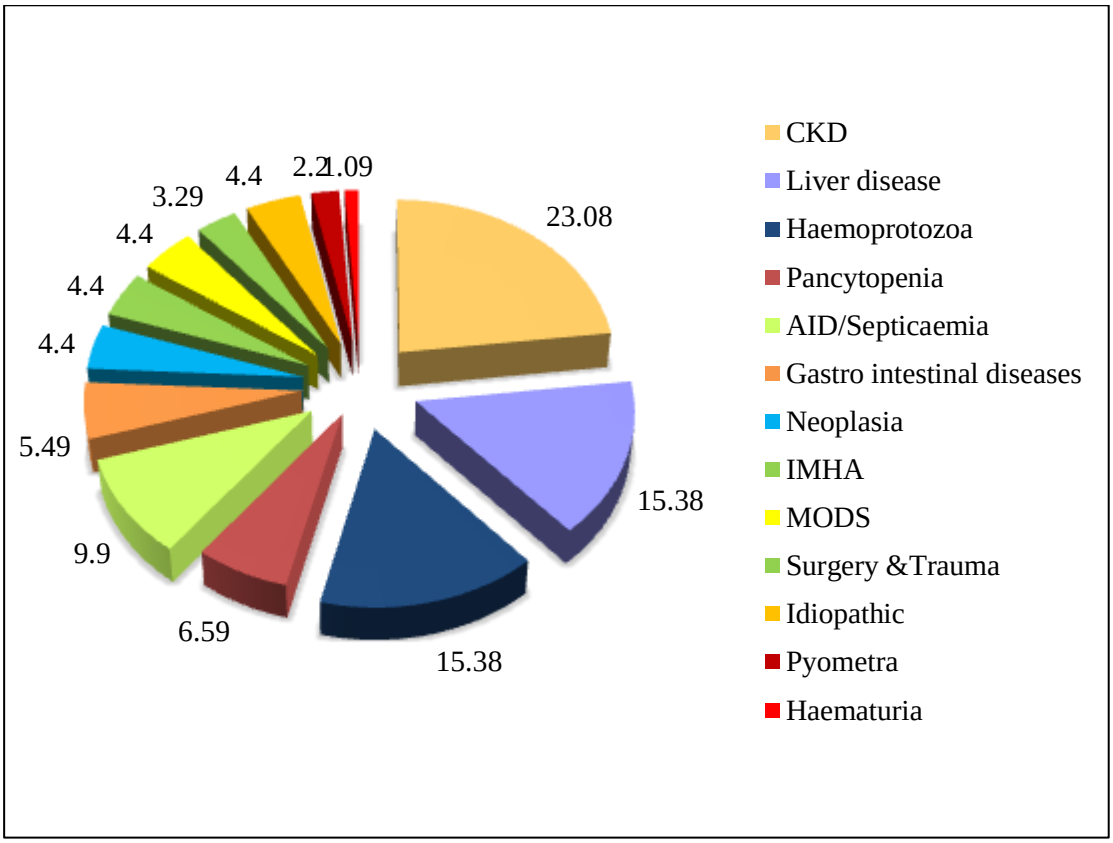


Fig. 5: Pie diagram showing etiology of anaemia in severely anaemic dogs

However, Singh et al. (2012) reported that prevalence of anaemia in dogs was high due to digestive disorders (30.76%) followed by ectoparasitic infestations (9.23%), hepatic insufficiency (9.23%), haemoprotozoan infection (7.69%) and renal disorder (1.57%). The haemoprotozoa was diagnosed in 15 cases of anaemia in dogs and *B. gibsoni* (n=8 57.14%) was the leading cause of anaemia in the current study, followed by *E.canis* (n=4 28.57%) and *H.canis* (n=2 14.29%). Liu and Su, (2015) also found *B.gibsoni* (83.7 percent, n = 36) as the most frequently diagnosed pathogen, followed by *E.canis* (11.6 %, n = 5), *B. canis* (2.3 %, n = 1), and Leptospira spp. (2.3%, n = 1). In their study as well cancer, infectious diseases, and renal failure are were most common causes of canine anaemia. Ognean et al. (2015) recorded coagulopathy (37.03%), haemorrhagic gastro-enteritis (29.62%), lymphoma or leukaemia (11.11%), trauma (14.81%), and kidney disease the most common causes of anaemia according to blood transfusion report (11.11%). In contrast to our study a retrospective analysis (n=41), by (Assarasakorn & Niwetpathomwat, 2006) noted parasitism (9.75%) thrombocytopenia (7.31%), surgery (4.87%), gastrointestinal disorder (4.87%), and pyelonephritis (4.87%) as major causes of anaemia. Similarly,

Useh et al. (2003), reported parasitic infestation (gastrointestinal and blood parasite) as 94.2 percent in cases of anaemia.

Means of haematological parameters revealed that IMHA had most severe effect Hb level (Hb 2.70±0.60 g/dL) followed by liver diseases (Hb 2.76±0.14 g/dL) and haemoprotozoan diseases (2.95±0.28) IMHA is considered to have a poor prognosis in dogs, with mortality rates of (50%–70%) (Klag et al., 1993 and Mason et al., 2003) and (30%–40%) (Goggs et al., 2015). Elhiblu et al. (2015) recorded anaemia in all the cases of liver diseases and that might be caused by a longer transit period of erythrocytes through the spleen due to decreased portal blood flow and/or the fragility of red blood cells due to elevated bile acid levels (Chikazawa et al.,2013; Rothuizen & Meyer, 2000; Bush, 2002). Gonde et al. (2017) observed significant decrease in the levels of Hb, PCV, TEC and thrombocytes indicating severe anaemia in *Babesia* positive dogs. Likewise, Brahma et al. (2019) also reported decreased levels of RBCs, Hb, PCV and platelets in canine babesiosis affected dogs. The destruction of circulating RBCs by auto antibodies which are directed against infected and non-infected red cell membranes resulting in intravascular and extravascular haemolysis. According to Taboada & Lobetti (2006), direct parasitic damage contributes to anaemia. Similarly, Das & Konar (2013) noted erythrogram changes in haemoprotozoan diaseases effected dogs were marked anaemia (80.95%), decreased RBC count and PCV. Xaxa & Kumar (2019) reported that the values of Hb, PCV, TEC, TLC, and total platelet count were significantly decreased in dog with *E.canis* infection. Similarly, Barman et al. (2014) recorded haemoglobin concentration 5.1 g/dL, RBC 3.13 m/mm3, thrombocyte count was 21 m/mm3 in dog that was affected with *E.canis.* Hasler (2012) observed severity of anaemia to be correlated with degree of renal failure and hence anaemia was severe in stage 3 and stage 4 renal diseases.

The highest improvement was recorded on 7th day of post-transfusion in the surgery and trauma group as (9.75±1.30) followed by the gastrointestinal diseases group (8.28±1.05) and haemoprotozoan group (Fig 6). The lowest improvement in the Hb indices was noted in pancytopenic group (5.30±0.36). The lowest improvement in this group was due to non-regenerative anaemia mainly and attributed to bone marrow depression. Following the trauma, the acute loss of blood was noted that needed an immediate transfusion as a supportive therapy. Similar to our findings

Chevallier & Love (2019) transfused blood during surgery in a dog and complete recovery was recorded after five days. Similarly, Xaxa and Kumar (2019) performed whole blood transfusion in severely anaemic *E.canis* positive dog and haemato-biochemical variables showed marked improvement after transfusion.

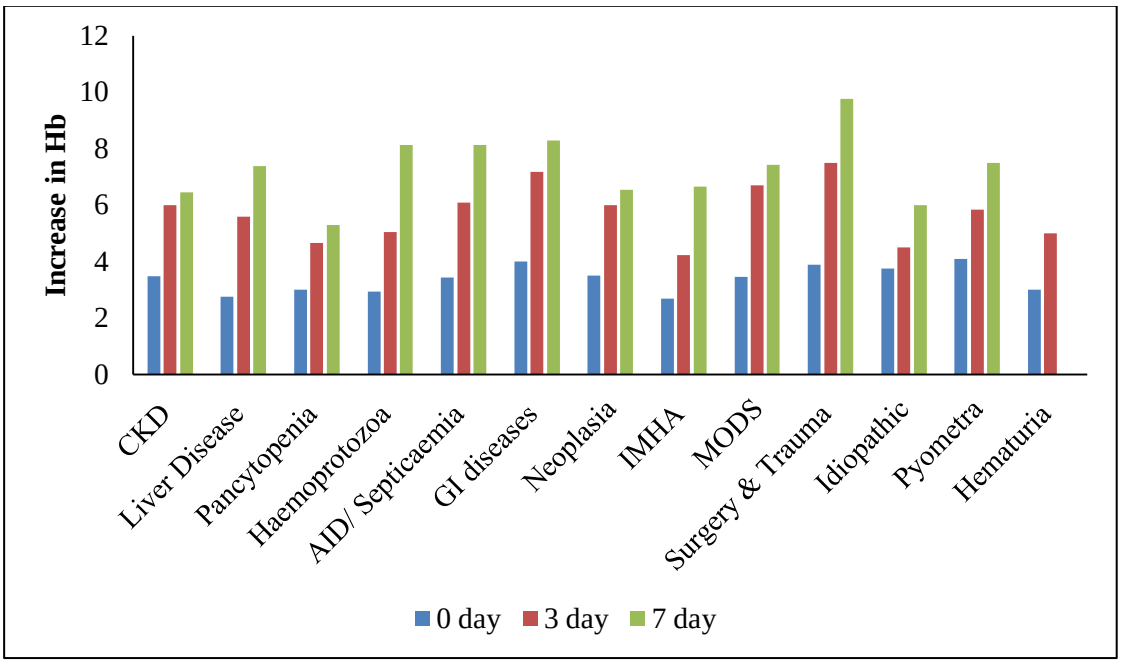


Fig. 6: Bar diagram showing improvement in Hb upon transfusion of whole blood or pRBCs in severely anaemic dogs

4.1.5 Evaluation of blood transfusion in severely anaemic dogs

In this study, blood transfusion was carried out in 91 dogs, while 26 cases in which donor dog could not be arranged served as a control group. Follow up on these dogs, either clinically or by enquiry, was carried out till one month after the initial presentation to ascertain their survival and haematobiochemical parameters, if possible.

4.1.6 Effect on haematological parameters

Table 8 shows a detailed analysis of haematological alterations upon blood transfusion. Over all, the initial mean Hb level was 3.28±0.10 g/dL which increased significantly ($P<0.005$) to 5.67±0.17 g/dL on the 3rd day and 7.28±0.22 g/dL on 7th day. ($P<0.005$). PCV showed a similar increase, with an initial value of 10.47±0.31 percent, followed by 19.03±0.54 percent on 3rd day and 23.41±0.70 percent on 7th day. TEC values increased post-transfusion, but TLC and platelet counts showed no

significant difference improvement at three day interval but decreased significantly ($p<0.05$) at seven day interval in pRBC transfusion group. The rise in Hb is almost similar to Pallavi (2019) observed mean Hb increase to 5.72±0.33 g/dL post-transfusion while Saini (2001) recorded a moderate increase. After the transfusion, Dietrich et al. (1990) found 2.2 g/ dL rise in Hb level. Sharma et al. (2015 b) transfused whole blood studied thrombocytopenic and anaemia cases of ehrlichiosis in canines and whole blood in canine ehrlichiosis cases for improvement in RBC and platelet counts. Kisielewicz et al. (2014) recorded that haemoglobin concentration, haematocrit increased significantly with transfusion while lactate concentration decreased significantly. The data clearly shows that blood transfusion has a beneficial impact on haematological parameters resulting in a substantial and radical improvement in the clinical condition of patient.

4.1.7 Comparison of Whole blood vs pRBCs transfusion

The patients given whole blood vs pRBCs (Table 8) were selected based on presentation of the cases .The patients with anaemia, thrombocytopenia and low levels of total plasma protein (TPP) were given whole blood while the patients with anaemia, normal platelet count and normal TPP were given pRBCs. The initial haemoglobin level in the whole blood group was 3.24±0.13 g/dL which increased significantly ($p<0.05$) to 5.51±0.21 g/dL on 3^{rd} day and 7.14±0.28 g/dL on 7^{th} day while the initial haemoglobin for pRBCs was 3.39±0.18 g/dL that increased significantly ($p<0.05$) to a higher level as compared to whole blood values (6.03±0.25 and 7.61±0.28 g/dL) on 3^{rd} and 7^{th} day respectively. Silvestrini et al. (2011) transfused pRBCs in dogs. The PCV before transfusion was 14.7 percent which increased to 21 percent post-transfusion and mean increase in PCV was 6.5 percent. Callan et al. (1996) reviewed transfusion of 658 units of RBCs, including 474 (72%) units of packed red blood cells (PRBCs) and 184 (28%) units of whole blood (WB) with the etiologies of haemorrhage, haemolysis and ineffective erythropoiesis, the modified mean post-transfusion PCV of dogs showed an increasing trend from (21±0.4, 13±0.9 and 18±0.9%) to (32±0.06, 30±1.3 and 31±1.3%) for haemorrhagic, haemolytic and in affective erythropoiesis cases of anaemia as compared to pre-transfusion PCV mean.

