# STUDIES ON ERYTHROCYTIC INDICES AND MORPHOLOGY VIS-À-VIS DISEASE CONDITIONS OF DOGS

**Thesis**

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

## MASTER OF VETERINARY SCIENCE
## in
## VETERINARY PATHOLOGY
**(Minor Subject: Veterinary Microbiology)**

**By**

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# CERTIFICATE – I

This is to certify that the thesis entitled, **"STUDIES ON ERYTHROCYTIC INDICES AND MORPHOLOGY VIS-À-VIS DISEASE CONDITIONS OF DOGS"** submitted for the degree of **M.V.Sc.**, in the subject of **Veterinary Pathology** (Minor Subject: **Veterinary Microbiology**) of the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, is a bonafide research work carried out by **Avantika Sharma (L-2017-V-65-M)** under my supervision and that no part of this thesis 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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# CERTIFICATE – II

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## ABSTRACT

The present study was undertaken to correlate hematological abnormalities observed in retrospective and prospective cases with different disease conditions in dogs. For retrospective studies, haematological values of 7,375 cases of dogs were analyzed and correlated with different disease conditions. The mean value of haemoglobin and TEC was lower than the normal range. The most common abnormality observed was normocytic normochromic anemia and it was associated with kidney failure. For prospective studies haematological and serum chemistry findings of 31 cases of apparently healthy animals were correlated with the findings of 363 clinical cases. Out of these, 259 were analyzed manually, whereas, 104 cases were analyzed using ADVIA 2120 Hematology System. The erythrocytic abnormalities were classified on the basis of abnormal colour, size and shape of RBC's and it was correlated with the different disease conditions. In the present study, out of the different erythrocytic abnormalities hypochromasia, microcytosis and spherocytosis were the most common abnormalities observed. Based on serum chemistry findings the cases were classified into liver and or kidney damage. A significant positive correlation ($p<0.05$) was observed between occurrence of polychromatophils and liver damage; acanthocytes, stomatocytes and polychromatophils and kidney damage; and occurrence of echinocytes and polychromatophils and concurrent liver and kidney damage. Mean HDW values were significantly higher in dogs suffering from liver damage. A good correlation was between presence of codocytes, schistocytes, echinocytes, polychromatophilic RBCs and leukocytosis; and stomatocytes and polychromatophilic RBCs with leukopenia. In addition, acanthocytes, echinocytes and schistocytes were common in cases of lymphoma.



**Keywords:** Blood, correlation, dogs, erythrocytic abnormalities, hematology, serum chemistry


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

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## ABBREVIATIONS

| | | |
|---|---|---|
| % | : | Percent |
| @ | : | At the rate of |
| < | : | Less than |
| = | : | Equals |
| > | : | Greater than |
| ALKP | : | Alkaline phosphatase |
| ALP | : | Alkaline phosphatase |
| ALT | : | Alanine aminotransferase |
| ARF | : | Acute renal failure |
| AST | : | Aspartate aminotransferase |
| B | : | Basophil |
| BM | : | Bone Marrow |
| BUN | : | Blood urea nitrogen |
| CBC | : | Complete blood count |
| CH | : | Mean hemoglobin content |
| CHCM | : | Hemoglobin concentration mean |
| CPV | : | Canine parvo virus |
| CRF | : | Chronic renal failure |
| DLC | : | Differential leukocyte count |
| E | : | Eosinophil |
| *et al* | : | And others |
| Fig. | : | Figure |
| FNAB | : | Fine needle aspiration biopsy |
| GADVASU | : | Guru Angad Dev Veterinary and Animal Sciences University |
| GGT | : | Gamma-glutamyl transferase |
| H & E | : | Hematoxylin and Eosin |
| Hb | : | Hemoglobin |
| Hct | : | Hematocrit |
| HDW | : | Hemoglobin concentration distribution width |
| IMHA | : | Immune-mediated hemolytic anemia |
| L | : | Lymphocyte |

| | | |
|---|---|---|
| M | : | Monocyte |
| MCH | : | Mean corpuscular hemoglobin |
| MCHC | : | Mean corpuscular hemoglobin concentration |
| MCV | : | Mean corpuscular volume |
| MPV | : | Mean platelet volume |
| N | : | Neutrophil |
| PCV | : | Packed cell volume |
| Q RBC | : | Quatrefoil red blood cell |
| RBC | : | Red blood cell |
| RDW | : | Red cell distribution width |
| SE | : | Standard Error |
| TB | : | Total bilirubin |
| TEC | : | Total erythrocyte count |
| TLC | : | Total leukocyte count |
| TP | : | Total protein |
| TVT | : | Transmissible venereal tumour |
| USG | : | Ultrasonography |
| Viz. | : | Namely |
| WBC | : | White blood cell |

## LIST OF MEASUREMENTS

| | | |
|---|---|---|
| µg | : | Microgram |
| dL | : | Deciliter |
| fL | : | Femtoliter |
| g/dL | : | Gram per deciliter |
| IU | : | International Unit |
| mg/dL | : | Miligram per deciliter |
| pg | : | Picogram |
| U/L | : | Units per liter |

# CHAPTER I

# INTRODUCTION

Hematological examination is often used to corroborate the physical examination and the medical history to provide excellent basis for diagnosis in both medical and veterinary practice (Harvey 2012). This evaluation when done encompasses the haematochemical parameters, blood metabolites and other components of the body and it serves as an important investigative tool in the clinical assessment of the patient (Aderemi 2004, Doyle 2006). Even in the age of molecular analysis, the blood smear examination remains an important diagnostic tool, whenever the results of the complete blood count indicate that the blood smear is essential for the validation or the further elucidation of a detected abnormality (Bain 2005).

Routine haematological examination includes complete blood cell count along with assessment of other leukocytic and erythrocytic abnormalities. Erythrocytic indices and morphology are important in establishing a diagnosis of not only the cause of anemia, but other disorders as well. There is an important correlation between pathologic processes and the presence of erythrocyte morphological abnormalities (Jones 2009, Barger 2010). However, the values are not always reliable since the physiological and haematological parameters of apparently healthy dogs are subject to considerable variations due to factors such as physiological state (e.g. lactation, pregnancy, age, sex, breed, nutrition, seasonal variations, sub-clinical diseases and climate (Awah and Nottidge 1998). Erythrocyte histogram of automated cell counters have been reported to be useful in finding the abnormalities of erythrocytes, asymmetry with a right shift indicated macrocytosis, whereas, asymmetry with a left shift indicated microcytosis (Athansiou *et al* 2018).

Abnormal RBC morphology and splenomegaly have been reported in dogs suffering from haemangiosarcoma (Johnson *et al* 1989). Acanthocytes in dogs have been reported due to changes in plasma lipids (Warry *et al* 2013). Disseminated intravascular coagulopathy (DIC) in dogs is characterized by microangiopathic haemolysis and presence of schistocytes in the blood smear (Hammer *et al* 1991). Vacuolation, acanthocytes, dacrocytes, codocytes, microspherocytes and bizarre shapes of RBC have been reported in dogs and horses suffering from *T. evansi*

infection (Silva *et al* 1995). Dogs suffering from canine pancytopenia have been reported to have normocytic-normochromic anemia (Maylina *et al* 2018).

Oxidative injury to the erythrocytes has been reported to cause hemolytic anemia in dogs (Fierro *et al* 2013,Walter *et al* 2014). Caldin *et al* (2005) reported that dogs having eccentrocytes indicate oxidant injury to red blood cells. Babesiosis in dogs may lead to thrombocytopenia and anisocytosis (Zygner *et al* 2007). Yogeshpriya *et al* (2018) reported decrease in haemoglobin, packed cell volume, total erythrocyte and platelet count with increase in globulin in dogs suffering from *Babesia gibsoni* infection as compared to healthy dogs. Paltrinieri *et al* (1998) studied the metabolic findings in the erythrocytes of cardiopathic and anaemic dogs and found a positive correlation between nucleated RBC's, pyruvate kinase activity and glucose-6-phospahte dehydrogenase activity. Intragastric administration of garlic in dogs has also been reported to be associated with eccentrocyte formation (Lee *et al* 2000). Nassiri *et al* (2005) obsereved that anisocytosis, spherocytosis, polychromasia, thrombocytopenia, hyperbilirubinemia and increased activity of ALT and ALKP were the important hematological and biochemical abnormalities in dog suffering from immune-mediated haemolytic anemia (IMHA). The haematological changes in dogs suffering from lymphoma included anemia along with lymphocytosis (Thangapandiyan *et al* 2013), whereas, thrombocytosis with increased activity of alanine transaminase and alkaline phosphatase along and hypercalcemia have been reported in dogs suffering from hepatocellular carcinoma (Leela-Arporn *et al* 2019).

Although, some studies have been conducted on anemia in dogs, but no comprehensive study has been conducted on erythrocyte indices and abnormalities, and their correlation with different disease conditions of dogs.

Therefore, the present study was envisaged with the following objectives:

- Retrospective evaluation of cases of dogs with erythrocytic abnormalities.
- Detailed prospective study of erythrocytic indices and morphology associated with specific disease condition(s) of dogs.

# CHAPTER II

# REVIEW OF LITERATURE

## 2.1 Prevalence of anemia

Paltrinieri *et al* (1998) recorded metabolic findings in the erythrocytes of cardiopathic and anemic dogs. They evaluated the membrane damage of RBC by measuring the osmotic fragility. They observed severe haemolytic anemia and neutrophilic leukocytosis in the affected dogs. They were of the opinion that values of pyruvate kinase, glucose-6-phosphate dehydrogenase and 2, 3 diphosphoglycerate were important in defining the clinico-haematological picture in clinical heart diseases and haemolytic anemia in dogs.

Useh *et al* (2003) determined the prevalence of anemia in dogs. Out of 5278 mongrel dogs investigated, they reported anemia in 2139 i.e. 40.5 per cent dogs with PCV values ranging from 7 to 36 per cent. They reported that most of the dogs with anemia had parasitic infestation; a few dogs were malnourished, whereas, in others, the cause of anemia could not be ascertained.

Comazzi *et al* (2004) performed 1022 consecutive canine blood tests and analyzed the frequency of associations of the abnormalities. They further compared the results in different age groups. They reported association of anemia with other abnormalities such as leukocytosis, decreased albumin or altered protein concentration which was found to be useful to identify the pathogenesis of anemia.

Singh *et al* (2006) reported that pyometra was one of the most common cause of kidney damage with nephritis in older female dogs. The hematological abnormalities seen in pyometric bitches included anemia, leukocytosis, increased neutrophil count, decreased lymphocyte count and monocyte count. They also observed high values of BUN and hyperproteinemia in the affected dogs.

Goddard and Leisewitz (2010) studied canine parvo virus (CPV) infection and found that during CPV enteritis, leukocyte count was generally depressed with a transient lymphopenia. Anemia was also among the common findings in CPV enteritis, especially in the later phases of the disease. Elevated ALKP activity was also detected in young animals.

Chervier *et al* (2012) studied the cause of anemia, other than acute blood loss, in dogs and determined whether severity of anemia provided clues to the diagnosis. They reported that inflammatory disease and cancer were the most common cause of anemia in dogs. The percentage of dogs with immune-mediated anemia increases with anemia severity, whereas, the percentage of dogs with anemia of inflammatory disease decreased with anemia severity. They concluded that the severity of anemia may provide clues to the diagnosis.

Thangapandiyan *et al* (2013) studied hemato-biochemical changes in canine lymphoma and observed that anemia was persistent finding with varying severity and lymphocytosis in peripheral blood smear.