Table 8: Overall evaluation of anaemic dogs upon transfusion of whole blood or pRBCs

Haematological parameters	Whole Blood or pRBCs	Day 0	Post transfusion	
			Day 3	Day7
Haemoglobin (g/dL)	Whole Blood	3.24±0.13[a] (0.90-5.00)	5.51±0.21[a] (2.30-11.40)	7.14±0.28 [a](1.80-13.00)
	pRBCs	3.39±0.18 [a] (1.50-5.00)	6.03±0.25 [a] (1.50-7.10)	7.61±0.28 [a] (5.20-11.30)
	Total	3.28±0.10[a] (0.90-5.00)	5.67±0.17[a] (1.50-11.40)	7.28±0.22 [a](1.80-13.00)
PCV (%)	Whole Blood	10.65±0.39[a] (3.10- 15.0)	18.92±0.63[a] (9.0-28.00)	23.06±0.92[a] (4.00-35.00)
	pRBCs	10.05±0.51 [a] (4.00-15.00)	18.53±1.25 [a] (5.00-30.4)	22.28±1.77 [a] (16.4-32.00)
	Total	10.47±0.31 [a] (3.10-15.00)	19.03±0.54 [a] (5.00-30.4)	23.41±0.70 [a] (4.00-35.00)
TEC($\times 10^6$/ µl)	Whole Blood	1.64±0.07 [a] (0.81-5.20)	2.74±0.13 [a] (0.81-5.20)	3.41±0.17 [a] (0.66-5.5)
	pRBCs	1.80±0.11[a](0.83-3.4)	3.52±0.16 [a] (1.5-5.29)	4.64±0.18 [a] (3.2-6.2)
	Total	1.69±0.06 [a] (0.50-4.20)	2.98±0.11 [a] (0.81-5.29)	3.76±0.14 [a] (0.66-6.20)
Platelet($x10^3$/µl)	Whole Blood	123.11±16.01(3-537)	157.92±18.28 (12-742)	-
	pRBCs	299.40±26.38 (34.00-539)	321.00±24.13(45-460)	-
	Total	182.20±16.35 (3-539)	209.53±16.97 (12-742)	-
TLC($\times 10^3$/µl)	Whole Blood	15.27±1.63 (0.22-56.00)	16.39±1.63 (0.16-45.00)	14.71±1.29 (0.11-35.00)
	pRBCs	25.37±2.85 [a] (7.00-63.15)	23.32±2.82 [a] (5.47-58.00)	16.03±2.82 [a] (8.00-35.00)
	Total	18.38±1.54(0.22-63.51)	18.58±1.50 (0.16-58.00)	15.09±1.03 (0.11 -35.00)

Figures in parenthesis indicate range. Number of transfusions at 0, 3 and 7 days were 63, 56, and 45 for whole blood transfusion; and 28, 25 and 18 for pRBCs. Values in columns with same superscript differ significantly ($p<0.05$)

4.1.8 Mean increase in Hb and PCV values post blood transfusion

A total of 65 transfusions were done in 63 dogs with whole blood intravenously and cases were divided into five groups based on body weight and dose rate of blood transfusion, as shown in Table 9. In our study highest increase was recorded in dogs with a body weight of 10 -20 kg in which the mean Hb and PCV increased to 6.15±0.44g/ dL and 19.54±0.92 percent transfusing 15.23 mL/kg Bwt., followed by 0-5 kg group 5.24±0.61 and 19.50±1.66 Most of the literature recommends that 2mL/kg Bwt. increases the PCV by one percent. Ognean et al. (2015) observed a 10.43 percent increase in PCV after transfusing 7.5 mL/kg whole blood while Chiaramonte (2004) recorded a 10 percent increase after transfusion of 20 mL/kg blood, and Assarasakorn & Niwetpathomwat (2006) recorded a 15.2 percent increase after transfusing 29 mL/kg whole blood. Transfusion of RBCs increased PCV in all groups, according to Callan et al. (1996), larger mean volume 33mL/kg Bwt. of WB administered had similar oxygen carrying capacity compared to the 19mL/kg Bwt. of pRBCs as PCV of WB is approximately 45-50 percent and that of pRBCs is about 75-80 percent.

Table 9: Increase in Hb and PCV according to dose rate of whole blood transfusion

Body wt (kg)	No. of dogs	Whole Blood (mL/ kg body wt)	Initial		Final (3rd day)	
			Hb (g/dL)	PCV (%)	Hb (g/dL)	PCV (%)
0-5 kg	8	16.34±0.55	3.21±0.21	10.81±0.96	5.24±0.61	19.50±1.66
5-10 kg	12	15.27 ±0.12	2.81±0.35	9.97±1.04	5.53±0.39	19.46±1.65
10-20 kg	16	15.23 ±0.20	3.33±0.26	10.95±0.91	6.15±0.44	19.54±0.92
20-30 kg	26	13.71±0.22	3.42±0.20	10.93±0.55	5.27±0.30	17.86±0.99
30-40 kg	1	11.56±0.00	2.50±0.00	9.80±0.00	4.40±0.00	18.00±0.00

4.1.9 Adverse reaction in dogs post transfusion

In this study, a total of two (2.19%) cases showed immediate transfusion reaction including hyperthermia, tachypnea and tachycardia. Callan et al. (1996) documented hyperthermia and emesis as the main transfusion reactions while as, Kerl and Hohenhaus, (1993) reported that acute reactions are characterized by emesis,

haemolysis, haematuria and jaundice. The frequency of the acute adverse reactions due to transfusions varies between different studies 3 to 8 percent according to Abrams-Ogg (2003) (7.6 %), Kerl and Hohenhaus (1993) (0.3 %), Harrell et al. (1997) and Callan et al. (1996) and (4.18 %) (Assarasakorn & Niwetpathomwat, 2006). Low level of adverse reactions in our study is maybe due to the fact that all of the cases except 1 dog had their first transfusion or it might be due to the fact that all dogs were crossmatched before transfusion or administration of diphenhydramine before transfusion contributes to decrease of inflammatory reactions.

There was no report of delayed adverse reaction confirmed by post-transfusion haemolysis check card (purchased from Korean Animal Blood Bank 513-86, Dongmyong-Dong, Sokcho-Si, Gangwfsondo, 217-020, Korea) on 3rd day after transfusion.

4.1.10 Effect of blood transfusion on survival

The comparison between the survival percentage and period of survival was carried out between transfusion and non-transfusion groups.

4.1.11 Comparison of survival between transfusion and non-transfusion cases

Table 10 shows that transfusions were done in 63 cases with whole blood, 45 (71.43%) survived while 18 (28.57%) could not survive. While 28 anaemic dogs were transfused with pRBCs out of which 18 (64.29%) survived and 10 (35.71%) could not survive. On the other hand out of 26 non-transfusion cases, 9 (34.62%) survived and 17 (65.38%) did not survive (Fig 7). Overall survival in transfusion cases (69.23%) was higher than in non-transfusion cases (34.62%). These findings clearly demonstrate the beneficial effects of transfusion in anaemic dogs when diagnosis is made and therapeutic effect is achieved. The survival rate of our study is similar to Ognean et al. (2015) who recorded recovery percentage as (66.6%) within 24 hour after transfusion. Silvestrini et al. (2011) reported (70%) recovery rate and Jutkowitz et al. (2002) recorded 47 to 61 percent. Waldrop et al. (2003) reported the survival on the basis of hospitalization as mean of 4 days (range, 1 to 23 days) in which 16 (29%) of the 55 dogs could not survive till discharge; 12 were euthanized and four died later so survival rate in that study is much lower than our study. It was observed that survival chances significantly increased with blood transfusion. Similarly, Lackritz et al. (1992) in a study between transfusion and non-transfusion groups reported that

blood transfusion in patients having Hb <5g/dL decreases the incidence of mortality. This all was attributed to the fact that transfusion extends the survivability of the patient till the diagnosis and treatment is done.

Table 10: Comparison of survival in transfusion and non-transfusion cases

Survival on basis of etiology	Whole blood (n=63,Mean Hb 3.24±0.13)	pRBCs (n=28,Mean Hb 3.39±0.18)	Total cases of anaemia (n=91,Mean Hb 3.28±0.10)	Non-transfusion (n=26, Mean Hb 3.20±0.23)
Day 0	63(100.00%)	28(100.00%)	91(100%)	26(100.00%)
3 Days	56 (88.88%)	25(89.28%)	81(89.01%)	20(76.92%)
7 Days	45(71.43%)	18(64.28%)	63(69.23%)	9(34.62%)

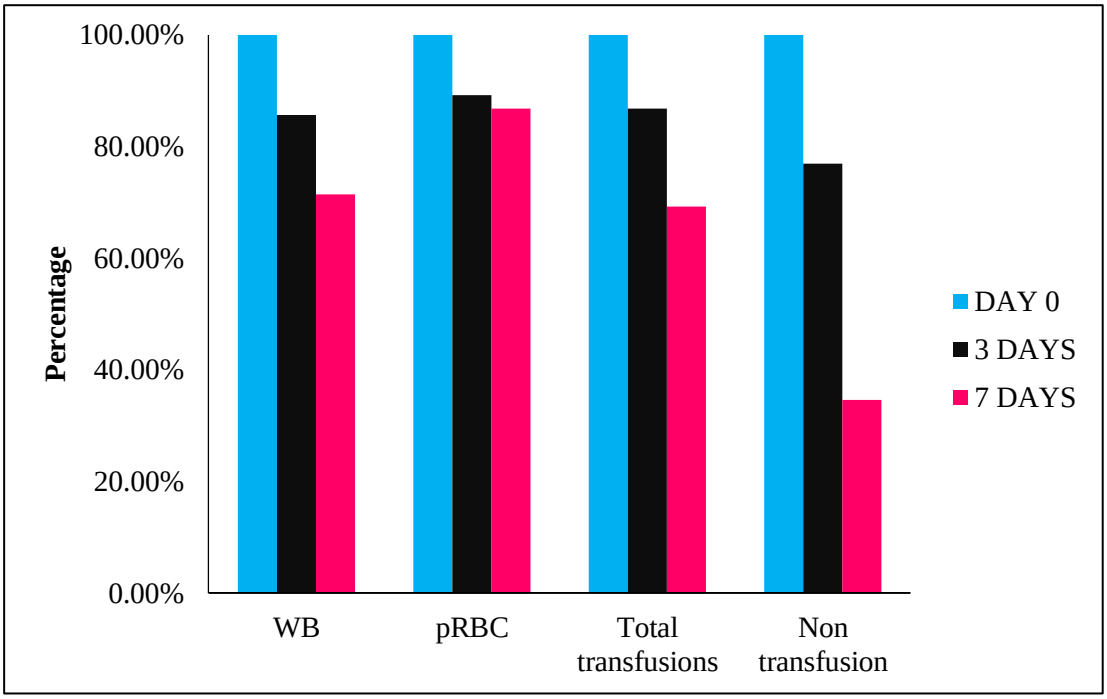


Fig. 7: Bar diagram showing the comparison of survival in transfused and non-transfused dogs

4.1.12 Comparative survival upon blood transfusion based on etiology

Table 11 shows survival among transfusion and non-transfusion cases on basis of etiology. In our study the highest survivability was recorded in gastrointestinal diseases, IMHA, neoplasia and pyometra. The survivability was higher in these diseases attributing to acute loss of blood and regenerative anaemia in these disorders. However, good improvement was noted in chronic conditions like tumour, chronic

kidney diseases as blood transfusion provides the immediate oxygen delivery improving the clinical conditions of transfused dogs. It is evident that blood transfusion has increased survival in all the etiology groups. Blood transfusion has been shown to improve survival in all etiology groups.

Table 11: Survival on basis of etiology in transfusion and non-transfusion cases

Etiology	Transfusion	Non-transfusion
CKD (26)	13/21 (61.90)	2/5 (40.00)
Liver disease (18)	12/14 (85.71)	2/4 (50.00)
Pancytopenia (10)	2/6 (33.33)	1/4 (25.00)
Haemoprotozoa (15)	8/14 (57.14)	0/1 0.00)
GI diseases (8)	5/5 (100.00)	1/3 (33.33)
AID/Septicemia (9)	7/9 (77.78)	0/0 (0.00)
Neoplasia (6)	4/4 (100.00)	1/2 (50.00)
IMHA(6)	4/4 (100.00)	0/2 (0.00)
MODS (6)	3/4 (75.00)	1/2 (50.00)
Surgery & Trauma (3)	2/3 (66.67)	0/0 (0.00)
Idiopathic (6)	1/4 (25.00)	0/2 (0.00)
Pyometra (3)	2/2 (100.00)	1/1 (100.00)
Haematuria(1)	0/1 (0.00)	0/0 (0.00)

4.2 Observation on severely thrombocytopenic dogs

4.2.1 Clinical signs

Melena was found to be most significant signs of severe thrombocytopenia (75.00%) followed by epistaxis (58.33%) and ecchymotic haemorrhages/purpura (50.00%) (Plate 10) (Table 12) (Fig 8). Kohn et al. (2000) concluded that classic signs of thrombocytopenia includes petechiation, ecchymosis, epistaxis, and gastrointestinal blood loss and the most severe thrombocytopenias, are due to Immune mediated

thrombocytopenia (IMT) often cause only mild haemorrhage. Kohn et al. (2000) recorded that clinical signs of haemorrhage were observed in 12 of 15 dogs with pITP and in 6 of 9 dogs with Evans' syndrome. Thrombocytopenia is the most commonly acquired haemostatic disorder in dogs and can become potentially life-threatening (Grindem et al., 1991; Bommer et al., 2008). The highest prevalence of thrombocytopenia was recorded for Labrador retriever (41.67%) followed by mixed breeds (25.00%) and German shepherd (16.67%) (Table 13). This finding can be attributed to over representation of Labrador retriever dogs at the hospital. However, Botsch et al. (2009) recorded the highest prevalence of thrombocytopenia in German shepherd followed by Bernese mountain dog and Golden retriever. However, according to Grindem et al. (1991) Doberman Pinschers were most presented breed. The difference in these findings can be attributed to the presentation of different breeds in a particular geographic area.