Paltrinieri (2014) studied that anemia was a frequent condition in dogs and cats. They found that it may be due to reduced production of RBC for primary or secondary bone marrow diseases or due to a reduced RBC lifespan (blood loss or hemolysis).

Salem (2014) found that anemia, leucopenia, neutropenia and lymphopenia as the most common hematologic alterations, along with increase in BUN levels in pups suffering from canine distemper virus (CDV) diarrhea.

Chikazawa and Dunning (2016) described the anemia of inflammatory disease in dogs and cats. Anemia of inflammatory disease (AID) was one of the most common cause in dogs and cats. It was characteristically non- regenerative, of mild–to-moderate severity and was associated with various chronic disorders including infectious, inflammatory, immune-mediated, and neoplastic diseases.

Andrea *et al* (2017) examined the hematological changes in dogs with parvo virus enteritis and reported that anemia was characteristic clinical finding in all dogs suffering from parvo virus enteritis, whereas, leukocyte and platelet abnormality differed in each case. They were of the opinion that hematological finding in parvo virus infected dogs may be helpful in the treatment of animals and improvement in the chances of survival.

### 2.1.1 Regenerative anemia

Ristic and Stidworthy (2002) observed inflammatory bowel disease characterized by severe regenerative microcytic hypochromic anemia.

Cowgill *et al* (2003) reported that the reticulocyte count upto 3-4 per cent can be seen in dogs during 6-8 weeks of age which decreases with age and reaches 1 per cent in adult dogs.

Mortier *et al* (2012) performed a retrospective study on canine lymphoma and found that 56 dogs had lymphoma based on the cytological and or histological results. Regenerative anemia and thrombocytopenia were evident, serum biochemistry values of ALKP showed increase in 50% of the cases.

Jardes *et al* (2013) described the clinical presentation and case management of a dog that developed hemolytic anemia and evidence of renal tubular dysfunction after ingestion of a natural hair dye containing Lawsonia inermis. The dog was showing signs of lethargy, vomiting, diarrhea, and weakness. A serum biochemistry profile, complete blood count, and urinalysis demonstrated evidence of renal tubular dysfunction and a regenerative anemia without spherocytosis. They concluded that henna ingestion was associated with the development of hemolytic anemia and acute kidney injury.

Moretti *et al* (2015) investigated the association between normoblastemia and other hematological changes potentially consistent with regenerative response to anemia. They observed that normoblastemia is detected in routine analysis of blood smears undergoing chemotherapy. They were of the opinion that morphological analysis of blood smears, followed by correction of total and differential WBC counts is mandatory.

Fuchs *et al* (2017) evaluated the clinical utility of reticulocyte hemoglobin equivalent (RET-He) in the diagnosis of different causes of iron-deficient erythropoiesis (IDE). They found that the majority of dogs with low RET-He values had anemia with microcytosis and hypochromasia.

Boyd and Best (2018) studied that Miniature Poodle was found to have a persistent erythrocyte macrocytosis and reticulocytosis with a normal and stable HCT. They reported that macrocytosis or Howell-Jolly bodies, and metarubricytosis, in the absence of anemia or cytopenias, along with bone marrow erythroid dysplasia, including megaloblastosis, binuclearity, increased mitotic activity, and nuclear fragmentation, were suggestive of congenital dyserythropoiesis also called as poodle macrocytosis.

Gori *et al* (2018) conducted retrospective study which include 90 dogs having Systemic Inflammatory Response Syndrome (SIRS), 50 healthy and 50 dogs with chronic diseases. They evaluated anemia and nucleated RBCs (NRBCs) in canine (SIRS) compared to the severity of illness and outcome. Mild-moderate, micro-normocytic normochromic anemia was a frequent finding in canine SIRS.

### 2.1.2 Non- regenerative anemia

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

Raskin and Messick (2012) studied the importance of bone marrow (BM) as a diagnostic tool and its various indications in small animal practice. They suggested that BM analysis should be done in following conditions- persistent, poorly non-regenerative anemia, thrombocytopenia and presence of abnormal cells in the peripheral blood smears.

Shah *et al* (2013) reported microcytic hypochromic, non-regenerative anemia, lymphopenia, sometimes associated with either leukopenia or leukocytosis often with shift to left and thrombocytopenia in dogs suffering from canine parvo virus infection. They also observed that the serum levels of blood urea nitrogen (BUN), creatinine, alkaline phosphatase (ALP) and alanine aminotransferase (ALT) were elevated, while the total protein (TP) and albumin levels were decreased in these dogs.

Lazo *et al* (2018) described the clinicopathological findings of dogs naturally infected with *Leishmania infantum*. They reported that the most frequent clinicopathological abnormalities included mild to moderate non-regenerative anemia, lymphopenia, hyperprotinemia, dysprotinemia and proteinuria. They concluded that anemia and protein related alterations were common in dogs infected with canine leishmania.

Maylina *et al* (2018) studied canine pancytopenia with normocytic-normochromic anemia and evaluated clinical studies, treatment, and survival of dogs. All the affected dogs showed a normocytic normochromic anemia and non-

regenerative anemia. In addition, three Shepherd dogs revealed pancytopenia along with normocytic anemia.

Stanley *et al* (2018) reported that megaloblastic, non-regenerative anemia in human was due to cobalamin or folate deficiencies but it was not observed in hypocobalaminemic or hypofolatemic dogs. They reported that there is no association between cobalamin and folate deficiency and megaloblastic non-regenerative in dogs as reported in humans.

### 2.1.3 Immune-mediated hemolytic anemia (IMHA)

Lobetti and schoeman (2001) reported an association between *Ancylostomum caninum* and IMHA. They observed moderate to severe anemia, reticulocytosis, spherocytosis and auto-agglutination in dogs suffering from *Ancylostomum caninum* infection.

Nassiri *et al* (2005) observed the prevalence of IMHA in anemic dogs by employing Coomb's test. They reported that the mean age of dogs with IMHA was 3.7 years affecting mostly Terriers and German Shepherd and more incidence of IMHA in female than the males. The main cause of IMHA was pyometra and drug toxicities.

Balch and mackin *et al* (2007) studied immune-mediated hemolytic anemia was a common type of anemia in dogs and cats that results from a type 2 hypersenstivity reaction. They suggested the presence of hemolytic anemia in a young adult or middle aged dog of a predisposed breed, autoagglutination and or spherocytosis, positive results from a direct antiglobulin (Coomb's test).

Ong *et al* (2015) concluded that IMHA was a potential complicating factor of elapid snake evenomation and its treatment in dogs, and should be considered as a differential diagnosis for a persistent or worsening anemia.

Zoia *et al* (2018) studied whether dogs with immune-mediated hemolytic anemia (IMHA) had low plasma mean platelet component (MPC) concentration and whether MPC was associated with outcome. They observed in dogs with IMHA, platelets appeared to have been activated to a greater degree, as determined by lower plasma MPC concentrations, than in healthy dogs or sick dogs without IMHA. Plasma MPC concentration at initial examination may be useful for predicting prognosis in dogs with IMHA.

## 2.2 Erythrocytic Indices

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

Bain (2005) concluded that even in the age of molecular analysis, the blood smear remains an important diagnostic tool. They were of the opinion that blood smear must be examined whenever the results of the complete blood count indicate that the blood smear was essential for the validation or the further elucidation of a detected abnormality.

Grimes and Fry (2014) developed the customized morphology flagging thresholds for canine erythrocyte volume and hemoglobin concentration on the ADVIA 120 hematology analyzer; compare automated morphology flagging with results of microscopic blood smear evaluation; and examine effects of customized thresholds on morphology flagging results. They were of the opinion that customized morphology flagging thresholds resulted in more sensitive detection of microcytosis, macrocytosis, and hypochromasia than default thresholds.

Adili *et al* (2017) investigated the influence of the age, the sex and the breed on the diameter, the circumference and the surface area of red blood cells in the dog. However, the breed seemed to have a significant effect only on the diameter of erythrocytes, in this case: red blood cells of local Sloughi dogs were bigger than those of the German Shepherd. They reported that the breed had no influence on the circumference and surface area of erythrocytes. They were of the opinion that new reference values should be used for the circumference and the surface of erythrocytes in Sloughi and German Shepherd dogs.

Silva *et al* (2017) discussed the relationship of MCV with surface area and diameters (vertical and horizontal) in 2D views of erythrocytes of clinically healthy dogs using confocal microscopy. Morphometry using a laser scanning confocal microscope showed that diameters and surface area of canine erythrocytes did not

correspond with the MCV value has limitations as an objective measurement of detecting anisocytosis.

### 2.3 Classification of Erythrocytic abnormalities

#### 2.3.1 On the basis of colour

Nassiri *et al* (2005) studied the hematological and biochemical findings of IMHA which included the anisocytosis, spherocytosis, polychromasia, thrombocytopenia, hyperbilirubinemia and increased activity of ALT and ALKP.

Zygner *et al* (2007) observed the hematological changes in blood samples from dogs infected with large babesia. The most common disorders were thrombocytopenia and anisocytosis. Anemia was present in 29 per cent, increased MCHC in 21 per cent, anisocytosis in 60.5 per cent, poikilocytosis in 25 per cent, polychromasia in 23.8 perc cent, hypochromasia in 19.7 per cent, erythroblastosis in 4.45 per cent, thrombocytopenia in 99.5 per cent, increased MCV in 15.3 per cent, neutropenia in 36.3 per cent, left shift in 21.85 per cent, lymphocytosis in 14.9 per cent and lymphopenia in 7.2 per cent cases.

#### 2.3.2 On the basis of size

Athanasiou *et al* (2018) studied the histograms of complete blood counts in dogs. They observed that erythrocyte histogram showed asymmetry with right shift, and macrocytosis; when it present a left shift, showed microcytosis and in case of blood transfusion, two peaks were founded in the curve. They were of the opinion that histogram helped in finding the abnormalities in the distribution curve that correspond to abnormalities in the size or number of cells, and made diagnostic or therapeutic decisions that were important in emergencies.

#### 2.3.3 On the basis of erythrocytic morphology

Hebbel *et al* (1980) studied that the abnormal shape and poor deformity of sickle erythrocyte have generally been held responsible for the microvasculature occlusions of sickle cell disease. They concluded that sickle RBCs adhere to vascular endothelial cells in vitro, perhaps caused by a calcium-induced aberration of membrane topography.

Hirsch *et al* (1981) studied a retrospective study on cases of canine hemangiosarcoma and found that it was most common in older dogs. They observed the association of acanthocytosis with hepatic disease noted in both man and the dog.

They further reported that the hepatic disease was not a constant feature in hemangiosarcoma-related acanthocytosis.

Rebar *et al* (1981) studied the cell fragmentation in the dogs. They reported the categories which include microangiopathic fragmentation, spherocytic fragmentation, Heinz body fragmentation, metabolic fragmentation associated with systemic disease, and artifactual fragmentation. They reported that all the types of red cell fragmentation observed in dogs were also observed and documented in man.

Smith *et al* (1983) studied the persistent elliptocytosis in a dog. The dog had membrane protein band 4.1 deficiency, microcytosis, shortened erythrocyte lifespan, increased osmotic sensitivity and a mild glutathione deficiency. Erythrocyte deformability and membrane stability were adversely affected. The dog had protien band 4.1 deficiency was associated with elliptocytosis. They observed that this disorder in dogs closely resembles human patients with band 4.1 deficiency and should provide a valuable animal model to study the erythrocyte membrane cytoskeleton.

Canfield *et al* (1987) examined the peripheral blood and revealed intraerythrocytic crystals, granulation of erythrocytes, nucleated erythroid cells, reticulocytosis and marked variation in erythrocyte morphology in the absence of anemia. They were of the opinion that bone marrow examination revealed sideroblasts and evidence of enhanced intramedullary destruction of erythroid cells.