Table 12: Clinical signs in severely thrombocytopenic dogs

Mucous membrane	Severely pale 1(8.33)
	Pale 2(16.66)
	Salmon pink 9(75.00)
General state	Recumbent 1(8.33)
	Able to stand but lethargic 3(25.00)
	Active 8(66.66)
Pulse quality	Weak 1(8.33)
	Bounding 4(33.33)
	Normal(58.33)
Haematochezia	2(16.66)
Melena	9(75.00)
Epistaxis	7(58.33)
Ecchymotic haemorrhages/Purpura	6(50.00)
Ticks	3(25.00)

Plate 10: Petechiation on abdomen due to severe thrombocytopenia

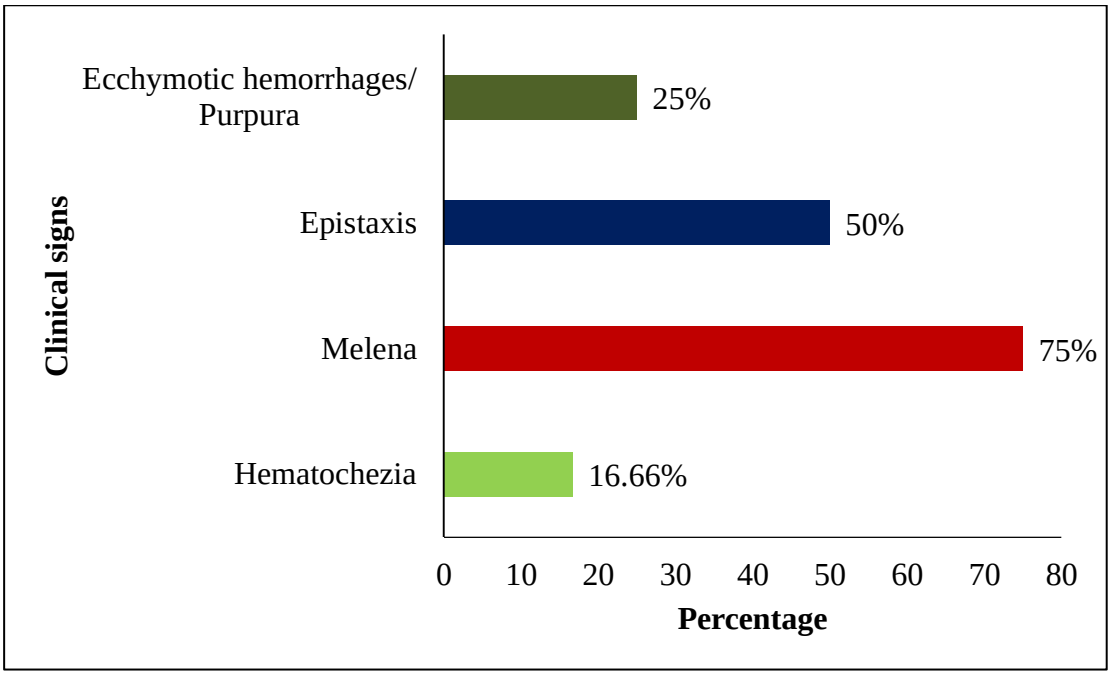


Fig. 8: Bardiagram showing clinical signs of severely thrombocytopenic dogs

Table 13: Epidemiological data analysis in severely thrombocytopenic dogs

Breed	
Labrador Retriever	5(41.67)
Other breeds	3(25.00)
German Shepherd	2(16.67)
Beagle	2(16.67)
Sex	
Male	11(91.67)
Female	1(8.33)

4.2.2 Haematological and biochemical alterations in thrombocytopenic dogs

A total of 12 thrombocytopenic cases were included in the study which were divided on the basis of etiology into three groups; the highest number of cases were recorded in haemoprotozoan group (50.00%) followed by renal failure (41.67%) and a single case of neoplasia (Table 14). Severe thrombocytopenia in dogs is mostly caused by immune-mediated thrombocytopenia; however, low platelet counts are also commonly associated with inflammatory, infectious or neoplastic diseases (Bommer et al., 2008; Botsch et al., 2009).

Table 14: Haematological and biochemical alterations in severely thrombocytopenic dogs based on etiology

Disease	Hb (g/ dL)	PCV (%)	TEC ($\times 10^6$/ µl)	Platelet ($\times 10^3$/ µl)	ALT (IU/L)	Total protein (g/ dL)	Albumin (g/ dL)	BUN (mg/dl)	Creatinine (mg/ dL)
CKD (n=5)	6.36±0.56 (4.2-7.80)	19.64±1.81 (12.8-25.00)	3.91±0.38 (2.33-4.80)	18.80±6.86 (6.00-48.00)	69.6±8.6 (50.00-104.00)	6.40±0.29 (5.50-7.50)	2.40±0.16 (1.80-2.90)	152.2±22.10 (96.00-245.00)	10.94±1.44 (5.70-15.00)
Haemo-protozoan (n=6)	6.20±0.64 (3.20-7.90)	19.07±1.78 (12.20-24.50)	3.52±0.45 (1.20-4.40)	13.00±3.89 (3.00-27.00)	42.67±3.96 (30.00-56.00)	6.55±0.17 (6.00-7.00)	2.43±0.08 (2.20-2.70)	23.83±1.98 (18.00-30.00)	0.97±0.83 (0.60-1.20)
Neoplasia (n=1)	6.10±0.00	18.90±0.00	3.54±0.00	2.00±0.00	50.00±0.00	6.50±0.00	2.50±0.00	15.00±0.00	0.80±0.00

The blood smear examination revealed no haemoprotozoa however, six patients were positive for haemoprotozoa on PCR including one case of *B. gibsoni* and five were positive for *E.canis*. The acute thrombocytopenia could be due to increased platelet consumption due to inflammatory changes in blood vessel endothelium, increased splenic sequestration of platelets, and immunologic destruction or damage resulting in a substantially reduced platelet life (Pierce et al., 1977 and Kakoma et al., 1978). All the thrombocytopenic groups showed moderate anaemia with low PCV and TEC. Similar findings were recorded by De Gopegui et al. (2007) reported that all dogs with babesiosis had thrombocytopenia and 20 percent had disseminated intravascular coagulation syndrome. Furlanello et al. (2005) also found that dogs presented with babesiosis had thrombocytopenia along with hyperfibrinogenemia and anaemia of variable severity. Birkenheuer et al. (1999) recorded the clinical signs of *Babesia* range from severe haemolytic anaemia and thrombocytopenia to subclinical infections. Bulla et al. (2004) reported thrombocytopenia in 146 samples (less than 200 000/mL) infected with *E.canis.* Similar to our study, Waner et al. (1995) concluded that thrombocytopenia is considered to be the most common and consistent haematological abnormality of dogs naturally or experimentally infected with *E. canis*. Smith et al. (1975) reported that platelet survival time decreased from a mean of 9 days to 4 days, 2 to 4 days after infection with *E. canis*. Thongsahuan et al. (2020) noted the similar findings as in our study as haematological alterations caused by Ehrlichia infections include anaemia, thrombocytopenia, monocytosis, and eosinophilia. Jacobson and Clark (1994) recorded haemolytic anaemia and thrombocytopenia as the predominant feature of canine babesiosis. Mechanisms of RBCs destruction include increased osmotic fragility, shortened RBC life span, and erythrophagocytosis.

In the current study, thrombocytopenia was recorded in patients with chronic kidney diseases. Supriya (2019) also observed a lower than normal level of platelet count in 24 dogs out of 90 dogs with renal failure. Dorgalaleh et al. (2013) also observed the presence of anaemia and thrombocytopenia in patients with renal failure. However, Mann, (2013) found the mean total platelet count to be $145.12\pm20\times10^3$/cu mm in dogs suffering from renal failure and reported thrombocytopenia in 10 out of 46 dogs. Devipriya et al. (2018) found that 77 dogs of the 150 with renal insufficiency had significantly lower PCV, Hb, TEC and higher TLC and high levels of BUN and creatinine. Mann, (2013) recorded the mean BUN to be 134.65±14.27 mg/ dL in renal

failure dogs. Singh, (2017) also found high levels of BUN (58.44 mg/ dL) in renal failure dogs, and Srinivasan et al. (1993) also reported high mean BUN (78.18 mg/d dL) in renal failure dogs. BUN elevation is associated with increased protein catabolism, oliguria, vomiting, or gastrointestinal bleeding (Bartges and Polzin, 2011).

In our study, the ALT and BUN in haemoprotozoan patients were unaffected. However, in contrast to our study, Boozer and Macintire (2003) noted the increased serum ALT, creatinine and BUN levels. Our results were in contrast to Burghen et al. (1971) & Harrus et al. (1999). They recorded hypoalbuminemia, hyperglobulinemia, and hypergammaglobulinemia are the predominant biochemical abnormalities found in dogs infected with *E.canis.* Our findings could be due to the reason that all the cases in our study had acute onset and presented immediately.

4.2.3 Evaluation of platelet transfusion in severely thrombocytopenic dogs

Platelet transfusion was carried out in 12 cases and follow up on these dogs either was carried out for about week after initial presentation to evaluate change in platelet counts at different intervals and to ascertain their survival. The platelet count showed an increasing trend within 1 hour of transfusion (Table 15 and Fig. 9). The platelet counts were significantly increased at 1 hour 3rd day and 7th day in haemoprotozoan group ($p<0.05$) while as non-significant increase was recorded in other two groups. The highest trend in increase was noted in haemoprotozoan group Callan et al. (2009) found that canine platelet apheresis allows collection of a concentrate with an elevated platelet yield, typically 3–4.5 $\times 10^{11}$ vs $<1\times 10^{11}$ for whole blood-derived platelets, improving the ability to provide sufficient platelets.

Table 15: Evaluation of thrombocytopenic dogs upon transfusion of platelet rich plasma

Disease	Pre Transfusion count	1 hour	3 day	5 day
CKD	18.80±6.86 (6-48)	29.25±9.09 (14-60)	34.00±2.55^{a} (28-40)	50.00±1.25^{a} (30-80)
Haemoprotozoan	13.00±3.89 ^{a} (3-27)	29.00±5.18 ^{a} (15-45)	132.50±15.15 ^{a} (80-150)	237.50±27.20^{a} (150-300)
Neoplasia	2.00±0.00	18.00±0.00	30.00±0.00	60.00±0.00
Total	14.50±3.87	28.00±4.83	77.33±18.89	145.00±38.21

***Mean platelet count of donor dogs was** 403.75±13.92 (Range 300-480$\times 10^{3/}$ µl)
Values in columns with same superscript differ significantly ($p<0.05$)

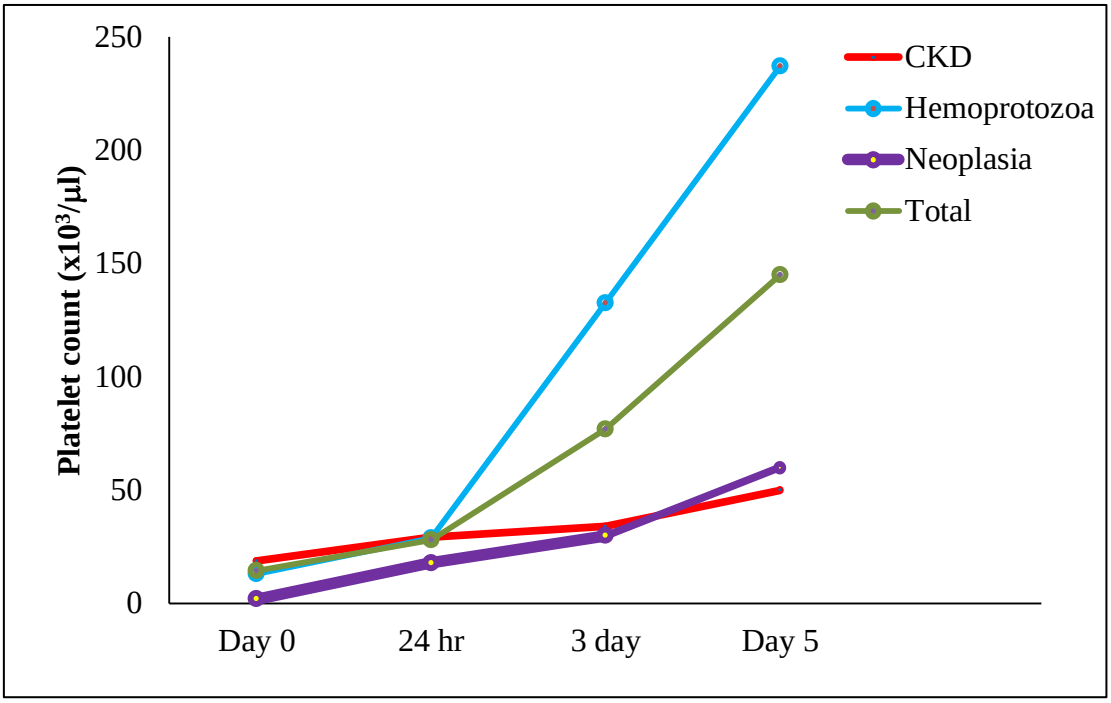


Fig. 9: Line diagram showing increase in platelet count after transfusion of platelet rich plasma

Abrams-Ogg et al. (1993) stated that platelet concentrates are beneficial to increase the platelet counts, if available. Crystal and Cotter (1992) reported increase of 13,000 platelets/ µL may be obtained by transfusing 12.5 mL/kg body weight of fresh whole blood from a donor with a platelet count of 200,000/µL. Abrams-Ogg, (2003) the recommended dose of PRP and PC to be 1 platelet unit per 10 kg Bwt. that should raise the platelet count by a maximum of 40,000/ µL.

4.3 Haematological and biochemical alterations in hypoproteinemia patients

A total of 10 cases with hypoproteinemia (total protein and albumin <4.5g/d dL or 2.0g/ dL) were included in the study, which were divided on the basis of etiology into three groups (Table 16). The highest number of cases were recorded in chronic liver disease (50.00%) followed by canine parvo viral enteritis (CPV) (30.00%) and acute hepatitis (20.00%). Haemoglobin, platelet count, AST, ALT and ALKP varied significantly ($p<0.005$) among these three groups. Plasma protein abnormalities are a significant biochemical finding that triggers various clinical and systemic effects and is associated with a number of disease processes. The levels of haemoglobin, PCV and platelet were low in the current study suffering from chronic liver diseases.