Akuzawa *et al* (1989) performed the hematologic examinations, osmotic fragility tests and scanning electron microscopy of erythrocytes in dogs given beta-acetylphenylhydrazine. They observed that echinocytes were increased with a peak level of 47.6% of treatment. They also detected increased numbers of acanthocytes and schizocytes.

Johnson *et al* (1989) studied haematological changes in dogs having splenic neoplasia which included anemia, nucleated RBC, abnormal RBC morphology and splenic rupture. They conducted ultrasonography which showed splenomegaly. They concluded that hemangiosarcoma was the most common cause of splenomegaly in dogs, and it was an important cause of abnormal RBC morphology.

Hammer *et al* (1991) observed haematological abnormalities in 24 dogs suffering from hemangiosarcoma and confirmed it through evaluation of

haematological profiles. They evaluated microangiopathic haemolysis through the presence of schistocytes and DIC. They reported that DIC is likely a complication of hemangiosarcoma resulting in fragmentation of RBC's.

Brown *et al* (1994) interpreted that the macrocytosis and histogram skewing were not likely due to accelerated erythrocyte regeneration. Although previously reported cases of this disorder in Miniature Schnauzers had mild reticulocytosis, and erythrocyte survival was moderately shortened in one other case of a clinically normal Miniature Schnauzer with stomatocytosis.

Silva *et al* (1995) studied the pathogenesis, haematological and clinical aspects of *Trypanosoma evansi* infection in dogs and horses. They reported that *T. evansi* caused pronounced leukopenia in dogs and also causes microcytic and hypochromic anemia. The pathological changes associated with it included vacoulation, acanthocytes, dacrocytes, codocytes, microspherocytes and bizarre shapes.

Turchetti *et al* (1997) evaluated the erythrocytic morphology in different pathologies which could affect flowing red cells. They found that morphological alterations of RBCs were seen in the patients suffering from chronic hepatopathies with cholestasis.

Walton *et al* (1997) studied the model of echinocytosis induced by *Crotalus atrox* venom. They characterized erythrocyte morphologic changes and investigated the potential mechanisms of echinocytic transformation. They concluded that echinocytosis induced by rattle snake venom is related to the degree of venom exposure and it was could be correlated clinically with the amount of venom absorbed.

Lee *et al* (2000) observed haematological changes associated with the appearance of eccentrocytes after intragastric administration of garlic extract to dogs. They found Heinz body formation, reduced glutathione concentration and eccentrocyte formation, but they did not observe haemolytic anemia. They were of the opinion that garlic had a potential to oxidize erythrocyte membranes and haemoglobin, inducing haemolysis associated with the appearance of eccentrocytes in dogs.

Berezina *et al* (2001) studied the sequence of morphological changes in RBC's during haemorrhage using microscopic morphometry and scanning electron microscopy. They reported that the morphological changes in RBC during haemorrhagic shock were similar to those observed in aging.

Hackett *et al* (2002) studied the retrospective study to identify the clinicaly relevant variables and treatments for dogs bitten by Prairie Rattlesnakes. Principal initial laboratory findings were echinocytosis, thrombocytopenia, leukocytosis and prolonged activated clotting time.

Bonfanti *et al* (2004) described the hematologic findings and RBCs characteristics in seven closely related Schnauzers with stomatocytosis. Percentage analysis suggested that stomatocytosis in Schnauzers was a hereditary component.

Caldin *et al* (2005) studied that eccentrocytes are RBCs that appear in a peripheral blood smear to have their hemoglobin shifted to one side of the cell. Eccentrocytes have been reported rarely in dogs and are associated with onion and garlic ingestion and the administration of oxidant drugs. They concluded the associations between eccentrocytes and diabetes mellitus, T-cell lymphoma and vitamin K antagonist intoxication in dogs.

Masserdotti (2009) studied that on bitten by viper snake, moderate numbers of unusual erythrocyte membrane-like structures ("erythroid loops") were observed. The loops were annular in shape and sometimes disrupted, appearing as thin pale blue bands. The unique appearance of the erythroid loops together with evidence for intravascular hemolysis and other erythrocyte morphologic changes suggest they may be a consequence of erythrocyte lysis. They observed echinocytes, spherocytes, and erythrocyte ghosts are known to result from the action of phospholipase in Viper venom.

Terlizzi *et al* (2009) evaluated persistent elliptocytosis in a dog. They detected a β- spectrin mutation in codon 2110 in which threonine was replaced by methionine. This mutation altered the molecular structure of the erythrocyte membrane leading to impared spectrin self-association and elliptocyte formation.

Fierro *et al* (2013) determined whether Skunk musk induces oxidative hemoglobin damage in vitro. They concluded that in vitro, Skunk musk causes Heinz body and methemoglobin formation in canine, feline, and Red Panda RBC. They

reported the clinical association between Heinz body hemolytic anemia and Skunk spray exposure.

Lubas *et al* (2013) documented that ‘‘Quatrefoil’’ red blood cells (RBCs) have been seen in blood smears using both optical and scanning electron microscopy. They compared with populations of dogs without ‘‘quatrefoil’’ RBCs, dogs with this RBC pattern are significantly more likely to exhibit lower total leukocyte and neutrophil counts. They described the origin of ‘‘quatrefoil’’ RBCs include artifact effects, dacryocyte overlapping, and the effect of adhesion forces between two circulating RBCs in the blood stream.

Warry *et al* (2013) studied the disease processes associated with the presence of acanthocytes in the peripheral blood of dogs. They observed that acanthocytosis was observed with a variety of neoplastic and non-neoplastic diseases. They suggested that the presence of acanthocytes in a blood smear should not be considered pathognomonic for hemangiosarcoma in dogs.

Gavazza *et al* (2014) observed the presence of two RBCs pattern on canine blood smear at optical microscope during evaluation of RBCs. They studied that q RBCs are associated with aging of dogs, total leukocyte and neutrophil count, anisocytosis, polychromasia and Howel-Jolly bodies.

Walter *et al* (2014) studied the oxidant-induced damage to equine erythrocytes from exposure to *Pistacia atlantica, Pistacia terebinthus, and Pistacia chinensis*. The findings suggested that *P. atlantica, P. terebinthus, and P. chinensis* can cause acute poisoning in horses.

Adekola *et al* (2015) evaluated the haematological parameters and morphological changes in the erythrocytes of dogs. They collected blood samples from 64 apparently healthy dogs and observed major morphological abnormalities which included echinocyte, macrocyte, spherocyte and eccentrocyte while other abnormalities such as acanthocyte, leptocyte, schizocyte and codocyte were also observed. They studied baseline information on the haematological parameters and studied the novel correlation of the associated erythrocyte abnormalities. They were of the opinion that the qualitative examination of the morphological changes when compared with the quantitative evaluation of the haematological parameters can also be employed as an important diagnostic tool in canine practice.

Krek *et al* (2015) studied the effect of carbon black agglomerated nanomaterial on biophysical properties of canine red blood cell and platelet membranes that are reflected in changes of cell shape. They observed the relative abundance of different erythrocyte shape types (discocytes, echinocytes, and spherically shaped erythrocytes) on populations of cells, in suspensions with added carbon black nanomaterial and in control suspensions. They were of the opinion that the two dimensional mathematical model of the erythrocyte shape was constructed to illustrate and explain erythrocyte swelling of intially discocytic/echinocytic shape to the final spherical shape, which preceeds membrane rupture.

Aniolek *et al* (2017) studied the frequency and intensity of asymptomatic anisocytosis using a three- grade scale in Japanese dog breeds with special emphasis on indices. They observed that in comparison to mixed breed dogs, dogs of Japanese breeds have reduced MCH, MCHC and significant anisocytosis in the blood smear and a higher RDW indicator.

Lenske *et al* (2018) observed severe hemolysis and spherocytosis in a dog envenomed by a red-bellied black snake and successful treatment with a bivalent whole equine IgG antivenom and blood transfusion.

Pindev and Krastev *et al* (2018) studied life-threatening anaemia in a dog, of the Pug breed, accompanied by splenomegaly. They observed single erythrocyte cells resembling target or cocardial cells and IMHA.

### 2.4 Serum Chemistry Findings

#### 2.4.1 In the cases of liver damage

Schlesinger and Rubin (1993) showed that the serum bile acids (SBA) test is a very sensitive and specific indicator of hepatic function. An increased concentration of SBA in the absence of jaundice or hepatic enzyme elevations suggests occult liver disease. Abnormal SBA in the presence of jaundice can distinguish between prehepatic (hemolysis) and hepatic jaundice. Abnormal serum concentrations of both hepatic enzymes and bile acids were found with hepatic necrosis, lipidosis, hepatitis, neoplasia, bile duct obstruction, and cholestasis.

Lester *et al* (2016) objective was to characterize the clinical presentation and outcome of dogs with acute liver failure. Common clinicopathologic abnormalities on presentation other than hyperbilirubinemia and increased serum liver enzyme activity

included thrombocytopenia (25/49, 51%), hypoalbuminemia (23/49, 46%), leukocytosis (17/49, 34%), anemia (14/49, 29%), hypokalemia (13/49, 27%), and hypoglycemia (10/49, 20%). The causes of ALF included neoplasia (13/49, 27%), presumptive leptosporosis (4/49, 8%) and ischemia (1/49, 2%). Canine ALF is associated with a grave prognosis. They suggested that besides targeting deteriorating hepatocellular function with specific antidotes and supportive care strategies, better characterization of the coagulopathy associated with ALF and strategies to prevent the development of ascites, gastrointestinal, and renal dysfunction are warranted.

**2.4.2 In cases having kidney damage**

Mandal *et al* (1991) examined that whether renal protection was due to prevention of red blood cell echinocyte formation and resultant renal PTC congestion. Echinocytes (burr cells) are poorly deformable, impart high viscosity to the blood, and may hinder reperfusion by increasing resistance to renal capillary blood flow. Splenectomized animals showed a marked and sustained decrease in creatinine clearance, consistently elevated serum creatinine levels and fractional excretion of sodium and diffuse PTC congestion with echinocytes. These animals had a peak in circulating echinocytes which showed an excellent negative correlation with creatinine clearance.

King *et al* (1992) evaluated the incidence, type and etiology of anemia in chronic renal failure (CRF). A non- regenerative, normochromic, normocytic anemia was seen and they found direct correlation between degree of anemia and extent of CRF as assessed by serum creatinine concentrations. They concluded that red blood cell morphology was normal, with no burr cells, echinocytes, or schistocytes seen along with platelets and WBC count was also in range.

Kogika (2014) studied the indicators of oxidative stress and intraerythrocytic antioxidant defense in dogs with anemia of CKD. Anemia of CKD dogs was nonregenerative (reticulocytes $\leq 0.2\%$ with scarce anisocytosis and poikilocytosis).

Bover *et al* (2018) analyzed the correlation and potential discrepancies with parathyroid hormone and bone turnover and most importantly the valuable recent association of alkaline phosphatases (ALKPs) with cardiovascular disease or vascular calcification. They suggested that ALKPs level cannot be used alone as an isolated primary target in the treatment of chronic kidney disease - mineral and bone disorders.

Rudinsky *et al* (2018) showed that chronic kidney disease (CKD) was associated with morbidity and mortality in dogs. Plasma fibroblast growth factor-23 (FGF-23) concentration is an independent predictor of CKD progression and survival in cats and people with CKD. To investigate the relationship among FGF-23, parathyroid hormone (PTH), vitamin D metabolites, and other clinical variables with survival time in dogs with CKD. In conclusion, increased FGF-23 concentration was associated with an increased risk of premature death in dogs with azotemic CKD as well as previously identified prognostic markers of proteinuria, hyperphosphatemia, advanced CKD stage and body composition. Future studies are necessary to determine the role of FGF-23 as a marker of disease severity versus mediator of disease progression in dogs with CKD and how it relates to prognosis.

### 2.4.3 On the basis of concurrent liver and kidney damage

Abinaya *et al* (2018) reported that fifteen obese Labrador dogs of more than 3 years age of either sex were selected and were grouped according to their age groups, viz., 3-5 years, 5-8 years and above 8 years. After physical examination, blood samples were collected and analysed for the biochemical parameters including glucose, cholesterol, alanine aminotransferase, BUN, creatinine, total protein, albumin and globulin. Compared with the established reference range, the results of the study revealed that the normal concentration of biochemical parameters including glucose, BUN, creatinine, albumin and ALT within the normal range. But the concentrations of cholesterol and globulin were gradually increased in obese animals as age advances. Aging had also increased serum total protein and decreased serum albumin gradually.

## 2.5 Hemoprotozoan and Ricketsial Infections

Chaudhuri *et al* (2008) studied the erythrocyte lipid peroxide and erythrocytic antioxidant levels in dogs with clinical babesiosis, caused by *Babesia gibsoni*, and impact of the disease on blood iron, zinc and copper levels. The study demonstrated oxidative damage in dogs naturally infected with *B. gibsoni*. Low level of blood iron, zinc and copper seems to have an additional role in the genesis of anemia and oxidative stress.

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

Shipov *et al* (2008) studied indicators for monocytic ehrlichiosis. The hematological findings were anemia, leucopenia, thrombocytopenia and serum biochemical changes like hypoalbuminemia, increased ALP and lactate dehydrogenase, hyperglobinemia, increased AST, hypokalemia and increased creatinine kinase.

Konto *et al* (2014) studied clinicopathological findings in dogs infected with *B.canis* and found that most clinical signs recoreded were ascites and edematous swelling of the whole body. There was also increase in ALP and ALT values.

Zamokas *et al* (2014) studied that babesiosis was characterized by marked thrombocytopenia, neutrophilic profile change to lymphocytic and monocytosis. In addition, there was a tendency of anemia and leukopenia.

Prashar *et al* (2015) studied clinical, biochemical and hematologic markers in natural infection of canine monocytic ehrlichiosis and found depression, anorexia, pyrexia, anemia, weakness, jaundice, melena, vomition and diarrhea as the main clinical symptoms. Hematological changes were pancytopenia mainly thrombocytopenia and significant changes in CBC and ALT values.

Islam (2017) reported that canine babesiosis was characterized by hemolytic anemia, thrombocytopenia, fever, and splenomegaly. The haematological examination revealed anaemia, thrombocytopenia and neutrophilia.

Kushwaha *et al* (2017) studied the microscopic examination of Giemsa stained blood smears revealed presence of small pear-shaped, oval or signet ring shaped *B. gibsoni* within the erythrocytes of 1.34 % dogs.

Ameldev and Tresamol (2018) reported that Mycoplasmas were one of the smallest bacteria and are divided into two groups, haemotropic and non-haemotropic mycoplasmas. Hemotrophic mycoplasmas (hemoplasmas) are uncultivable cell wall-less bacteria, previously known as *Haemobartonella* and *Eperythrozoon species*. Hemoplasmas can cause acute hemolytic anemia in dogs and cats.

Dear *et al* (2018) studied that *Babesia conradae* is an intraerythrocytic piroplasm infecting dogs in the southern United States. *Babesia conradae* infection dogs lead to anemia, leukopenia, thrombocytopenia, hypoalbuminemia and hyperglobulinemia. As with *B. gibsoni,* aggressive interactions with other canids may

play a role in *B. conradae* transmission. Infection with *B. conradae* should be considered a differential diagnosis for any dog with these signs, particularly when associated with a history of exposure to coyotes or potentially other wild canids.

Lannino *et al* (2018) studied that Bartonellae are haemotropic gram‑negative bacteria that parasitize the erythrocytes and endothelial cells of mammalian hosts and are highly adapted to facilitate intracellular persistence. The exception to this rule is *B. bacilliformis*, which triggers massive haemolysis of colonised human erythrocytes, giving rise to an often fatal haemolytic anemia on intracellular Bartonella. In vitro infection of human CD34 +progenitor cells with *B. henselae* suggests that these bacteria are capable of infecting bone marrow progenitor cells, which may contribute to ongoing erythrocytic infection.

Petrov *et al* (2018) studied that thrombocytopenia, anemia and hypoalbunimea were detected in patients with canine monocytic ehrlichiosis. They observed that treatment with doxycycline results in increase of RBCs, platelets, hemoglobin, hematocrit and albumin in dogs naturally infected with *E.canis*.

Yogeshpriya *et al* (2018) reported the changes in clinical signs, haematology, serum biochemistry and ultrasonographic changes in dogs affected with *Babesia gibsoni*. Splenomegaly was a common finding in most of the cases. Haematology and serum biochemistry revealed a significant decrease in haemoglobin, packed cell volume, total erythrocyte and platelet count, significant increase in globulin in affected dogs when compared to healthy dogs.

**2.6 Neoplastic Conditions**

Senthil *et al* (2015) studied the case of a *Hepatozoan canis* infection in a dog with a sexually transmissible venereal tumour was reported. Haematological examination revealed marked decrease in haemoglobin, PCV and RBC counts and the blood smear revealed rouleaux formation of RBCs, hypochromasia, leptocytes and neutrophilia.

El-Baky (2017) reported that the canine lymphoma was the most common hematologic malignancy in dogs, and in many aspects comparable to non-Hodgkin lymphoma in humans. It was characterized by the involvement of multiple lymph nodes and the infiltration of various organs especially liver and spleen by neoplastic lymphocytes.

Das *et al* (2017) evaluated the hemato-biochemical alterations, urinalysis along with histomorphological and histological changes of prostate glands in dogs affected with benign prostatic hyperplasia. The serum biochemical analysis revealed non-significant increase in creatinine and BUN with a significant decrease in total protein, albumin, globulin and A: G ratio.

Duda *et al* (2017) identified paraneoplastic laboratory abnormalities in several types of cancers in dogs and cats. They evaluated hematological, biochemical, and hemostatic abnormalities and correlated them with mammary tumor staging in female dogs with mammary cancer. They observed anemia, neutrophilic leukocytosis, monocytosis, eosinophilia, thrombocytosis, hypoalbunimea, hypocalcemia, hypoglycemia and low blood urea nitrogen in the affected animals.

Banerjee *et al* (2018) conducted the usefulness of hemato-biochemical study as a reliable prognostic factor in canine malignant mammary tumour. A total twenty-six bitches with malignant mammary tumours were studied. Hematological parameters like Hb, PCV, TEC and biochemical parameters viz. total protein, albumin, globulin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALKP) were analyzed.

Hristov *et al* (2018) studied the hematological studies in dogs with mammary gland carcinoma at different stages of development. They showed anemia with erythropenia, without significant changes in erythrocyte indices, thrombocytopenia and leukocytosis and slight variation in differential white blood cell count.

Arporn *et al* (2019) studied that hepatocellular carcinoma (HCC) was the most common primary liver tumour in dogs. They investigated the clinical features and risk factors for canine HCC. Thrombocytosis, increased activity of alanine transferase and alkaline phosphatase, and hypercalcemia were observed in cases of HCC.

# CHAPTER III

# MATERIALS AND METHODS

## 3.1 Analysis of the retrospective data for erythrocytic abnormalities

The retrospective analysis of clinical data of 7,375 blood samples of dogs presented to Small Animal Clinics of the Department of Veterinary Clinical Services Complex, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU) Ludhiana w.e.f. 1st January, 2016 to 31st December, 2017 was done. The hematological parameters viz. Hemoglobin (Hb), Packed cell volume (PCV), Total erythrocyte count (TEC), Total leucocyte count (TLC) and Differential leucocyte count (DLC) were recorded and mean and median values of each parameters were calculated. In addition, on the basis of record available, important disease conditions in the dogs were recorded.

## 3.2 Prospective studies on erythrocytic abnormalities

### 3.2.1 Source of Samples

The study was conducted on blood samples of dogs presented to Centralized Clinical Diagnostic Laboratory in the department of Teaching Veterinary Clinical Complex, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU) Ludhiana, w.e.f. from 1st July, 2018 to 31st March 2019. A total of 363 cases of dogs were analyzed. Out of these, 259 were analyzed manually and 104 cases were analysed using ADVIA 2120 Hematology System (Siemens, USA).

### 3.2.2 Signalment and patient history

Complete information about each case was recorded and the following details were noted.

a) Name and address of the owner.
b) Breed.
c) Age of the dog.
d) Sex.
e) Time since affection was observed/ duration of illness.
f) Clinical signs observed by the owner.
g) Diet of the dog.
h) Earlier treatment if any, vaccination status and deworming status.

In addition serum chemistry, ultrasound and radiography findings, were also recorded wherever possible.

### 3.3 Analysis of blood samples

#### 3.3.1 Hematology

The hematological parameters analyzed manually included hemoglobin (Hb), total leucocyte count (TLC) and differential leucocyte count (DLC). The DLC was performed manually on blood smear stained by Leishman's method (Jain 1986). In 104 cases, complete blood cell count was determined using ADVIA 2120 Hematology System and different parameters viz. White blood cell count (WBC), Red blood cell count (RBC), Hematocrit (HCT), Mean corpuscular volume (MCV),Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Hemoglobin concentration mean (CHCM), Mean hemoglobin content (CH), Red cell volume distribution width (RDW), Hemoglobin concentration distribution width (HDW) and Mean platlet volume (MPV) were recorded. However, the DLC was done manually as per the method described above.

In addition, thirty one samples of apparently healthy animals presented for vaccination and routine health check up to the Centralized Clinical Diagnostic Laboratory in the department of Teaching Veterinary Clinical Complex, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU) Ludhiana were also analyzed and all the above parameters were recorded. The values were taken as control values for the comparision with dogs suffering from disease conditions.

#### 3.3.2 Blood smear examination

A thorough examination of all the 363 stained blood smears was done to study the morphological and staining characteristic of erythrocytes and abnormal inclusions. In each case a total of 500 RBC's were counted from the 10 random fields and then percentage of each erythrocytic abnormality was calculated. The morphological abnormalities with respect to change in colour of RBC i.e. polychromasia and hypochromasia and the abnormalities on the basis of changes in size of erythrocytes viz. microcytic, macrocytic and normocytic were also recorded. In addition, the blood smears were also screened for various abnormalities in shape viz. schistocytes, keratocytes, acanthocytes, echinocytes, spherocytes, eccentrocytes, codocytes,

stomatocytes, elliptocytes, blister cells, incomplete Spherocytes, nucleated RBCs, polychromatophilic RBC, quatrefoil RBCs (q RBCs) and dacrocytes.

### 3.3.3 Special Staining

#### 3.3.3.1 Staining for Reticulocyte

Reticulocyte count was also done using New Methylene Blue stain (Merck company). Two or three drops of the staining solution were added to the blood containing pipette and was mixed. The mixture was kept at 37º C for 15-20 minutes, then the red cells was resuspended by gentle mixing and the smear were made on clean glass slide, air dried. Smears were also counterstained with Leishmans solution for better visualization. The reticulocytes numbers were counted per 1000 RBC under oil immersion and their percentage was calculated. A corrected reticulocyte count was calculated as per the method described by Cowgill *et al* 2003.