Table 16: Haematological and biochemical alterations in hypoproteinemic dogs based on etiology

Disease	Hb (g/dL)	PCV (%)	Platelet ($\times 10^3$/ µl)	ALT (IU/L)	AST (IU/L)	ALKP (IU/L)	Total protein (g/dL)	Albumin (g/dL)
Acute hepatitis (n=2)	8.30±0.70[a]	23.60±1.83	225.0±17.68 [a]	375.0±17.68[a]	280.0±35.36[a]	550.0±35.36[a]	4.35±0.10	1.90±0.07
Chronic liver disease (n=5)	7.14±0.23[a]	19.60±0.47	161.40±6.50 [a]	58.40±7.14[a]	77.4±3.50[a]	144.4±23.15[a]	4.32±0.06	1.76±0.04
CPV (n=3)	7.50±0.04[a]	22.33±0.72	290.00±30.92[a]	-	-	-	4.20±0.04	1.76±0.05

Values in rows with same superscript differ significantly ($p<0.05$)

Similar findings were observed by Elhiblu et al. (2015) who recorded that levels of haemoglobin, lymphocytes, and platelet count were significantly lower in liver cirrhosis group, while total protein and albumin were significantly lower. Canine parvo viral enteritis group had moderate anaemia with Hb (7.5±0.04) g/ dL this is due to blood loss in feces. Besides, the virus affects bone marrow and is cytotoxic to haematopoietic cells, causing myeloid and erythroid hypoplasia in the early stages of infection. Similar, findings were noted by Shah et al. (2013) who noted moderate anaemia in CPV patients with mean Hb (7.31 ± 0.58) g/ dL in CPV dogs. Weiss et al. (1999) recorded anaemia, leukocytopenia, neutropenia and thrombocytopenia in 10 cases of canine parvovirus. CPV enteritis group had low levels of albumin and total protein (4.50±0.16 and 1.76±0.02)g/ dL

Similar findings were supported by (Ramprabhu et al., 2002 and Dharmadheeran et al., 2003). Shah et al. (2013) recorded mean total protein of 4.68 ± 0.58 g/dL and albumin level 2.55 ± 0.46 g/dL in CPV patients. Hypoproteinemia may be caused by serum protein leakage through impaired capillaries in the intestine's villi and reduced absorption through the damaged villi. Biswas et al. (2005) & Sagar et al. (2008) also reported protein loss via the gastrointestinal tract and hypoalbuminemia was found more specific in canine parvovirus. Anorexia, vomiting, and diarrhoea causing fluid and electrolyte loss in CPV-infected dogs results in hypoalbuminemia and a reduction in plasma proteins throughout the disease. These were primarily due to a combination of intestinal haemorrhage followed by rehydration.

4.3.1 Evaluation of plasma transfusion (total protein and albumin in severely hypoproteinemia dogs

In this study, plasma transfusion was carried out in 10 cases and follow up was carried out for about a week after the initial presentation to determine the change in total protein and albumin. The total protein and albumin were monitored at 3rd and 7th day (Table 17). The concentration of total protein and albumin showed a beneficial effect in all groups on day three. However, both dogs with acute hepatitis collapsed before day seven, while in dogs with chronic liver diseases, total protein and albumin levels remained the same as on day three. Patients with CPV showed the best improvement in terms of TP and albumin levels and clinically. Patients with acute hepatitis collapsed within the 5th days of treatment and all three patients with CPV

improved completely within 5^{th} days of treatment. This study was in accordance with Logan et al. (2001) who administered fresh frozen plasma in 74 dogs and fifty (68%) dogs were alive at the time of discharge from the hospital they concluded that fresh frozen plasma plays an important role in the cure of critically ill dogs. Moore (1998) suggested that the doses of fresh frozen plasma for albumin replacement should range from 6 to 10 mL/kg given every 8 hours to increase albumin concentration by 0.5 g/dL. However, Snow et al. (2010) conducted a study on plasma transfusion in 298 dogs. They concluded that fresh frozen plasma administration for the treatment of hypoalbuminemia or pancreatitis was associated with a significant reduction in prothrombin time and activated partial thromboplastin time yet did not significantly alter albumin concentration. Similarly, Weatherton and Streeter (2009) concluded that the mortality rate for dogs receiving plasma was higher than those that did not receive FPP and no benefit of FFP administration was noted. Desborough and Stanworth (2012) concluded that plasma is effective in patients with coagulation factor defects but higher doses of fresh frozen plasma (15-30 mL/kg) are needed to provide adequate factors to reverse coagulopathy from Von Willebrand diseases or Haemophilia A in dogs (Stokol and Parry, 1998).

Table 17: Evaluation of plasma therapy in hypoproteinemic dogs

Disease	**Pre transfusion**		**Post transfusion day 3**		**Post transfusion day 7**	
	Total protein	**Albumin**	**Total protein**	**Albumin**	**Total protein**	**Albumin**
Acute hepatitis	4.35±0.10	1.90±0.07	5.06±0.00	1.80±0.00	0	0
Chronic liver disease	4.32±0.15	1.76±0.07	4.90±0.08	1.85±0.03	4.77±0.05	1.75±0.04
CPV	4.20±0.05	1.76±0.02	5.13±0.10	2.00±0.09	6.23±0.05	2.30±0.05

4.3.2 Adverse reaction in dogs post transfusion

No adverse reaction to the plasma transfusions were recorded in the current study that might be due to the fact in the current study received the transfusion therapy for the first time.

4.4 Coagulation factor levels in healthy and diseased dogs

The liver is required to maintain haemostasis as the liver produces pro-coagulant, anti-coagulant, and fibrinolytic proteins. Liver parenchymal cells synthesize most of the clotting factors, including fibrinogen and factors II, V, VII, IX, X, XI, and XIII, whereas factor VIII is thought to be produced mainly within the liver vascular endothelium (Mischke et al., 2003). In the current study, coagulation factors II, V, VII, VIII, IX, X and vWF were studied in trauma (n=10), diabetes mellitus (n=10), bleeding patients (n=10), acute hepatitis (n=5) and chronic liver disease (n=15). (Table 18).

Studies on coagulation factors in veterinary are not available. The levels of coagulation factors were healthy serum, healthy plasma (n=20). The severity of coagulation disorders is determined by the level of liver dysfunction. Patients with hepatic failure may have a wide range of factor deficiencies, as well as they may develop DIC. Patients with liver cirrhosis have the most serious changes in humans (Kemkes-Matthes et al., 1991; Mammen, 1992). Our study found that all the coagulation factors decreased in chronic liver disease. However, vWF was not decreased as hepatocytes produce fibrinogen, prothrombin and factors V, VII, IX, X, XI and XIII, as well as are responsible for the activation of the vitamin K-dependent factors (II, VII, IX and X) and protein C (Prater , 2000). Factor II was detected at high levels as compared to other coagulation factors. Prins et al. (2010) determined the mean value of Factor II as 83 (68–95) that is slightly lower than our study. The level of coagulation factor II in our study was higher in acute hepatitis and low in chronic hepatic diseases or cirrhosis. Our results show that FII, the main cause for hypercoagulable state, is directly induced by FX and FVIII in normal individuals and by FX, FXI, FV and vWF cofactors in diabetic patients. Elmahgoub et al. (2014) reported that in human diabetes mellitus, FII neither increases in concentration (more than 10mg/dL) nor is a risk factor for vascular events.

The activity of factor X was lower in diabetes in dogs in our study. Similar findings were seen in human diabetes patients by (Ostermann &Van de Loo 1986 & Ceriello, 1993). Hepatic degeneration in dogs was associated with a decrease in factor XI, hepatic cirrhosis with a decrease in factors IX, X, and XI and an increase in von Willebrand's factor, while hepatic neoplasia was associated with a reduction in

Table 18: Coagulation factor levels in healthy and diseased dogs

	Healthy serum (n=20)	Healthy plasma (n=20)	Trauma (n=10)	Diabetes mellitus (n=10)	Bleeding patients (n=10)	Acute hepatitis (n=5)	Chronic liver disease(n=15)
FACTOR II	91.68±9.17 (21.5-158.80)	104.08±11.98 (37.26-187.65)	91.04±15.80 (33.80-198.80)	150.50±19.98 (25.34-227.65)	79.28±24.11 (39.19-281.11)	182.65±39.24 (88.80-306.0)	50.39±4.62 (2.65-71.88)
FACTOR V	38.19±5.62 (7.44-111.62)	44.43±9.11 (14.72-179.77)	41.59±9.69 (12.10-127.34)	50.26±25.95 (7.64-294.14)	27.31±3.02 (13.75-51.52)	131.01±52.53 (31.52-299.77)	21.45±1.52 (5.31- 29.97)
FACTOR VII	8.94±3.20 (0.28-29.26	23.17±8.89 (1.70-88.37)	17.87±8.03 (2.00-28.00)	6.04±2.06 (1.02-13.01)	59.25±0.88 (58.37-60.13)	62.12±15.08 (43.05-81.18)	19.33±9.24 (10.09-28.58)
FACTOR VIII	81.83±18.29 (35.76-280.38)	88.70±19.83 (42.11-156.92)	64.19±20.29 (15.00-105.76)	48.73±15.41 (8.46-110.57)	95.26±30.12 (10.96-454.80)	168.80±75.49 (67.30-454.80)	55.20±14.25 (84.23-36.53)
FACTOR IX	61.31±14.04 (12.04- 229.08	75.49±14.78 (18.34-290.44)	33.19±5.13 (11.06-58.71)	41.58±10.14 (1.18-114.64)	54.72±22.77 (14.51-54.72)	102.02±63.20 (24.04-351.55)	12.44±1.15 (4.39-19.58)
FACTOR X	18.71± 4.08 (3.73-68.98)	31.62±6.03 (5.52-100.95)	9.99±1.46 (3.33-17.68)	12.52±2.89 (0.35-34.52)	63.92±20.07 (4.37-108.68)	30.06±17.21 (3.92-105.85)	3.98±0.42 (1.32- 7.23)
vWF	6.30±1.70 (0.24-20.24)	10.08±6.22 (0.71-120.13)	11.49±6.18 (0.19-54.35)	23.74±19.23 (0.24-138.05)	3.97±1.311 (0.47-12.50)	3.05±0.80 (1.23-5.47)	15.27±13.02 (0.01-145.39)

factor VIII and an increase in von Willebrand's factor (Prins et al., 2010). In our study, von Willebrand factor was found to be the lowest among all the coagulation factors however, in trauma patients and diabetic patients, it was at higher levels, while chronic liver diseases had not much effect on vWF. vWF is produced by endothelial cells and megakaryocytes and is stored in the alpha granules in platelets and in special organelles called Weibel-Palade bodies in endothelial cells. Both alpha-granules and Weibel-palade bodies serve as intracellular storage organelles of vWF. Dogs have a very small percentage (3%) of vWF in platelets as compared to cats or human beings (20%) (Waters et al., 1989; Parker et al., 1991). In our study, diabetic patients had higher level of vWF factor similar to reported by Ceriello (1993), Madan et al. (2010) and Chen et al. (2013) in human diabetic patients. Prins et al. (2010) found that mean percentage of factor activities II (48%), V (47%), VII (54%), VIII (89%), IX (69%), X (46%), XI (69%), and XII (80%) in dogs with chronic hepatitis plus cirrhosis were low. Inflammation of the liver was associated with a rise in von Willebrand's factor (Badylak et al., 1983). In trauma patients, results similar to our study were recorded by De Oliveira et al. (2007) in human trauma patients and found mean plasma vWF concentration to be significantly higher in the patients with severe traumatic brain injury.

4.5 Coagulation time in dogs with haemostatic disorders

Interpretation of the PT and APTT is useful for assessment of haemostatic disorders in dogs. Table 19 and Fig. 10 shows the assessment of coagulation time using secondary haemostatic tests in healthy dogs as well as in different haemostatic conditions. Prothrombin (PT) and activated partial thromboplastin (APTT) time in healthy dogs was 11.50±0.70 and 18.66±0.76 sec, respectively. Similarly, Sumathi et al. (2012) recorded mean value for PT range as 12.12±0.57 and APTT as 43.17±2.39 sec in healthy dogs. However, Geffre et al. (2010) recorded the PT range of 6.9–8.8 sec and the APTT range of 13.1–17.2 sec in citrated plasma in 139 healthy fasting purebred dogs lower than that of those of others the current study. Giurgiu et al. (2009) recorded range value for APTT in 1.5 years old dogs as 16.74± 0.35 sec, while 12.36 ± 0.68 sec for the adult dogs and PT range of 5.3±0.63 sec in adult dogs and 5.7±0.4 sec in young dogs.