Corrected reticulocyte count = % Reticulocyte count × TEC

A reticulocyte count of more than 1.5 per cent was used to indicate regenerative anemia and a reticulocyte count below 1 per cent indicate non- regenerative anemia (Tvedten 2004).

In addition, the blood smears were also screened for the presence of abnormal inclusions and hemoprotozoa.

### 3.4 Ancillary diagnostic techniques used

#### 3.4.1 Leukogram analysis

Leukogram analysis was carried out in all the 363 cases and detailed leukocytic abnormalities were recorded. The cases were categorized into those having normal total leukocyte count, leukopenia, leukocytosis and finally the cases having leukemoid response.

#### 3.4.2 Serum chemistry analysis

Attempts were made to collect blood samples in gel vaccutainers from each case for serum chemistry analysis. The vaccutainers were kept undisturbed at an angle of 30 degree, at a room temperature for 30-60 minutes and blood was allowed to clot. Serum was harvested by centrifugation at 3000 rpm for 15 minutes in (R-8CBL Laboratory Centrifuge, Remi company) and stored at -20 degree until analysis. Serum concentration of different parameters like total bilirubin (TB), aspartate transaminase

(AST), alanine transaminase (ALT), alkaline phosphatase (ALKP), total protein (TP), albumin, Gamma-glutamyl transferase (GGT), Blood urea nitrogen (BUN) and creatinine were determined by VITROS 350 Chemistry System, Ortho- Clinical Diagnostics, (Johnson and Johnson, USA) available in the Centralized Clinical Diagnostic Laboratory in the department of Teaching Veterinary Clinical Complex, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU) Ludhiana using standard kits. The values of serum biochemicals of diseased dogs were compared with normal refrence values. On the basis of serum chemistry findings the cases were segregated into cases having liver damage, kidney damage and the cases having concurrent liver and kidney damage.

**Table 1: Serum Biochemical Parameters and their normal ranges in dogs**

| **Parameter** | **Range (Units)** |
|---|---|
| Total bilirubin | 0.1-0.6 (mg / dL) |
| Total protein | 5.5-7.5 (g/ dL) |
| Albumin | 2.6-4 (g/ dL) |
| Aspartate aminotransferase (AST) | 8.9-49 (U/L) |
| Alanine aminotransferase (ALT) | 8.2-57 (U/L) |
| Alkaline phosphatase (ALKP) | 10.6-101 (U/L) |
| Gamma-glutamyl transferase (GGT) | 1.9-2.7 (U/L) |
| Blood urea nitrogen (BUN) | 8.8-25.9 (mg/ dL) |
| Creatinine | 0.5-1.6 (mg/ dL) |

### 3.4.3 Ultrasonography

The ultrasound examination of selected dogs was performed using Philips Affinity 70 machine with 5-12MHz pure wave linear transducer available in the Department of Veterinary Surgery and Radiology, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU) Ludhiana. Ultra-sonographic examination was performed and the detailed ultra sonographic findings in respect of shape (round, oval or irregular), margin (circumscribed, not circumscribed), echogenicity (hypoechoic, isoechoic, hyperechoic or anechoic), presence of distal acoustic enhancement or shadowing were recorded, wherever possible.

### 3.4.4 Radiographic examination

Radiographic examination of dogs was also performed in selected cases using X-ray facilities available in Department of Veterinary Surgery and Radiology. Detailed radiographic abnormalities in different organs were recorded. The detailed findings were recorded, wherever possible.

### 3.4.5 Cytological examination

Cytological examination of the smears submitted to the Centralized Clinical Diagnostic Laboratory was also performed. The smears were stained by Leishman stain. On the basis of cytological examination, the cases were classified into neoplastic and non-neoplastic conditions.

### 3.4.6 Post-Mortem examination

Five dogs died during the present study but post-mortem examination could be conducted only in one case as the owners did not give consent for post-mortem examination in four cases. A thorough post-mortem examination was conducted in one case and gross lesions in different organs were recorded.

### 3.4.7 Histopathological examination

At necropsy the tissue samples from the organ showing lesions were collected and preserved in 10% neutral buffered formalin. The samples were processed for paraffin section and were stained by routine Hematoxylin and Eosin technique (Luna 1968). The slides were examined using Olympus BX61 microscope (Japan) available in the department and detailed microscopic changes in different organs were recorded.

## 3.5 Statistical analysis

The data pertaining to different parameters was analyzed using Statistical Analysis Software (SAS-version 9.3, Institute, CARY, USA) available in the Department of Animal Genetics and Breeding, GADVASU, Ludhiana.

The comparison between the normally distributed quantitative variables was done using ANOVA along with Duncan correction. In addition, the correlation and regression analysis was also carried out and the association between different erythrocytic abnormalities and disease conditions of dogs was calculated.

# CHAPTER IV

# RESULTS AND DISCUSSION

## 4.1 Retrospective analysis of the clinical cases

During the present study, retrospective data analysis of clinical cases of 7, 375 dogs presented to Small Animal Clinics of the Department of Veterinary Clinical Services Complex, GADVASU, Ludhiana from 1st January, 2016 to 31st December, 2017 was done. In addition, the prospective study was conducted on blood samples of dogs presented to Small Animal Clinics of the Department of Teaching Veterinary Clinical Complex, Guru Angad Dev Veterinary and Animal Sciences University, GADVASU, Ludhiana, w.e.f. from 1st July, 2018 to 31st March, 2019. A total of 363 samples of dogs were analyzed.

### 4.1.1 Hematological parameters in clinical cases of dogs interpreted manually

The retrospective data of clinical cases (n= 7, 375) w.e.f. 1st January, 2016 to 31st December, 2017 was analyzed. The hematological parameters are depicted in the (Table 2). The hemoglobin value varied from 1.00 to 22.90 (g/dL), with a mean value of 11.08 and median value of 11.30 which were less to the normal value, suggestive of anemia. The packed cell volume varied from 2.29 to 75.80 (%), with a mean value of 33.06 and median value of 33.70 which was found to be within normal range. The total erythrocyte count varied from 0.45 to 12.1 ($\times 10^6$/ µl) with a mean value of 5.21 and median value of 5.35 which was slightly lower than the normal range. Similarly, the mean values of Hb, PCV and TEC i.e. 8.13 ± 0.17 g/dl, 27.33 ± 0.61 per cent and 4.69 ± 0.09 × $10^6$ / µl respectively have reported by Abiramy *et al* (2003) in 12 natural cases of anemia in dogs.

**Table 2: Retrospective analysis of the hematological parameters in clinical cases of dogs interpreted manually**

| Variable | Range | Mean value | Median value | Normal value |
|---|---|---|---|---|
| Hemoglobin (Hb) (g/dl) | 1.00 - 22.90 | 11.08 | 11.30 | 12-18 |
| Packed cell volume (PCV) (%) | 2.29 - 75.80 | 33.06 | 33.70 | 33-55 |
| Total erythrocyte count (TEC) ($\times 10^6$/ µl) | 0.45 – 12.1 | 5.21 | 5.35 | 5.5-8.5 |

### 4.1.2 Classification of anemia on the basis of severity

The anemia was further classified into mild (2191), moderate (735) and severe (731) on the basis of hemoglobin concentration and blood smear examination, whereas, hemoglobin concentration was found to be normal in 3202 cases and 124 cases of hemoconcentration were observed (Table 3). The regenerative anemia was observed in severe anemia cases as reported earlier by (Barger 2003).

In addition, on the basis of blood smear examination, anemia was further classified into regenerative anemia (230) and non-regenerative anemia (70). The main reason for regenerative anemia can be red cell lysis due to hemoprotozoa and IMHA (Klag *et al* 1993, Irizarry-Rovira *et al* 2001 and Nassiri *et al* 2005). On the other hand King *et al* (1992), Niwetpathomwat *et al* (2006) and Amude *et al* (2007) reported that renal failure, canine distemper, parvovirus infection and ehrlichiosis were the predominant causes for non-regenerative anemia.

**Table 3: Retrospective classification of anemia on the basis of severity**

| S. No. | Severity | Number of cases | Range (g/dL) |
|---|---|---|---|
| 1 | Mild | 2191 | 8.1-11.5 |
| 2 | Moderate | 735 | 6.1-8.0 |
| 3 | Severe | 731 | <6 |

### 4.1.3 Classification of erythrocytic abnormalities on the basis of size and staining intensity of RBCs

The erythrocytic abnormalities were classified on the basis of RBC size and staining intensity of RBC (Table 4). The cases were further classified into normocytic normochromic (187), normocytic hypochromic (183), macrocytic hypochromic (137), microcytic hypochromic (118), hypochromasia (60), anisocytosis (28), polychromasia and microcytic normochromic in two cases each. The incidence of normocytic and normochromic anemia was higher in the present study because in most of the cases, the etiology of anemia was renal failure, parvovirus and *E.canis* infections which usually cause normocytic normochromic anemia (King *et al* 1992, Weiss *et al* 1999 and Niwetpathomwat *et al* 2006), whereas, Inflammatory bowel disease has been

associated with regenerative microcytic hypochromic anemia (Ristic and Stidworthy 2002).

**Table 4: Retrospective classification of erythrocytic abnormalities on the basis of size and staining intensity of RBCs**

| S. No. | Conditions | Number of cases |
|---|---|---|
| 1 | Normocytic normochromic | 187 |
| 2 | Normocytic hypochromic | 183 |
| 3 | Macrocytic hypochromic | 137 |
| 4 | Microcytic hypochromic | 118 |
| 5 | Hypochromasia | 60 |
| 8 | Anisocytosis | 28 |
| 6 | Polychromasia | 2 |
| 7 | Microcytic normochromic | 2 |

### 4.1.4 Classification of erythrocytic abnormalities on the basis of variation in shape of RBCs

The erythrocytic abnormalities observed on the basis of shape have been depicted in (Table 5), whereas, variation in shape of RBC viz. codocytes were observed in 76 cases, spherocytes in 16 cases, schistocytes in 11 cases, acanthocytes in 6 cases, leptocytes in 5 cases and dacrocytes in one case were observed.

**Table 5: Retrospective classification of erythrocytic abnormalities on the basis of variation in shape of RBCs**

| S. No. | Conditions | Number of cases |
|---|---|---|
| 1 | Codocytes | 76 |
| 2 | Spherocytes | 16 |
| 3 | Schistocytes | 11 |
| 4 | Acanthocytes | 6 |
| 5 | Leptocyte | 5 |
| 6 | Dacrocyte | 1 |

### 4.1.5 Other abnormalities recorded in retrospective cases

The other abnormalities observed have been depicted in (Table 6). IMHA was observed in 28 cases, pancytopenia in 26, roulex in 20, agglutination of RBCs in 5 cases and rickettsia and Heinz body in one case each. In IMHA, red cells are destroyed as a result of anti-erythrocyte antibody mediated hemolysis caused by intravascular complement activation and it results in intravascular hemolysis (Frank *et al* 1977). Anemia, neutrophilia, leukocytosis and eosinopenia were reported in canine hepatozoonosis (Mumdim *et al* 2008).

**Table 6: List of other conditions recorded in retrospective cases**

| S. No. | Conditions | Number of cases |
|---|---|---|
| 1 | IMHA | 28 |
| 2 | Pancytopenia | 26 |
| 3 | Roulex | 20 |
| 4 | Agglutination of RBCs | 5 |
| 5 | Rickettsia | 1 |
| 6 | Heinz body | 1 |

### 4.1.6 Gastro-intestinal affections

Maximum number of cases were of hepatitis (24), followed by ascites (19), gastroenteritis (11), jaundice (7), gastritis (6), acute gastritis (5), chronic hepatitis, liver damage and perineal hernia (4), intussusception (3) and enteritis, haemorrhagic enteritis, infectious canine hepatitis, dental tartar and hernia were observed in 2 cases each (Table 7). In addition, adhesive peritonitis, anal sacculitis, perineal fistula, hemorrhagic gastro-enteritis, pancreatitis, periodontitis, abdominal haemorrhage, gastric ulcer, hepatic encephalopathy, intestinal obstruction, chronic liver disease, hepatomegaly, mega eosophagus and oesophageal obstruction were observed in 1 case each.

**Table 7: Gastro-intestinal affections observed in retrospective studies in dogs**

| SNo. | Name of the affections | No. of cases |
|---|---|---|
| 1 | Hepatitis | 24 |
| 2 | Ascites | 19 |
| 3 | Gastro-enteritis | 11 |
| 4 | Jaundice | 7 |
| 5 | Gastritis | 6 |
| 6 | Acute gastritis | 5 |
| 7 | Chronic hepatitis | 4 |
| 8 | Liver damage | 4 |
| 9 | Perineal hernia | 4 |
| 10 | Intussuception | 3 |
| 11 | Enteritis | 2 |
| 12 | Hemorrhagic enteritis | 2 |
| 13 | Infectious canine hepatitis | 2 |
| 14 | Dental tartar | 2 |
| 15 | Hernia | 2 |
| 16 | Adhesive peritonitis | 1 |
| 17 | Anal sacculitis | 1 |
| 18 | Hemorrhagic gastro-enteritis | 1 |
| 19 | Perineal fistula | 1 |
| 20 | Pancreatitis | 1 |
| 21 | Periodontitis | 1 |
| 22 | Abdominal haemorrhage | 1 |
| 23 | Gastric ulcer | 1 |
| 24 | Hepatic encephalopathy | 1 |
| 25 | Intestinal obstruction | 1 |
| 26 | Chronic liver disease | 1 |
| 27 | Hepatomegaly | 1 |
| 28 | Mega eosophagus | 1 |
| 29 | Oesophageal obstruction | 1 |

### 4.1.7 Important tumors recorded

The highest number of cases were of TVT (15), followed by lymphoma (9), tumour (8), mammary tumour (7), mast cell tumour (2), whereas, adrenal tumor, unclassified carcinoma, hemangiosarcoma, joint carcinoma, melanoma, oral tumour, splenic neoplasm, squamous cell carcinoma were observed in 1 case each only (Table 8). Decreases in Hb, PCV, TEC and albumin concentration similarly have been reported in dog suffering from mammary tumours (Banerjee *et al* 2018).

**Table 8: Important tumors observed in retrospective cases**

| S. No. | Types of tumors | Number of cases |
|---|---|---|
| 1 | Transmissible venereal tumour | 15 |
| 2 | Lymphoma | 9 |
| 3 | Unclassified tumours | 8 |
| 4 | Mammary tumour | 7 |
| 5 | Mast cell tumour | 2 |
| 6 | Adrenal gland tumour | 1 |
| 7 | Unclassified carcinoma | 1 |
| 8 | Hemangiosarcoma | 1 |
| 9 | Joint carcinoma | 1 |
| 10 | Melanoma | 1 |
| 11 | Oral tumour | 1 |
| 12 | Splenic tumour | 1 |
| 13 | Squamous cell carcinoma | 1 |
| 14 | Vaginal tumour | 1 |

### 4.1.8 Eye and ear affections

The highest cases were of cataract (7), followed by corneal ulcer (6) and Otitis (2) whereas, neuclear cataract, bilateral blindness, anterior uveitis and ulcer, keratitis, desmetocoele, lipid keratopathy were observed in one case each (Table 9).

**Table 9: Eye and ear affections observed in retrospective cases**

| SNo. | Name of the affections | No. of cases |
|---|---|---|
| 1 | Cataract | 7 |
| 2 | Corneal ulcer | 6 |
| 3 | Otitis | 2 |
| 4 | Neuclear cataract | 1 |
| 5 | Bilateral blindness | 1 |
| 6 | Anterior uveitis and ulcer | 1 |
| 7 | Keratitis | 1 |
| 8 | Desmetocoele | 1 |
| 9 | Lipid keratopathy | 1 |

### 4.1.9 Respiratory tract affections

The highest numbers of cases were of pneumonia (6), followed by pleural effusions and sinusitis which were observed in 2 cases each. URT infection, laryngitis, pharyngitis and trachea bronchitis were observed in one case each (Table 10).

**Table 10: Respiratory tract affections observed in retrospective cases**

| S. No. | Name of the affections | No. of cases |
|---|---|---|
| 1 | Pneumonia | 6 |
| 2 | Pleural effusions | 2 |
| 3 | Sinusitis | 2 |
| 4 | Upper respiratory tract infection | 1 |
| 5 | Laryngitis | 1 |
| 6 | Pharyngitis | 1 |
| 7 | Trachea bronchitis | 1 |

### 4.1.10 Urinary tract affections

Among urinary tract affections, maximum cases were of renal failure (37), followed by cystitis (13), chronic kidney disease (14**)**, urolith (8) and cystolith (3). Acute renal failure, renal hyperparathyroidism and urinary obstruction were observed in two cases each. Acute nephritis and nephritis were observed in 1 case each (Table 11). Rudinsky *et al* (2018) reported that chronic kidney disease was a common cause

of morbidity and mortality in dogs. Uma *et al* (2018) reported that the renal calculi were a common problem in dogs.

**Table 11: Urinary tract affections in retrospective cases**

| S. No. | Urinary tract conditions | No. of cases |
|---|---|---|
| 1 | Renal failure | 37 |
| 2 | Cystitis | 13 |
| 3 | Chronic kidney disease | 14 |
| 4 | Urolith | 8 |
| 5 | Cystolith | 3 |
| 6 | Acute renal failure | 2 |
| 7 | Renal hyperparathyroidism | 2 |
| 8 | Urinary obstruction | 2 |
| 9 | Acute nephritis | 1 |
| 10 | Nephritis | 1 |

### 4.1.11 Reproductive affections

Maximum cases observed were of pyometra (85) followed by endometritis (2). Aspermia, dystocia, fetal resorption, vaginal hyperplasia, vaginal hypertrophy and prostrate enlargement were observed in one case each (Table 12).

**Table 12: Reproductive affections in retrospective cases**

| S. No. | Name of the conditions | No. of cases |
|---|---|---|
| 1 | Pyometra | 85 |
| 2 | Endometritis | 2 |
| 3 | Aspermia | 1 |
| 4 | Dystocia | 1 |
| 5 | Fetal resorption | 1 |
| 6 | Vaginal hyperplasia | 1 |
| 7 | Vaginal hypertrophy | 1 |
| 8 | Prostrate enlargment | 1 |

### 4.1.12 Cardiac affections

Maximum cases were of cardiomegaly (4), followed by heart failure (2), chronic heart failure, pericardial effusion and persistent right aortic arch were

observed in one case each (Table 13). Cardiomegaly was often seen in older dogs as compared to younger one. Cardiac diseases are often a diagnostic problem and determination of the heart size is important for evaluation of the patient with cardiac disease (Mourya *et al* 2018).

**Table 13: Cardiac affections observed in retrospective cases**

| S. No. | Name of the affections | No. of cases |
|---|---|---|
| 1 | Cardiomegaly | 4 |
| 2 | Heart failure | 2 |
| 3 | Chronic heart failure | 1 |
| 4 | Pericardial effusion | 1 |
| 5 | Persistent right aortic arch | 1 |

### 4.1.13 Musculoskeletal disorders

The cases observed were of femur fracture, mandible fracture and spondylitis in two cases each. Left patellar luxation, right tibia fracture, arthritis, lameness and osteomalacia were observed in one case each (Table 14). O'Nell *et al* (2016) observed that canine patellar luxation was one of the most common orthopaedic disorders of dogs and was a potential welfare concern because it can lead to lameness, osteoarthritis and pain**.**

**Table 14: Musculoskeletal disorders in retrospective cases**

| S. No. | Name of the affections | No. of cases |
|---|---|---|
| 1 | Femur fracture | 2 |
| 2 | Mandible fracture | 2 |
| 3 | Spondylitis | 2 |
| 4 | Left patellar luxation | 1 |
| 5 | Right tibia fracture | 1 |
| 6 | Arthritis | 1 |
| 7 | Lameness | 1 |
| 8 | Osteomalacia | 1 |

### 4.1.14 Cutaneous affections

Maximum number of cases were of dermatitis (4), followed by acute dermatitis, abcess and pyoderma which were observed in two cases each, cellulitis and scabies were observed in one case each (Table 15).

**Table 15: Cutaneous affections in retrospective cases**

| S. No. | Name of the affections | No. of cases |
|---|---|---|
| 1 | Dermatitis | 4 |
| 2 | Acute dermatitis | 2 |
| 3 | Abcess | 2 |
| 4 | Pyoderma | 2 |
| 5 | Cellulitis | 1 |
| 6 | Scabies | 1 |

### 4.1.15 Parasitic affections

Maximum cases were of hemoprotozoa (54) which included, *E.canis* (23), *H. canis* (15), *Babesia* sp. (10), unclassified hemoprotozoan (5) and trypanosome (1). Hookworm, demodex and mange infestation were present in three cases each (Table 16).

**Table 16: Parasitic affections in retrospective cases**

| S. No. | Name of the conditions | No. of cases |
|---|---|---|
| 1 | Hemoprotozoa | 54 |
| 2 | Hookworm | 3 |
| 3 | Demodex | 3 |
| 4 | Mange infestation | 3 |

### 4.1.16 Miscellaneous affections

Maximum cases were of canine distemper (35), followed by fever of unknown origin (7), brucellosis (6), parvo virus (4), foreign body (3) and atopy, tetnus and hypothyroidism were observed in two cases each. In addition, secondary hyperparathyroidism, dermatophytosis, diffuse encephalopathy, fibrotic myopathy, idiopathic epilepsy, polydipsia, rabies and lymph node hyperplasia were observed in one case each (Table 17).

**Table 17: Miscellaneous affections in retrospective cases**

| S. No. | Name of the affections | No. of cases |
|---|---|---|
| 1 | Canine distemper | 35 |
| 2 | Fever of unknown origin (FUO) | 7 |
| 3 | Brucellosis | 6 |
| 4 | Parvo virus | 4 |
| 5 | Foreign body | 3 |
| 6 | Atopy | 2 |
| 7 | Tetnus | 2 |
| 8 | Hypothyroidism | 2 |
| 9 | Secondary hyperparathyroidism | 1 |
| 10 | Dermatophytosis | 1 |
| 11 | Diffuse encephalopathy | 1 |
| 12 | Fibrotic myopathy | 1 |
| 13 | Idiopathic epilepsy | 1 |
| 14 | Polydipsia | 1 |
| 15 | Rabies | 1 |
| 16 | Lymphnode hyperplasia | 1 |

### 4.1.17 Erythrocytic abnormalities associated with different disease conditions

In retrospective cases having respiratory affections, maximum cases were of pneumonia. Earlier, Sherman and Karagiannis (2017) reported that aspiration pneumonia was one of the common diagnosis in canine patients and can occurr secondary to various underlying predisposing factors and conditions. In retrospective cases having cutaneous affections, maximum cases were of dermatitis. Earlier, Botoni *et al* (2019) concluded that soft-coated wheaten terrier, american staffordshire terrier, english bulldog and labrador retriever were over-represented for the atopic dermatitis. In retrospective cases having parasitic affections, maximum cases were of *E.canis*. Earlier, Dagnone *et al* (2003) determined that ehrlichiosis was associated with anemia, thrombocytopenia or ticks in dogs.