Table 19: Coagulation time in dogs with haemostatic disorders

	Prothrombin time/sec	Prothrombin Index/sec	Activated partial pro thrombin time /sec
Healthy (n=6)	11.50±0.70 (8-13)	108.45± 2.85 (100-118.1)	18.66±0.76 (15-21)
Chronic liver diseases (n=15)	16.67±0.76 (13-19)	79.2±3.87 (68.4-100)	35.47±2.31 (27-45.5)
Thrombo-cytopenia (n=10)	14.00±0.70 (9-18)	94.10±4.62 (78.2-132)	31.44±1.39 (26-38)
Bleeding (n=5)	13.8±0.58 (12-15)	91.50±2.76 (86.6-100)	31.00±1.28 (28-34.2)

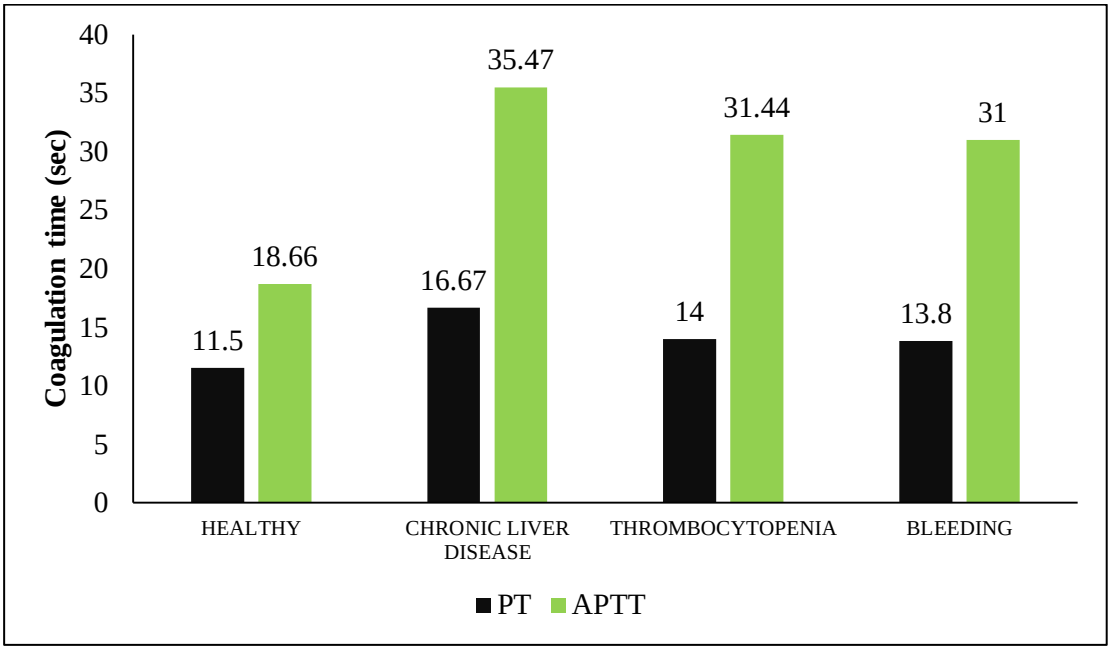


Fig. 10: Comparison of coagulation time in dogs with haemostatic disorders

The mean value of PT (16.67±0.76 sec) and APTT (35.47±2.31 sec) increased in patients with chronic liver diseases. Similarly, Badylak et al. (1983) found that 93 percent of dogs with naturally occurring hepatic disease had at least one abnormal coagulation test value, with PT and APTT abnormal in 50 percent and 75 percent of cases, respectively. Prins et al. (2010) recorded both mean PT and APTT concentrations were above upper reference values in chronic hepatitis, chronic hepatitis plus cirrhosis and the miscellaneous group. Elhiblu et al. (2015) observed that PT and APTT were significantly higher in dogs with liver cirrhosis than the

control values. The values of PT in bleeding as well as thrombocytopenic group were within the normal range however, the APTT values were slightly above the normal range. It can be attributed to the low platelet counts causing internal bleeding activating the intrinsic pathway of coagulation. Besides, APTT is more sensitive to *in-vitro* activation of clotting factors than the PT and may be prolonged with difficult venepuncture or i insufficient blood collected in citrate. Over-dilution of plasma with citrate will prolong the clotting times.

4.6 Blood smear and PCR analysis of recipient and healthy donor dogs for haemoprotozoa

Blood samples of 200 dogs (recipient dogs with anaemia and thrombocytopenia, n=100) and healthy donor dogs (n=100) were stained with Leishman stain and examined under microscope for the presence of various haemoprotozoan and rickettsial infections, such as *Babesia gibsoni, Babesia canis vogeli, Hepatozoon canis, and Ehrlichia canis.* Out of these, 100 samples were used to extract DNA for PCR analysis. Upon standardization of the respective parasite specific PCR assays, the amplicons for the respective parasites were observed in horizontal agarose gel (1.5%) electrophoresis at 672 bp (*B. gibsoni*), 601 bp (*B. canis vogeli*), 380 bp (*E. canis*) and at 666 bp (*H. canis*) (Table 20).

Out of 200 smear examination of donor as well as dogs with haematological disorders dogs, 11 cases were positive on blood smear examination. Out of these, *Babesia gibsoni* revealed as small intraerythrocytic piroplasms, was observed in six anaemic dogs (6.00%) while in one dog in donor group (5.00%), while *Hepatozoon canis* infection in neutrophil was observed in two cases of anaemia and two donor dogs(Plate 11a).

Out of 100 dogs, 23 (23.00%) were found to be positive for haemoprotozoa and rickettsial infection by PCR based assays (Table 20). *Babesia gibsoni* was observed in nine (11.25%) (Plate 11b) cases of anaemia and one donor dog (5.00%), *Ehrlichia canis* in nine cases (11.25%) (Plate 11c), *Hepatozoon canis* in two anaemic dogs (2.50%) (Plate 11d) and two donor dogs (10.00%) while *Babesia canis vogeli* could not be found in any case. PCR-based assays are more sensitive and specific for haemoprotozoa and rickettsia than coventional tests and hence in our study more cases could be diagnosed with PCR compared to conventional blood smear

examination. Supriya (2019) in a study on 90 dogs found that, five cases of renal failure were positive for haemoprotozoa and rickettsial infection, with *Babesia gibsoni* in four cases (4.44%) and *Ehrlichia canis* in one case (1.11%) on blood smear examination; whereas on PCR, *Babesia gibsoni* was found in 11 (12.22 %), *Babesia canis vogeli* in 2 (2.22 %), *Ehrlichia canis* in one (1.11 %), *Hepatozoon canis* in 11 (12.22 %), and mixed infection of *E. canis* and *H. canis* in one case (1.11%). A Study conducted by Singh et al. (2014) on 214 blood samples from suspected canine babesiosis, revealed an overall prevalence of (7.47%) (16/214) on blood smear comprising (0.93%) (2/214) of large *Babesia* and (6.54%) (14/214) of *Babesia gibsoni*. While, molecular diagnosis revealed (15.42%) (33/214) samples positive for *B. gibsoni* infection indicating higher sensitivity of PCR as compared to blood smear examination. Similarly studies by Singh (2017) on *H. canis* infection, reported the DNA of parasite in (13.78%) samples whereas, routine blood smear examination revealed gamonts in (5.78%) samples.

Table 20: Examination of healthy donor and sick recipient dogs for haemoprotozoa

	Blood smear		**PCR**	
Haemo-protozoa	**Recipient dogs (n=100)**	**Donor dogs (n=100)**	**Recipient dogs (n=80)**	**Donor dogs (n=20)**
Babesia gibsoni	6(6.00%)	1(1.00%)	9(11.25%)	1(5.00%)
Babesia canis	0(0.00%)	0(0.00%)	0 (0.00%)	0(0.00%)
Ehrlichia canis	0(0.00%)	0(0.00%)	9(11.25%)	0(0.00%)
Hepatozoon canis	2(2.00%)	2(2.00%)	2(2.50%)	2(10.00%)
Total	8(8.00%)	3(3.00%)	20(25.00%)	3(15.00%)

4.7 Retrospective studies on canine thrombocytopenia

4.7.1 Prevalence

A total of 24,370 dogs were examined in the OPD, and 3840 were analyzed for blood platelet counts. Of these, 1209 were found to be thrombocytopenic. The overall hospital prevalence of thrombocytopenia was (4.96 %) (1209/24370), while the overall prevalence out of the suspected cases was found to be (31.48%, n=1209/3840). The hospital prevalence in the current study was similar to Grindem et al. (1991), who reported hospital prevalence of (5.2%),However, it was lower than as

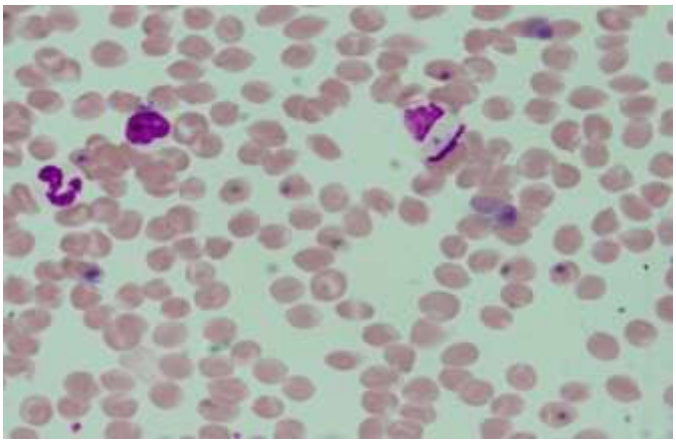


Plate 11a: *Hepatozoon canis* in a neutrophil (100X)

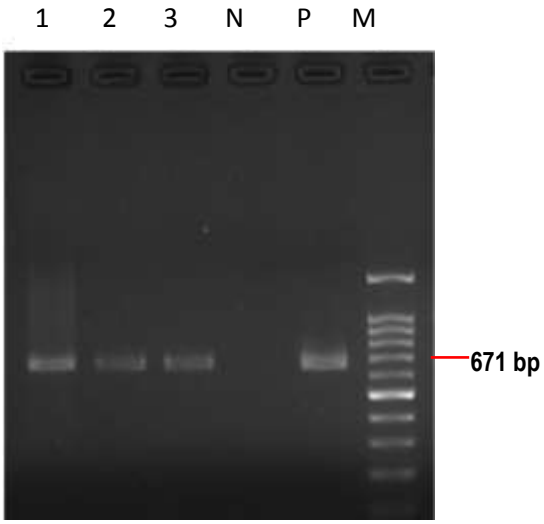


Plate 11b: *B. gibsoni*

M –Marker, P- Positive control, N- Negative control and 1,2,3 samples

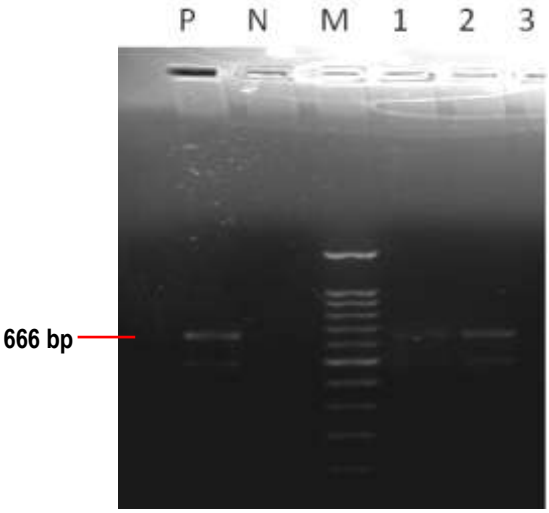


Plate 11c*: H. canis*

M- Marker, P- Positive control, N- Negative control and 1,2 samples

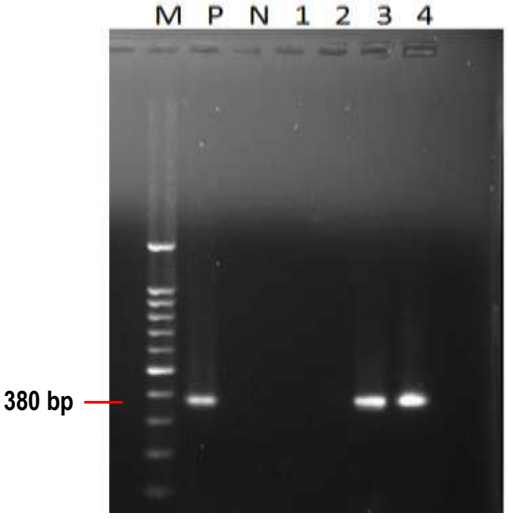


Plate 11d: *E. canis*

M- Marker, P- Positive control, N- Negative control and 1, 2, 3, 4 samples

Plate 11: Diagnosis of haemoprotozoan diseases in dogs

reported (6·7%) by Botsch et al. (2009) that might be due to low platelet analysis carried out in our study as compared to total number of cases. The proportion of mild, moderate and severe thrombocytopenia was (47.06%), (24.90%) and (28.04%) respectively. Thrombocytopenia is reported to be the most common acquired haemostatic disorder in dogs (Grindem et al., 1991). Thrombocytopenia in dogs can be caused by a primary pathology, such as reduced platelet development in conditions like aplastic bone marrow, or it can be caused by a secondary pathology, such as many other etiologies.

4.7.2 Season-wise prevalence

Table 21 describes the season-wise prevalence of thrombocytopenia. The highest prevalence was recorded in monsoon (36.49%) followed by (29.93%) in summer and (26.94%) in the winter. The prevalence of thrombocytopenia in monsoon season can be attributed to more presence of ticks.

4.7.3 Age-wise prevalence

On the basis of age, higher prevalence of thrombocytopenia was noticed in the middle aged group (5-10 yr) 33 percent (430/1303) followed by young group (1-5yr) 32.76 percent (512/1560) and lowest prevalence in less than > 6 month age group 23.91 percent (71/297) (Table 22 and Fig. 11). Similar, to our study Paras et al. (2019) stated that it is common middle-aged dogs.