**Table 18: Erythrocytic abnormalities associated with different disease conditions in retrospective cases**

| S. No. | Disease conditions | Associated erythrocytic abnormalities |
|---|---|---|
| **Kidney affections** | | |
| 1 | Kidney failure | • Normocytic normochromic and or normocytic hypochromic anemia and thrombocytopenia.<br>• Schistocytes and *H.canis* infection. |
| | Chronic kidney disease | • Microcytic hypochromia anemia |
| | Chronic renal failure | • Microcytic hypochromic and non-regenerative anemia |
| **Liver affections** | | |
| 2 | Ascites | • Moderate normocytic hypochromic or microcytic hypochromic anemia with target cells. |
| | Hepatomegaly | • Regenerative anemia |
| | Hepatitis | • Microcytic hypochromic anemia<br>• Regenerative anemia<br>• Target cells |
| | Jaundice | • Anemia |
| **Hemoprotozoa** | | |
| 3 | Hemoprotozoa | • Normocytic hypochromic anemia.<br>• Pancytopenia. |
| | *E. canis* | • Microcytic hypochromic anemia. |
| **Reproductive affections** | | |
| 4 | Pyometra | • Neutrophilic leukocytosis<br>• Normocytic hyochromic anemia<br>• Normocytic normochromic, microcytic hypochromic and macrocytic normochromic was also observed.<br>• Panleucopenia |
| **Viral conditions** | | |
| 5 | Canine distemper | • Normocytic hypochromic anemia |
| | Parvo virus | • Anemia |
| **Cutaneous affections** | | |
| 6 | Dermatitis | • Mild anemia |
| | Demodex | • Anemia |
| **Inflammatory affections** | | |
| 7 | Gastritis | • Microcytic hypochromic |
| | Haemorrhagic enteritis | • Anemia |
| | Gastroenteritis | • Anemia |
| | Endometritis | • Anemia |
| **Eye affections** | | |
| 8 | Cataract | • Normocytic hypochromic anemia |
| | Corneal ulcer | • Anemia |
| **Tumors** | | |
| 9 | TVT | • Anemia |
| | Lymphoma | |
| | Vaginal tumors | |
| | Joint tumor | |
| **Other conditions** | | |
| 10 | Perineal hernia | • Anemia |
| | Fractures | |
| | Pneumonia | |
| | Congestive heart failure | |
| | Arthritis | |

### 4.2 Prospective analysis of the clinical cases

For prospective studies, 363 cases of dogs were analyzed. Out of these, 259 were analyzed manually, 104 cases were analyzed using ADVIA 2120 Hematology System and blood samples from 31 apparently healthy animals were also analyzed for comparison purpose.

#### 4.2.1 Mean and median values of hematological parameters in apparently healthy dogs

Blood samples of 31 apparently healthy animals were analyzed for comparision of clinical cases in prospective studies. The hematological values are depicted in the (Table 19). The value of haemoglobin (g/dL) varied from 2.50 to 19.80 with a mean value of 12.72 and median value of 13.20 which was within the normal range. The value of total leucocyte count varied from 2500 to 56900 with a mean value of 15519.35 and median value of 14580.00 which was within the normal range. The absolute value of neutrophils (µL) varied from 1350 to 39830 with a mean value of 13360.52 and median value of 10780.00 which was above the normal range. The absolute value of eosinophils varied from 0 to 4374 with a mean value of 312.58 which was within the normal range. The absolute value of lymphocytes varied from 220 to 17070 with a mean value of 1822.25 and median value of 1303.40 which was within the normal range. The values of apparently healthy animals were compared with reference values.

**Table 19: Prospective analysis showing mean and median values of hematological parameters in apparently healthy dogs**

| Parameter | Range | Mean | Median | Reference Values |
|---|---|---|---|---|
| Haemoglobin (g/dL) | 2.50- 19.80 | 12.72 | 13.20 | 12-18 |
| Total leucocyte count | 2500.00- 56900.00 | 15519.35 | 14580.00 | 6000-17000 |
| Neutrophils (µL) | 1350.00- 39830.00 | 13360.52 | 10780.00 | 3600-12240 |
| Eosinophils (µL) | 0-4374.00 | 312.58 | 0 | 120-1700 |
| Lymphocytes (µL) | 220.00- 17070.00 | 1822.25 | 1303.40 | 720-5100 |

### 4.2.2 Hematological parameters in clinical cases

Blood samples of 363 cases were analyzed. The hematological values are depicted in the (Table 20). In the clinical cases, the hemoglobin value varied from 1.50 to 17.70 with a mean value of 9.41 and median value of 9.20 which was below the normal range. The total leucocyte count varied from 500.00 to 173790.00 with a mean value of 20935.15 and median value of 15800.00 which was above the normal range. The absolute count of neutrophil varied from 0 to 161700.00, with a mean value of 18619.28 and median value of 13176.00 which was above the normal range. The absolute count of lymphocyte varied from 0 to 29330.00, with a mean value of 2031.07 and median value of 1568.00 which was within the normal range. The absolute count of eosinophil varied from 0 to 2845.80, with a mean value of 122.15 which was within the normal range. Similarly, Ravnik *et al* (2010) reported the median values of the hematological parameter viz. WBC, RBC, HCT, platelet, neutrophils, lymphocytes, monocytes in the cases of dogs suffering from diseases.

**Table 20: Prospective analysis of the hematological parameters in clinical cases**

| Variable | Range | Mean | Median | Reference Range |
|---|---|---|---|---|
| Hemoglobin (Hb) | 1.50- 17.70 | 9.41 | 9.20 | 12-18 g/dL |
| Total Leucocyte count (TLC) | 500.00- 173790.00 | 20935.15 | 15800.00 | 6000-17000 |
| Neutrophils (µL) | 0-161700.00 | 18619.28 | 13176.00 | 3600-12240 |
| Lymphocytes (µL) | 0- 29330.00 | 2031.07 | 1568.00 | 720-5100 |
| Eosinophils (µL) | 0- 2845.80 | 122.15 | 0 | 120-1700 |

### 4.2.3 Classification of erythrocytic abnormalities observed in dogs on the basis of colour of RBC

On the basis of staining intensity for RBC, the erythrocytic abnormalities were classified into hypochromasia and polychromasia (Table 21). Out of 363 cases analyzed hypochromasia (Fig 1) were observed in 157 cases and polychromasia (Fig 2) was detected in 40 cases. In 104 cases analyzed using ADVIA 2120 Hematology System, the hypochromasia was further classified into grade (+) and grade (++), a total of six cases of hypochromasia were detected which consisted of five cases of

grade + and one case was of grade ++ hypochromasia. Niwetpathomwat *et al* (2006) reported that microcytic hypochromic anemia and thrombocytopenia were the common findings in dogs suffering from babesia infection.

**Table 21: Classification of erythrocytic abnormalities observed in dogs on the basis of colour of RBC in prospective studies**

| On the basis of color of RBC | Number of cases | Grade (ADVIA) | Number of cases |
|---|---|---|---|
| Hypochromasia | 157 | + | 5 |
| | | ++ | 1 |
| Polychromasia | 40 | - | - |

### 4.2.4 Classification of erythrocytic abnormalities observed in dogs on the basis of size of RBC

On the basis of size, erythrocytes were classified into macrocytic and microcytic RBCs (Table 22). In 104 cases analyzed using ADVIA 2120 Hematology System, the macrocytic RBCs were further classified into grade + and grade +++ macrocytic. A total of four cases of macrocytic RBCs (Fig 3) were observed which consisted of three cases of grade + and one case of grade +++. Similarly, microcytic RBCs (Fig 4) were detected in 24 cases, in which 13 were of grade +, five were of grade ++ and six were of grade +++. In addition, using hematology analyzer, anisocytosis was detected in 20 cases (Fig 5). Swenson and Reece 1993 reported that the dogs suffering from iron deficiency, had decreased hemoglobin and were characterized by microcytic hypochromic anemia.

**Table 22: Classification of erythrocytic abnormalities observed in dogs on the basis of size of RBC in prospective cases**

| On the basis of size of erythrocytes | Grade | Number of cases |
|---|---|---|
| Macrocytic RBCs | + | 3 |
| | +++ | 1 |
| Microcytic RBCs | + | 13 |
| | ++ | 5 |
| | +++ | 6 |

### 4.2.5 Predominant erythrocytic abnormalities observed on the basis of shape of RBC

In the present study, out of total 363 cases analyzed, the predominant erythrocytic abnormalities are depicted in the (Table 23). The maximum number of cases had spherocytes (111), followed by echinocytes (95), codocytes (88), schistocytes (39), acanthocytes (14), incomplete spherocytes (12), polychromatophils (3) and least cases had elliptocyte (1).

**Table 23: Predominant erythrocytic abnormalities observed on the basis of shape of RBC in prospective cases**

| Maximum predominant erythrocyte | Number of cases |
|---|---|
| Spherocyte | 111 |
| Echinocyte | 95 |
| Codocyte | 88 |
| Schistocyte | 39 |
| Acanthocyte | 14 |
| Incomplete spherocyte | 12 |
| Polychromatophilic RBC | 3 |
| Elliptocyte | 1 |

### 4.2.6 Common erythrocytic abnormalities on the basis of mean occurrence

The table 24 depicts the mean values of the erythrocytic abnormalities on the basis of occurrence. In the present study, out of 363 cases analyzed, the most common erythrocytic abnormalities observed on the basis of mean values was echinocytes (Fig 6), followed by spherocytes (Fig 7), codocytes (Fig 8), schistocytes (Fig 9), incomplete spherocytes (Fig 10), acanthocytes (Fig 11), polychromatophils, eccentrocytes (Fig 12), dacrocytes and stomatocytes (Fig 13), q RBCs (Fig 14), blister cells (Fig 15), elliptocytes, keratocytes (Fig 16) and nucleated RBCs (Fig 17). Bonfanti *et al* (2004) reported that stomatocytes were induced by abnormal membrane pumps. Caldin *et al* (2005) reported that eccentrocytes are RBCs that appear in a peripheral blood smear which have their hemoglobin shifted to one side of the cell. Harvey (2012) suggested that echinocytes form when the surface area of the outer lipid monolayer increases relative to the inner monolayer.