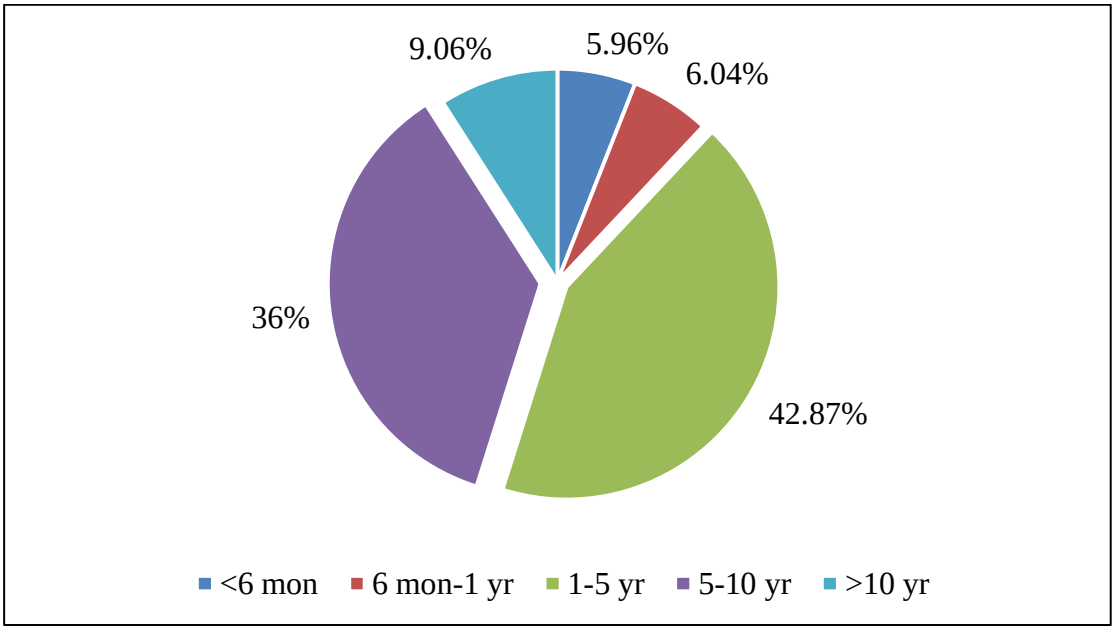


Fig. 11: Age-wise proportion of thrombocytopenia in dogs

Table 21: Season-wise case prevalence of thrombocytopenia in dogs

Season	Total CBC Reports	Mild		Moderate		Severe		Total	
		Dogs affected	Case prevalence	Affected	Case prevalence	Dogs affected	Case prevalence	Dogs affected	Case Prevalence
Monsoon	1181	184	15.58%	119	10.08 %	128	10.84%	431	36.49%
Summer	2065	303	14.67%	149	7.22%	166	8.03%	618	29.93%
Winter	594	82	13.80%	33	5.56%	54	7.58%	160	26.94%
Total	3840	569	14.82%	301	7.84%	339	8.83%	1209	31.48%

Table 22: Age-wise case prevalence of thrombocytopenia in dogs

Age	Total CBC reports	Mild		Moderate		Severe		Total	
		Dogs affected	Case prevalence	Dogs affected	Case prevalence	Dogs affected	Case prevalence	Case prevalence	Case prevalence
<6 mon	297	26	8.75 %	25	8.42 %	20	6.74%	71	23.91%
6 mon-1 yr	261	28	10.73%	25	9.58%	19	7.28%	72	27.59%
1-5 yr	1560	238	15.26%	122	7.82%	151	9.68%	511	32.75%
5-10 yr	1303	210	16.12%	103	7.90%	117	8.98%	430	33.00%
>10 yr	368	66	16.22%	22	5.98%	30	8.15%	108	26.35%
Total	3789	558	14.72%	297	7.84%	337	8.90%	1192	31.45%

4.7.4 Breed-wise prevalence

Out of the 3842 samples submitted from dogs for platelet counts, breed data was available for 2692 dogs (Table 23 and Fig. 12). Prevalence of thrombocytopenia was higher in other breeds group comprising of Boxer, Bull Terrier, Grey Hound, Great Dane, Himalayan Shepherd, Bordeaux, Chow Chow, Doberman, Gaddi Siberian Husky and Daschund (38.25%) followed by Labrador (38.07%), German shepherd (35.11%). Botsch et al. (2009) recorded the highest prevalence of thrombocytopenia in German shepherd followed by Bernese mountain dog and Golden retriever. However, as per Grindem et al. (1991) Dobeman Pinscher was most presented breed.

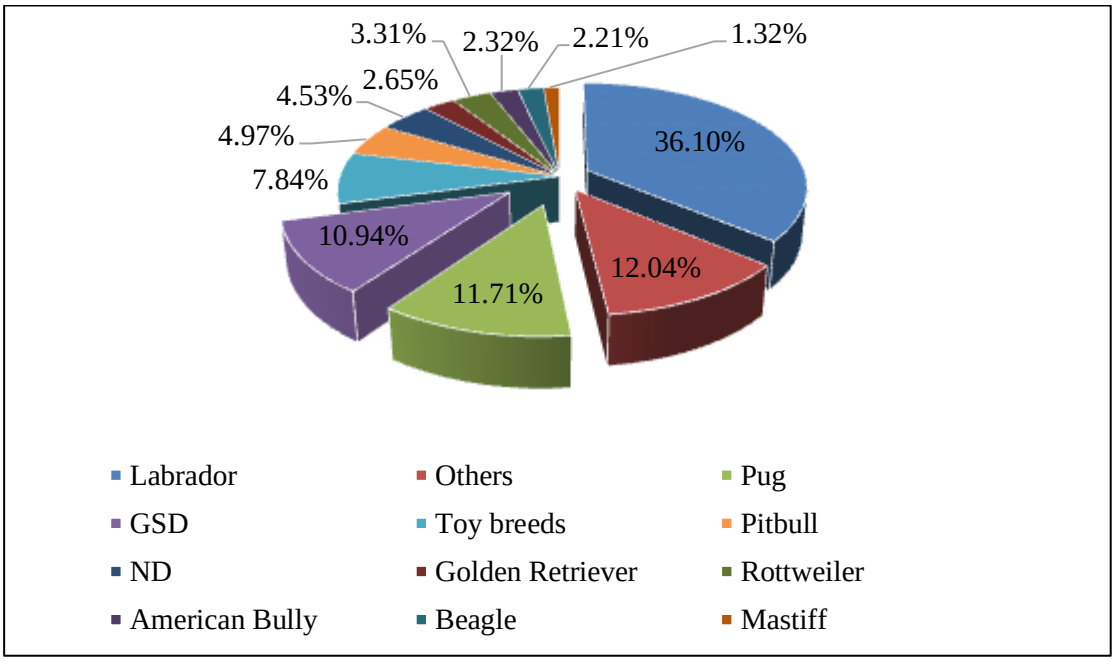


Fig. 12: Breed-wise proportion of thrombocytopenia in dogs

4.7.5 Sex-wise prevalence of thrombocytopenia

Sex-wise prevalence (Table 24) was higher in males (33.17%) as compared with females (28.90%). Our study is in contrast to Paras et al. (2019) who stated that thrombocytopenia was more prevalent in females and Botsch et al. (2009) reported higher proportion of immune-mediated thrombocytopenia in female dogs. Additionally, females and small breed dogs, such as Poodles and Cocker Spaniels, appear pre- disposed to immune-mediated thrombocytopenia (Williams & Maggio-Price, 1984).

Table 23: Breed-wise case prevalence of thrombocytopenia in dogs

Breed	Total CBC reports	Mild (90-165 x10^3)		Moderate (50-90x10^3)		Severe(<50 x 10^3)		Total	
		Dogs affected	Breed Prevalence	Dogs Affected	Breed Prevalence	Dogs affected	Breed Prevalence	Dogs affected	Breed Prevalence
American Bully	98	10	10.20%	6	6.12%	5	5.10%	21	21.43%
Beagle	70	11	15.71%	5	0.55%	4	5.71%	20	28.57%
Golden Retriever	73	11	15.07%	9	0.99%	4	5.47%	24	32.88%
GS	282	54	19.15%	23	8.16%	22	7.80%	99	35.11%
Labrador	859	145	16.88%	86	10.01%	96	11.18%	327	38.07%
Mastiff	42	6	14.29%	2	4.76%	4	9.52%	12	28.57%
ND	145	22	15.17%	14	9.66%	5	3.44%	41	28.28%
Other Breeds	285	57	6.30%	21	2.32%	31	3.42%	109	38.25%
Pitbull	128	18	14.06%	13	10.16%	14	10.94%	45	35.16%
Pug	398	47	11.81%	31	7.79%	28	7.04%	106	26.63%
Rottweiler	108	15	13.89%	5	4.63%	10	9.26%	30	27.77%
Toy breeds	204	33	16.18%	18	8.82%	2	9.80%	71	34.80%
Total	2692	429	47.40%	233	25.75%	243	26.85%	905	

(Others – Boxer, Bull Terrier, Grey Hound, Great Dane, Himalayan Shepherd, Bordeaux, Chow Chow, Doberman, Gaddi and Siberian Husky)

Table 24: Sex-wise prevalence of thrombocytopenia in dogs

Sex	Total CBC cases	Mild		Moderate		Severe		Total		
		Dogs affected	Case Prevalence	Dogs affected	Case Prevalence	Dogs affected	Case Prevalence	Dogs affected	Case Prevalence	Sex wise
Females	1360	200	14.70%	98	7.20%	95	6.99%	393	28.90%	34.68%
Male	2231	330	14.79%	184	8.25%	226	10.12%	740	33.17%	65.31%
Total	3591	530	14.76%	282	7.86%	321	8.93%	1133		100%

4.7.6 Haemoprotozoa prevalence

The prevalence of haemoprotozoan disease was very low (3.70%). It is due to reason that all the blood samples were examined by microscopy. Observation of a large proportion of negative results in the present study cannot rule out haemoprotozoa in dogs (Table 25). Microscopy technique requires lot of experience and there may be possibility of false negative results (Solano-Gallego et al., 2016). Although, with the advancement of nucleic acid amplification techniques like PCR, the diagnostic sensitivity has increased manifold (Mandal et al., 2015 & Mittal et al., 2019). Among all the detected haemoparasites, *Babesia gibsoni* showed the highest prevalence (2.73%) followed by *H.canis* (0.78%) and least by *E.canis* (0.19%). Botsch et al. (2009) also observed thrombocytopenia in 11·9 percent dogs with babesiosis, 4·8 per cent with ehrlichiosis, while high incidence of thrombocytopenia caused by infectious diseases. Subapriya et al. (2020) stated that in canine practice, thrombocytopenia caused by haemoprotozoans and rickettsial species is common. Thrombocytopenia is also caused by the haemoprotozoans *Babesia vogeli* and *Babesia gibsoni* found in RBCs, as well as the extracellular parasite *Trypanosoma evansi*.

4.7.7 Disease wise comparison on the basis of etiology

The diseases were diagnosed on the basis of diagnostic aids such as haematology, serum biochemistry, histopathology, cytology, parasitology, and radiology (Table 26 and Fig 13). The highest prevalence of thrombocytopenia was recorded in liver disease (53.60%), followed by MODS (multiple organ dysfunction syndrome, 42.59%), and haemoprotozoa (40.00%) and the least in patients with skin affections (14.29%). However, Grindem et al. (1991) noted miscellaneous thrombocytopenia as leading cause (59%) comprising of nephropathy (19·4%), other diseases (19·4%), hepatopathy (15·3%), neuropathy (11·3%), cardiopathy (10·8%), intoxication (8·5%), chemotherapy (8·5%), endocrinopathy (6·8%) and neoplasia-associated thrombocytopenia; (23%). Botsch et al. (2009) in a retrospective study observed inflammatory/ infectious thrombocytopenia (34·9%) as leading cause of thrombocytopenia followed by neoplasia associated immune-mediated thrombocytopenia (5·6%) and thrombocytopenia caused by miscellaneous disorders (25·5%).

Table 25: Occurrence of blood haemoprotozoa in thrombocytopenic dogs

	Blood smear examinations	Mild		Moderate		Severe		Total	
		No.	%age	No.	%age	No.	%age	No.	%age
Haemoprotozoa(+)	*B.gibsoni* (2.73%), *H.canis* (0.78%), *E.canis* (0.19%)	7	0.55%	4	0.31%	8	0.62%	19	3.70%

Table 26: Occurrence of thrombocytopenia in different canine disorders

Disease	CBC Reports	Mild		Moderate		Severe		Total	
		Dogs affected	Case prevalence	Dogs affected	Case prevalence	Dogs affected	Case prevalence	Dogs affected	Case prevalence
CKD	**400**	**69**	17.25%	**38**	9.50%	**35**	8.75%	**142**	35.5%
GI	**345**	**49**	14.20%	**25**	7.25%	**30**	8.70%	**104**	30.14%
Gynae and obstetrics	**140**	**27**	19.29%	**11**	7.86%	**5**	3.58%	**43**	30.71%
Healthy	**85**	**9**	10.59%	**4**	4.71%	**1**	1.18%	**14**	16.47%
Haemoprotozoa	**30**	**4**	13.33%	**0**	0.00%	**8**	26.67%	**12**	40.00%
Infectious diseases	**116**	**18**	15.52%	**7**	6.03%	**10**	8.62%	**35**	30.17%
Integumentary system	**224**	**17**	7.58%	**3**	1.34%	**12**	5.36%	**32**	14.29%
Liver diseases	**222**	**56**	25.23%	**25**	11.26%	**38**	17.12%	**119**	53.60%
MODS	**54**	**10**	18.52%	**9**	16.67%	**4**	7.41%	**23**	42.59%
Neoplasia	**121**	**17**	14.05%	**7**	5.79%	**5**	4.13%	**29**	23.97%
Pyometra	**87**	**18**	20.69%	**7**	8.05%	**2**	2.30%	**27**	31.04%
Traumatic	**83**	**12**	14.46%	**4**	4.82%	**7**	8.43%	**23**	27.71%
Idiopathic	**1342**	**175**	13.04%	**113**	8.42%	**133**	9.92%	**421**	31.37%
Cardiopulmonary	**145**	**29**	20.00%	**13**	8.97%	**9**	6.21%	**51**	35.17%
Miscellaneous	**446**	**59**	13.23%	**35**	7.85%	**40**	8.97%	**134**	30.04%
Total	**3840**	**569**	14.82%	**301**	7.84%	**339**	8.82%	**1209**	31.48%

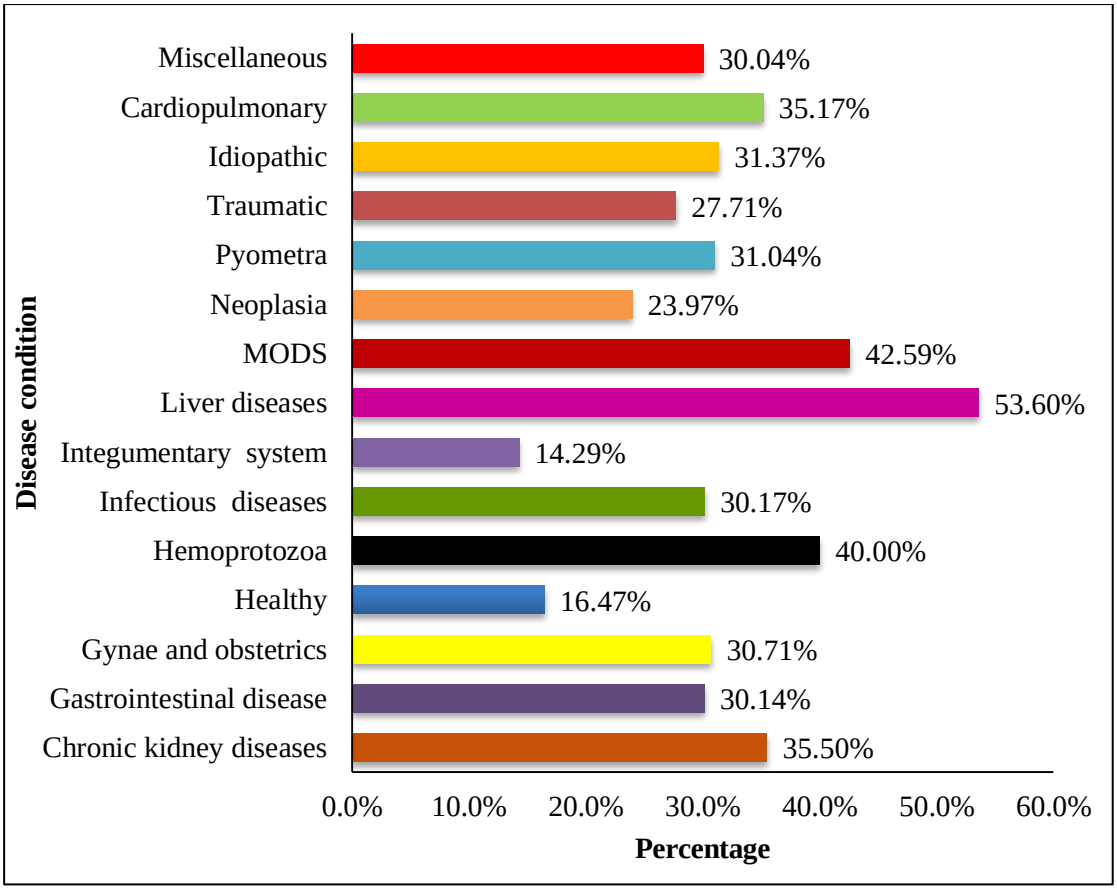


Fig. 13: Bar diagram showing etiology of thrombocytopenia in canines

4.7.8 Haematobiochemical alteration in thrombocytopenic dogs

On the basis of platelet count, grading was done into three groups as mild, moderate and severe (Table 27). The mean haemoglobin showed the significant difference and dogs with thrombocytopenia also had moderate anaemia. The PCV, TEC and platelet also showed the significant difference ($p<0.005$). However, no significant difference was noted in the TLC counts on comparison. Overall, the biochemical parameters showed no significant difference within the groups.

4.7.9 Clinical signs of thrombocytopenia

Thrombocytopenia is associated with signs like melena, haematochezia, haematuria, haemetemesis, epistaxis and pyrexia as well as bleeding in the internal cavities like haemoabdomen or haemoperitoneum. In current study, melena was found in highest number of cases (6.70%) followed by haematochezia (2.81%). Overall, the signs of external bleeding were recorded in (13.90%) cases (Table 28).

Table 27: Haematobiochemical changes in thrombocytopenic dogs

Variable	Mild 128.96±0.90(91.00-165.00)	Moderate 70.27±0.64(51.00-90.00)	Severe 30.27±0.69(2.00- 50.00)
Haemoglobin(g/dL)	10.62±0.16[a](2.10-20.10)	9.93±0.21[a](2.10-18.20)	9.11±0.22[a](1.20-20.30)
PCV (%)	31.48±0.53[a](5.70-76.40)	28.48±0.69[a](5.75-75.50)	26.71±0.69[a](4.00-60.40)
TLC(×10^3/ µl)	18.28±1.10(3.20-53.14)	15.38±8.73(5.38-119.10)	14.12±8.94(1.50-124.59)
TEC(×10^6/ µl)	5.13±0.083[a](0.99-10.63)	4.63±0.11[a](0.92-8.65)	4.32±0.11[a](0.16-9.30)
Total bilirubin(mg/ dL)	2.08±0.53(0.10-30.00)	2.56±0.61(0.10-24.10)	2.48±0.57(0.10-23.50)
ALT(IU/L)	93.06±7.57(4.00-991.00)	104.30±12.40(4.00-900.00)	123.1±13.10(5.00-900.00)
AST(IU/L)	94.6±14.3(18.0-900.00)	69.63±7.27(27.00-189.00)	101.0±23.2(23.0-410.00)
ALKP(IU/L)	281.00±23.00(23.0-1700.0)	268.60±39.00(15.0-1700.0)	370.10±34.50(29.0 -1700.0)
Total protein(g/dL)	5.61±0.12(2.00-10.90)	5.96±0.23(1.90-14.00)	5.37±0.17(1.20-9.60)
Albumin(g/dL)	2.11±0.05(1.10-3.80)	2.32±0.10(0.90-4.20)	2.05±0.07(0.80-3.60)
BUN(mg/dL)	44.39±3.05(4.00-283.00)	47.85±4.17(4.00-195.00)	43.60±3.68(4.00-285.00
Creatinine(mg/dL)	3.30±0.25(0.20-21.50)	3.58±0.39(0.30-20.50)	2.23±0.21(0.20-20.00)

(Values in parenthesis represent range and Values having different superscript in same row differ significantly at ($p<0.005$)

Table 28: Clinical signs of thrombocytopenia in dogs

Clinical signs of bleeding	Case affected	%age
Melena	81	6.70%
Haematochezia	34	2.81%
Haematemesis	22	1.82%
Epistaxis	19	1.57%
Pyrexia	132	10.92%
Haematuria	12	0.99%

Botsch et al. (2009) recorded haematoma (1·8%), ecchymosis (2·1%) and petechiae (2·4%) haemoperitoneum (4·0%), bleeding from the prepuce or vulva (2·0%) and epistaxis (2·0%) and further found a significant correlations between the platelet count and haemorrhagic diarrhoea, haematuria, dermatorrhagia and epistaxis. However, Giger (2006) concluded that classic signs of thrombocytopenia include petechiation, ecchymosis, epistaxis, and gastrointestinal blood loss.

4.8 Prevalence of canine abnormal protein disorders

A total of 24,370 dogs were examined in the OPD unit, out of which 1257 were analyzed for total serum protein and albumin. Of these, 642 were found to have abnormal protein concentration. The overall hospital prevalence was (2.63%) based on cases presented and (51.07%) in suspected cases. Hypoproteinemia in dogs can be caused by liver disorders, enteritis, cardiac disorders and parvoviral enteritis. Acute enteritis in young animals and chronic liver failure in adult animals is the leading cause of hypoproteinemia. The grouping of the cases was done and categorized into three groups as hypoproteinemia and hypo albuminemia (n=393, 31.26%), Hypo proteinemia and normo albuminemia (n=78, 6.21%) and normo proteinemia and hypo albuminemia (n=171, 13.60%).

4.8.1 Abnormal protein concentrations in different canine disorders

The highest prevalence of abnormal protein concentrations (Table 29) as hypoproteinemia + hypoalbuminemia group was recorded in liver diseases (50.75%) followed by infectious diseases (40.00%) and cardiac diseases (34.29%). While highest prevalence as hypo proteinemia+normoalbuminemia was recorded in gastrointestinal diseases (20.37%) followed by cardiac diseases (17.14%).

Table 29: Abnormal protein concentrations in different canine disorders

Disease	Total biochemical reports	Hypoproteinemia + hypoalbuminemia		Hypo proteinemia + normoalbuminemia		Normoproteinemia & hypo albuminemia	
		Dogs affected	Case prevalence	Dogs affected	Case prevalence	Dogs affected	Case prevalence
Chronic kidney diseases	249	51	20.48%	13	5.22%	45	18.07%
Chronic liver disease	201	102	50.75%	7	3.48%	24	11.94%
Gastrointestinal diseases	54	12	22.22%	11	20.37%	2	3.70%
Healthy	13	2	15.38%	2	15.38%	2	15.38%
Cardiopulmonary	35	12	34.29%	6	17.14%	7	20.00%
Haemoprotozoa	16	4	25.00%	0	0.00%	6	37.50%
Infectious	15	6	40.00%	0	0.00%	1	6.67%
Miscellaneous	126	26	20.63%	9	7.14%	16	12.70%
Multiple organ dysfunction syndrome	86	27	31.40%	7	8.14%	18	20.93%
Neoplasia	51	14	27.45%	5	9.80%	4	7.84%
Pyometra	13	1	7.69%	1	7.69%	2	15.38%
Idiopathic	398	136	34.17%	17	4.27%	44	11.06%
Total	1257	642(51.07%)					

The highest prevalence as normoproteinemia+hypoalbuminemia was recorded in haemo-protozoan (37.50%) followed by cardiac group (20.00%). Regmi & Shah (2017) recorded hypoproteinemia and hypoalbuminemia in a German shepherd dog in liver diseases. The protein-losing enteropathies (PLE) include a group of intestinal, generally small intestinal, diseases commonly associated with weight loss, hypoproteinemia caused by hypoalbuminemia or panhypoproteinemia.

4.8.2 Haematobiochemical variables in dogs with abnormal protein concentrations

The mean values of total protein and albumin in three groups were recorded as 4.27±0.03, 1.63±0.01 g/dL in hypoproteinemia + hypoalbuminemia group; 4.92±0.05, 2.56 ±0.02 g/dL in hypoproteinemia+normoalbuminemia; and 6.04±0.04 and 1.96±0.01g/dL in normoproteinemia + hypoalbuminemia. All the three groups of hypoproteinemic patients showed moderate anaemia attributed to secondary pathological conditions like liver diseases, cardiac diseases, infectious and inflammatory diseases (Table 30). The serum ALT, AST and ALKP levels in all three groups were higher. Center (1996) recorded that increases in ALT and AST occurs due to secondary leakage from damaged hepatocytes. Cooper et al. (2006) stated that increase in ALKP in liver diseases occur as alkaline phosphatase is present on both the hepatocyte biliary membrane as well as bile duct cells.

4.8.3 Breed wise prevalence of abnormal protein concentrations in dogs

The highest prevalence of hypoproteinemia+hypoalbuminemia was recorded in Golden retriever (45.16%) followed by other breed group 42/96 (43.75%) and the Mastiff breed 10/23 (43.48%) while, the highest prevalence of hypoproteinemia + normoalbuminemia was recorded in Mastiff breed (21.74%) followed by American bully (12.50%) and that of normoproteinemia+hypoalbuminemia (21.62%) in Rottweiler and (17.65%) in Pitbull (Table 31). This data could be due the prevalence of particular breed at particular geographical area.

Table 30: Haematobiochemical variables in dogs with abnormal protein concentrations

	Hypoproteinemia + hypo albuminemia	**Hypo proteinemia + normo albuminemia**	**Normo proteinemia + hypo albuminemia**
	n=393	**n=78**	**n=171**
Total Protein (mg/dL)	4.27±0.03 (1.70-5.30)	4.92±0.05 (3.00-5.30)	6.04±0.04 (5.40-7.50)
Albumin (mg/dL)	1.63±0.01 (0.70-2.20)	2.56±0.02 (2.30-3.10)	1.96±0.01 (1.20-2.20)
Hb (g/dL)	8.00±0.15 (1.50-17.20)	10.66±0.40 1.50-18.50	9.27±0.27 (1.50-19.00)
PCV (%)	24.7±10.43 (0.22-121)	21.22-15.99 (3.20-78.64)	21.22±13.41 (6.90-109.43)
TLC ($\times 10^3$/ µl)	22.89±0.57 (5.40-59.10)	30.90±1.87 (14.00-58.40)	26.51±1.00 (8.10-47.80)
TEC ($\times 10^6$/ µl)	3.88±0.10 (1.02-9.64)	4.80±0.24 (1.91-7.48)	4.17±0.16 (0.99-7.86)
Platelet ($\times 10^3$/ µl)	238.56±11.93 (3.00-955.00)	337.60±29.25 (57.00-890.00	227.38±19.41 (7.00-991.00)
Total bilirubin (mg/dL)	2.25±0.27 (0.10-23.50)	2.01±1.06 (0.10-30.00)	1.78±0.40 (0.10-21.50)
AST (IU/L)	116.47±25.77 (21.00-900)	62.16±9.62 (35.00-102.00)	72.53±12.39 (34.00-209.00)
ALT (IU/L)	117.52±8.71 (7.00-971.00)	123.09±18.22 (8.00-725.00	97.84±9.99 (7.00-900.00)
ALKP (IU/L)	332.80±20.68 (19.00-1700.00)	290.04±54.55 (29.00-1700.00)	384.55±38.54 (34.00-1700.00)
BUN (mg/dL)	293.00±37.73 (1.00-293.00)	45.69±5.98 (6.00-45.69)	45.58±3.96 (4.00-160.00)
Creatinine (mg/dL)	2.12±0.17 (0.10-20.00)	3.20±0.55 (0.40-20.00	3.33±0.31 (0.30-20.00)

Table 31: Breed-wise prevalence of abnormal protein concentrations in dogs

Breed	Total biochemical reports	Hypoproteinemia + hypo albuminemia		Hypo proteinemia + normo albuminemia		Normo proteinemia + hypo albuminemia	
		Dogs affected	Case prevalence	Dogs affected	Case prevalence	Dogs affected	Case Prevalence
American bully	16	6	37.50%	2	12.50%	0	0.00%
Beagle	28	15	53.57%	2	7.14%	2	7.14%
German Shepherd	96	25	26.04%	9	9.38%	13	13.54%
Labrador Retriever	334	76	22.75%	19	5.69%	55	16.47%
Mastiff	23	10	43.48%	5	21.74%	2	8.70%
Non-descript	35	14	40.00%	0	0.00%	1	2.86%
Others	96	42	43.75%	4	4.17%	10	10.42%
Pit bull	34	10	29.41%	2	5.88%	6	17.65%
Pug	118	48	40.68%	8	6.78%	18	15.25%
Golden retriever	31	14	45.16%	1	3.23%	1	3.23%
Rottweiler	37	11	29.73%	2	5.40%	8	21.62%
Toy breeds	58	11	18.97%	6	10.34%	8	13.79%

4.8.4 Sex- wise case prevalence of abnormal protein concentrations in dogs

The highest prevalence of hypoproteinemia + hypoalbuminemia was recorded in females (34.63%) and males (30.27%) while that of hypoproteinemia+ normoalbuminemia was (8.59%) in females and (4.84%) in males and normoproteinemia+hypo albuminemia (13.30%) in females and (13.77%) in males (Table 32).

4.8.5 Age-wise prevalence of abnormal protein concentrations in dogs

The age wise prevalence of abnormal protein concentrations has been presented in Table 33. The highest prevalence of hypoproteinemia+ hypoalbuminemia was recorded in less than one year age group (47.01%) followed by 1-5 year group (37.83%) and >10 year age group (20.99%). The prevalence of hypoproteinemia + normoalbuminemia was highest in less than one year age group (11.19%) followed by 5 -10 year age group (5.33%) and that of normoproteinemia + hypoalbuminemia was highest in >10 year age group (41.98%) followed by less than one year age group (18.66%).

Table 32: Sex-wise case prevalence of abnormal protein concentrations in dogs

Sex	Total biochemical reports	Hypoproteinemia + hypo albuminemia		Hypo proteinemia + normo albuminemia		Normo proteinemia + hypo albuminemia	
		Dogs affected	Case prevalence	Dogs affected	Case prevalence	Dogs affected	Case prevalence
Male	806	244	30.27%	39	4.84%	111	13.77%
Female	361	125	34.63%	31	8.59%	48	13.30%

Table 33: Age-wise prevalence of abnormal protein concentrations in dogs

Age	Total biochemical reports	Hypoproteinemia + hypo albuminemia		Hypo proteinemia + normo albuminemia		Normo proteinemia + hypo albuminemia	
		Dogs affected	Case prevalence	Dogs affected	Case prevalence	Dogs affected	Case prevalence
Less than 1 year	134	63	47.01%	15	11.19%	25	18.66%
1-5 year	563	213	37.83%	34	6.04%	65	11.55%
5 -10 year	469	94	20.04%	25	5.33%	78	16.63%
>10 year	81	17	20.99%	3	3.70%	34	41.98%

CHAPTER IV

SUMMARY AND CONCLUSIONS

Haematological disorders mainly comprise of anaemia, thrombocytopenia and coagulation disorders. Anaemia in dogs may occur as a result of loss of blood, destruction of RBCs or decreased production from bone marrow. Thrombocytopenia mainly occurs as a result of *Ehrlichia canis*, immune-mediated or due to bone marrow depression. The main haemostatic disorders include coagulation factor deficiencies, Von Willebrand disease and acquired coagulation disorders.

In prospective studies on 91 severely anaemic dogs, the severely pale mucous membrane was found to be the most significant sign of severe anaemia (78.02%) followed by tachypnea (72.52%)**,** lethargic but able were to stand (68.10%), inappetence/anorexia (61.53%), tachycardia (57.14%), vomition (54.94%), weak pulse (51.64%) and melena (30.76%). The highest prevalence of severe anaemia was observed in Labrador retrievers (29.67%); more in males (74.73%) may be due to overrepresentation of this breed and sex in the hospital. The highest prevalence of anaemia was observed in the age group of 0-3 year (45.05%), followed by the adult age group of 6-9 year (26.38%). The cases of anaemia were classified into 13 etiology groups with maximum anaemic cases associated with kidney disease (23.08%) followed by liver disease (15.38%) and haemoprotozoan infections (15.38%). IMHA had the most severe effect on haematocrit levels, followed by liver diseases.

Transfusion of whole blood or pRBCs resulted in the highest improvement in haematological parameters in surgery and trauma group patients followed by gastrointestinal diseases and haemoprotozoan infections, while the minimum improvement was noticed in the pancytopenia group. Overall, an increase of 2.27 g/dL in Hb level and 8.27 percent in PCV level was recorded after whole blood transfusion, and that of 2.64 g/dL in Hb level and 8.48 percent was observed after transfusion of pRBCs at 3rd day after transfusion. After transfusion of whole blood, 45 (71.43%) out of the 63 severely anaemic cases survived, while transfusion of pRBCs could save 18 (64.29%) out of 28 dogs. Of the 26 dogs which could not undergo transfusion due to lack of donors, only 9 (34.62%) survived on 7th day of the observation period. It was quite evident that blood transfusion could increase survival in all the etiology groups.

In thrombocytopenic dogs, melena was the most significant sign (75.00%) followed by epistaxis (58.33%) and ecchymotic haemorrhages/purpura (50.00%). The cases were divided into three groups based on etiology; the highest cases were recorded in haemoprotozoan group (50.00%) followed by renal failure (41.67%) and neoplasia (8.33%). Twelve severely thrombocytopenic dogs (mean platelet count of 14.50 ± 38.75× 10^3) were subjected to platelet transfusion, and mean platelet count showed an increasing trend within 1 hour of transfusion (mean platelet count of 28.00 ± 48.35× 10^3).

The clinical cases of hypoproteinemia+hypoalbuminemia (n=10) were divided into three groups based on etiology as chronic liver disease (50.00%), canine parvoviral enteritis (30.00%) and acute hepatitis (20.00%). These dogs underwent plasma therapy and total protein and albumin were monitored on the 3rd and 7th day post-transfusion. There was a marked improvement in total protein and albumin concentration and all cases suffering from parvoviral enteritis survived.

The levels of coagulation factors (II, V, VII, VIII, IX, X and vWF) in healthy serum (n=20), healthy plasma (n=20) as well as in patients affected with trauma (n=10), diabetes mellitus (n=10), bleeding patients (n=10), acute hepatitis (n=5) and chronic liver disease (n=15) were studied. All the coagulation factors decreased in chronic liver diseases except vWF as hepatocytes produce fibrinogen, prothrombin and factors V, VII, IX, X, XI and XIII besides having a role in activation of vitamin K-dependent factors and protein C. Diabetic patients were found to have a higher level of vWF factor.

The mean prothrombin time (PT) and activated partial thromboplastin time (APTT) was 11.50±0.70 sec and 18.66±0.76 sec in healthy dogs; while it increased to 16.67±0.76 sec and 35.47±2.31sec respectively in dogs suffering from chronic liver diseases. The values of PT and APTT in bleeding and thrombocytopenic groups were within the normal range.

Based on retrospective studies carried out on 24,370 dogs from January 2018 to December 2019, overall hospital prevalence of thrombocytopenia was 4.96 percent and 31.48 percent based on blood reports in suspected cases 28.04 percent cases were severely thrombocytopenic. The highest prevalence of thrombocytopenia was noticed in the middle-aged dogs (33.00%); males (33.17%); during monsoon season (36.49%)

and group comprising of other breeds (38.25%). The prevalence of haemoprotozoan diseases based on blood smear examination was very low (3.70%); *Babesia gibsoni* showed the highest prevalence (2.73%) followed by *Hepatozoon canis* (0.78%) and *Ehrlichia canis* (0.19%). The highest prevalence of thrombocytopenia was recorded in liver diseases (53.60%) followed by multiple organ dysfunction syndrome (42.59%), and haemoprotozoa (40.00%). Thrombocytopenia was mainly associated with melena (6.70%), haematochezia (2.81%), haematuria, haematemesis, epistaxis and pyrexia.

A total of 1257 were analyzed for total serum protein and albumin in clinical cases, and of these, 642 were found to have abnormal protein concentration (51.07%). The cases were categorized into three groups as hypoproteinemia + hypoalbuminemia (n=393, 31.26%), Hypoproteinemia+normoalbuminemia (n=78, 6.21%) and normoproteinemia + hypoalbuminemia (n=171, 13.60%). The highest prevalence of hypoproteinemia + hypoalbuminemia group was recorded in liver diseases (50.75%) followed by infectious diseases (40.00%) and cardiac diseases (34.29%). The highest prevalence of hypoproteinemia + hypoalbuminemia was recorded in Golden retrievers (45.16%); females (34.63%) and < 1 year age group (47.01%).

Conclusions:

- Chronic Kidney disease was the most common cause of anaemia followed by liver disease and haemoprotozoan infections.
- Transfusion of whole blood or pRBCs resulted in highest improvement in surgery/ trauma group followed by gastrointestinal diseases and haemoprotozoan infections, while the minimum improvement was noticed in pancytopenia group.
- Mean increase of 2.27 g/dL in Hb level and 8.27 percent in PCV level was recorded after whole blood transfusion, and that of 2.64 g/dL in Hb level and 8.48 percent in PCV level was observed after transfusion of pRBCs.
- Transfusion of whole blood or pRBCs improved survivability (69.23%) in comparison to the non-transfusion group (34.62%).
- Mean platelet count increase of 13.50×10^3/µl was recorded in thrombocytopenic dogs one hour after transfusion of platelet- rich plasma.
- Transfusion of plasma proved to be more beneficial in enhancing survival in canine parvoviral enteritis than dogs suffering from chronic liver disease and acute hepatitis.

- All the coagulation factors decreased in chronic liver diseases except vWF, while vWF factor was found to increase in diabetic patients.
- PT and APTT increased in dogs with chronic liver diseases, but remained within normal range or slightly higher in bleeding and thrombocytopenic groups.
- PCR analysis revealed haemoprotozoan infections in 25.00 percent recipient dogs with *Babesia gibsoni* (11.25%), *Ehrlichia canis* (11.25%) and *Hepatozoon canis* (2.50%).
- Based on retrospective studies on 24,370 dogs, the hospital prevalence of thrombocytopenia was 4.96 percent.
- Based on 1257 suspected cases analyzed for total serum protein and albumin, 642 were found to have abnormal protein concentration (51.07%) with maximum suffering from hypoproteinemia + hypoalbuminemia (n=393, 31.26%).

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APPENDIX - I

Risk note for whole blood transfusion/ pRBCs/ FFP in dogs

Date- Case no-

Name and address of the owner –

Contact no -

Species - Breed-

Sex- Age-

I accept and understand that my pet has severe anemia, which, according to the doctor, can lead to serious complications, including death. Blood transfusion risks as well as other complications have been explained to me. The donor dog has been arranged by me/ NGO for collection and administration of blood In case of eventuality during or after transfusion, I will not seek compensation from the university hospital, and no one from the university staff will be held liable.

WARNING – In spite of laboratory tests and careful donor selection, the risk of transmitting infectious agents to the patient is present. Also, blood may contain immunizing substance that can cause reaction even in typed and cross matched transfusions. Such reactions are rare but may be life threatening.

Signature (Dog owner)

APPENDIX – II

Transfusion record

Identification number and name of the patient –

Address and phone no - Species- Breed -

Age - Sex - Weight -

Patient blood type - Previous transfusion (yes/no) -

If yes, date of previous transfusion – Relevant history -

Diagnosis – Volume of whole blood transfused –

Unit ID number – Date of collection and expiry –

Donor ID - Donor blood type -

Parameter	**Pre- tx**	**0 Min**	**10 min**	**20 min**	**30 min**	**1 hr**	**2 hr**	**3 hr**	**4 hr**
Start time									
Stop time									
Rate (ml/hr)									
MM									
Temp									
Resp rate									
Heart rate									
Vomiting (+/-)									
Dyspnea (+/-)									
Shivering									
Any other complication									
Hb									
PCV									

VITA

Name of the student	:	Iqra Shafi Khan
Father's name	:	Mohmad Shafi Khan
Mother's name	:	Marefat Jan
Nationality	:	Indian
Date of birth	:	12-02-1992
Permanent home address	:	House No 26-A Lane No. 4, Pamposh Colony Natipora, Srinagar, Kashmir
E-mail Address	:	khaniqrashafi@gmail.com

EDUCATIONAL QUALIFICATIONS

Bachelor degree	:	B.V.Sc. and A.H.
University	:	SKUAST-K (Sher-e- Kashmir University of Agricultural Sciences and Technology)
Year of award	:	2015
OGPA/OCPA/% marks	:	6.84/10.00
Master's degree	:	M.V.Sc. (Veterinary Medicine)
University	:	Guru Angad Dev Veterinary and Animal Sciences University Ludhiana
OGPA/OCPA/% marks	:	8.06/10.00
Year of award	:	2017
Title of thesis	:	Disease profile in dairy calves at an organised dairy farm
Ph.D.	:	Ph.D. (Veterinary Medicine)
OGPA/OCPA/% marks	:	8.42/10.00
Awards/Distinctions/Fellowships/ Scholarship :	:	▪ MOMA scholarship throughout B.V.Sc. & A.H. Degree (2010-2015) ▪ National Talent Scholarship (ICAR) M.V.Sc. degree (2015-2017) ▪ University Merit Scholarship throughout Ph.D. degree (2017-2021)