**Table 24: Prospective analysis of common erythrocytic abnormalities on the basis of mean occurrence**

| S. No. | Erythrocytic abnormality | Mean ± SE |
|---|---|---|
| 1 | Echinocytes | 47.22 ± 3.60 |
| 2 | Spherocytes | 44.62 ± 2.42 |
| 3 | Codocytes | 33.39 ± 2.09 |
| 4 | Schistocytes | 21.66 ± 1.76 |
| 5 | Incomplete spherocytes | 10.19 ± 1.51 |
| 6 | Acanthocytes | 7.51 ± 1.07 |
| 7 | Polychromatophils | 2.61 ± 7.37 |
| 8 | Eccentrocytes | 1.30 ± 4.33 |
| 9 | Dacrocytes | 1.09 ± 3.95 |
| 10 | Stomatocytes | 0.96 ± 0.17 |
| 11 | Q RBCs | 0.86 ± 0.11 |
| 12 | Blister cells | 0.49 ± 0.12 |
| 13 | Elliptocytes | 0.42 ± 0.37 |
| 14 | Keratocytes | 0.24 ± 0.06 |
| 15 | Nucleated RBCs | 0.16 ± 0.05 |

### 4.2.7 Hematological parameters in dogs using ADVIA hematology system

The median value of CH varied from 11.4-25.4 (pg) and the median value was 20.20 (pg), which was below the normal range (Table 25). The median value of CHCM varied from 23.3-37.5 (g/dL) and the median value of 32.40 (g/dL), which was within the normal range. The median value of RBC count varied from 1.17 to 8.84 × $10^6$ cells/µL and the median value was 4.74× $10^6$ cells/µL, which was below the normal range. Andrea (2017) similarly reported lower mean value of RBC in dogs suffering from parvo viral enteritis. The median value of hematocrit varied from 9.6 to 53.3 % and the median value was 30.25%, which was below the normal range. Anderson (1958) reported that the PCV of pregnant bitches was found to decrease from baseline of 55 percent to 32 per cent at pregnancy term, whereas, Lynch *et al*

(2016) reported that general anaesthesia and sedation may lower PCV and institution of fluid therapy also lowers PCV in dehydrated patients, and effectively unmasks anemia. The median value of MCV varied from 6.7 to 102.1 fL and the median value was 62.20 fL, which was within the normal range. It has been reported that hypochromic microcytic erythrocytes in iron- deficiency show decreased MCV when determined electronically, but showed normal diameter (Thrall 2006). Some Japanese breeds like Akita and Shiba normally have MCV values less than refrence value for other breeds of dogs, but these dogs are not considered anemic (Gookin *et al* 1998). In hyperglycemia, red cells are transiently hypertonic in relation to the isotonic diluting fluid, resulting in swollen cells and an elevated MCV (Sharma 1990). The median value of MCH varied from 6.3 to 24.8 pg, and the median value was 19.75 pg, which falls within the normal range. The median value of MCHC varied from 15.5 to 42.5 g/dL, and the median value was 32.10 g/dL, which was within the normal range. Sharma 1990 reported that in megaloblastic anemias, there is normal hemoglobin content, but the MCV and MCH increased, while the MCHC remains normal. The median value of HDW varied from 1.55 to 5.86 g/dL and the median value was 2.27 g/dL, which was within the normal range. The median value of RDW varied from 10.8 to 25 % with a median value of 14.70 %, which was within the normal range. Mazzotta *et al* (2016) reported that RDW was a quantitative measurement of anisocytosis. Paltrinieri (2014) reported that RDW may detect regeneration earlier than MCV. Palterinieri (2014) reported that reticulocytosis associated with early regeneration may increase the RDW. The WBC count varied from 1.73 to 124.5 × $10^3$ cells/µL with a median value of 16.00, which was within the normal range. Singh *et al* (2006) reported that anemia, leukocytosis along with increased neutrophil count, decreased lymphocyte count and monocyte count in dogs suffering from pyometra. The median of MPV varied from 1.73 to 124.5 fL with a median value of 15.54 fL, which was above the normal range.Gawlita *et al* (2015) reported that higher MPV was associated with increased risk of coronary artery disease in dogs. Sharma 1990 reported that when the values of hemoglobin, red cell count, and MCV are affected, MCH and MCHC also become abnormal, since these indices are calculated and are not directly measured.

**Table 25: Hematological parameters in dogs in prospective study using ADVIA hematology system**

| S. No. | Hematological parameters | Units | Range in clinical cases | Median value of clinical cases | Normal range |
|---|---|---|---|---|---|
| 1 | Mean hemoglobin content (CH) | (pg) | 11.4-25.4 | 20.20 | 24.0-35.0 |
| 2 | Hemoglobin concentration mean (CHCM) | (g/dL) | 23.3-37.5 | 32.40 | 29.0-34.0 |
| 3 | Red blood cell count (RBC) | ($\times 10^6$ cells/µL) | 1.17-8.84 | 4.74 | 5.5-8.5 |
| 4 | Hematocrit (HCT) | (%) | 9.6-53.3 | 30.25 | 37.0-55.0 |
| 5 | Mean corpuscular volume (MCV) | (fL) | 6.7-102.1 | 62.20 | 60.0-77.0 |
| 6 | Mean corpuscular hemoglobin (MCH) | (pg) | 6.3-24.8 | 19.75 | 19.5-24.5 |
| 7 | Mean corpuscular hemoglobin concentration (MCHC) | (g/dL) | 15.5-42.5 | 32.10 | 31.0-34.0 (Wintrobe) |
| 8 | Hemoglobin concentration distribution width (HDW) | (g/dL) | 1.55-5.86 | 2.27 | 1.9-3.0 |
| 9 | Red cell volume distribution width (RDW) | (%) | 10.8-25 | 14.70 | 12.0-15.0 |
| 10 | White blood cell count (WBC) | ($\times 10^3$ cells/µL) | 1.73-124.5 | 16.00 | 6-17 |
| 11 | Mean platelet volume (MPV) | (fL) | 1.73-124.5 | 15.54 | 6.00-11.00 |

### 4.2.8 Mean and median values of leukocytes using ADVIA hematology system

The mean value of neutrophils was higher than the normal range. The mean value of lymphocytes and eosinophils was within the normal range. The mean value of monocytes was below the normal range (Table 26). Paltrinieri *et al* (1998) reported that severe anemia and neutrophilic leucocytosis were found in dogs suffering from hemolytic anemia and to a lesser degree, in those suffering from severe heart failure. Comazzi *et al* (2004) reported that young dogs exhibited lower values for hemoglobin concentration with higher number of lymphocytes and neutrophilia in uremic patients.

**Table 26: Mean and median values of leukocytes in the prospective cases using ADVIA hematology system**

| Parameter (µL) | Mean ± SE | Median | Normal range |
|---|---|---|---|
| **Neutrophils** | 20848.25 ± 2311.45 | 13355.30 | 3600-12240 |
| **Eosinophils** | 167.00 ± 45.85 | 0 | 120-1700 |
| **Lymphocytes** | 1723.12 ± 194.90 | 1353.20 | 720-5100 |
| **Monocytes** | 3.55 ± 3.55 | 0 | 150-1350 |

#### 4.2.8.1 Reticulocytes

The cases in which polychromasia was detected, the additional blood smears were stained with new methylene blue stain and the cases were further classified into regenerative and non-regenerative anemia on the basis of reticulocyte counts. Reticulocytes (Fig 18) were counted and then counter stained with Leishman stain for better visulalization (Fig 19). In the present study, regenerative response was observed in five cases having values more than 1.5% i.e. 1.8, 2.2, 2.9, 4.1 and 5.9 % whereas, two cases showed non- regenerative response having reticulocyte count below 1% i.e. 0.7 and 1 %. Similarly, Tvedten (2004) reported that a reticulocyte count of more than 1.5 per cent was used to indicate regenerative anemia and a reticulocyte count below 1 per cent indicate non- regenerative anemia.

#### 4.2.8.2 Immune-mediated hemolytic anemia

In addition, IMHA was observed in five cases. Blood smears of these cases revealed spherocytosis and agglutination of the RBCs was observed (Fig 20). The agglutination was further confirmed by saline agglutination test (Fig 21) which

showed clumping of the erythrocytes in isotonic saline. The auto-agglutination of the RBCs along with spherocytosis was suggestive of IMHA. Carr *et al* (2002) reported spherocytosis and auto-agglutination in dogs having immune-mediated hemolytic anemia.

#### 4.2.8.3 Hemoprotozoan affections

In the present study, the correlation of erythrocytic abnormalities with hemoprotozoan infection was carried out. A total of 9 cases of *Babesia gibsoni* was studied (Fig 22), out of which three cases showed codocytes and three cases showed spherocytes, followed by echinocytes which were present in two cases and one case showed polychromatophilic RBCs. In two cases, *Hepatozoon Canis* was also detected (Fig 23). Similarly, Loretti and Barros (2005) observed spherocytosis in dogs having babesiosis, whereas, Holovakha *et al* (2018) reported that dogs having babesiosis showed echinocytes in the blood smears.

### 4.2.9 Correlation of morphological abnormalities of erythrocytes with leukogram findings

In the present study the erythrocytic abnormalities observed were correlated with the findings of the leukogram in all 363 cases (Table 27). The leukogram was classified into animals having normal TLC, leukopenia, leukocytosis and leukemoid response. The mean value of acanthocytes was more in dogs showing leukemoid response, followed by those having leukocytosis, normal TLC and leukopenia respectively. However, the mean values of acanthocytes were 6.23 in normal TLC, 5.11 in leukopenia, 9.13 in leukocytosis and 10.16 in leukemoid response. The mean values of blister cells was more in dogs showing normal TLC, followed by leukocytosis and no blister cells were observed in leukopenia respectively. However, the mean value of blister cells was 0.76 in normal TLC, 0.11 in leukopenia, 0.24 in leukocytosis and 0.00 in leukemoid response. The mean value of codocytes was more in dogs showing leukemoid response, followed by leukocytosis, normal TLC and leukopenia. However, the mean value was 30.88 in normal TLC, 24.96 in leukopenia, 36.68 in leukocytosis and 48.33 in leukemoid response. The mean value of dacrocytes was more in dogs showing leukopenia, followed by normal TLC, leukocytosis. However, the mean value was 1.23 in normal TLC, 2.25 in leukopenia, 0.75 in leukocytosis and 0.00 in leukemoid response.

**Table 27: Correlation of morphological abnormalities of erythrocytes with leukogram findings**

| Erythrocytic morphology | Normal TLC | Leukopenia | Leukocytosis | Leukemoid response |
|---|---|---|---|---|
| Acanthocytes | $6.23^{a} \pm 1.35$ | $5.11^{a} \pm 2.91$ | $9.13^{a} \pm 1.90$ | $10.16^{a} \pm 6.86$ |
| Blister cells | $0.76^{a} \pm 0.24$ | $0.11^{a} \pm 0.11$ | $0.24^{a} \pm 0.12$ | $0.00^{a} \pm 0.00$ |
| Codocytes | $30.88^{a} \pm 3.01$ | $24.96^{a} \pm 5.60$ | $36.68^{a} \pm 3.39$ | $48.33^{a} \pm 7.89$ |
| Dacrocytes | $1.23^{a} \pm 0.31$ | $2.25^{a} \pm 1.26$ | $0.75^{a} \pm 0.25$ | $0.00^{a} \pm 0.00$ |
| Echinocytes | $45.09^{a} \pm 5.29$ | $38.18^{a} \pm 9.89$ | $52.58^{a} \pm 5.73$ | $18.5^{a} \pm 12.35$ |
| Elliptocytes | $0.11^{a} \pm 0.11$ | $0.00^{a} \pm 0.00$ | $0.86^{a} \pm 0.85$ | $0.00^{a} \pm 0.00$ |
| Incomplete spherocytes | $11.83^{a} \pm 2.06$ | $19.51^{a} \pm 13.39$ | $7.29^{a} \pm 1.39$ | $0.00^{a} \pm 0.00$ |
| Keratocytes | $0.23^{a} \pm 0.08$ | $0.37^{a} \pm 0.17$ | $0.24^{a} \pm 0.12$ | $0.00^{a} \pm 0.00$ |
| Nucleated RBCs | $0.15^{a} \pm 0.08$ | $0.07^{a} \pm 0.07$ | $0.20^{a} \pm 0.07$ | $0.00^{a} \pm 0.00$ |
| Polychromatophils | $1.65^{a} \pm 0.44$ | $0.40^{a} \pm 0.19$ | $4.01^{a} \pm 0.72$ | $4.5^{a} \pm 4.11$ |
| Q RBCs | $0.87^{a} \pm 0.17$ | $0.40^{a} \pm 0.31$ | $0.90^{a} \pm 0.18$ | $1.5^{a} \pm 0.84$ |
| Schistocytes | $18.36^{a} \pm 2.26$ | $12.11^{a} \pm 2.55$ | $26.83^{a} \pm 3.15$ | $28^{a} \pm 7.22$ |
| Spherocytes | $50.84^{a} \pm 3.39$ | $41.40^{a} \pm 11.53$ | $39.13^{a} \pm 3.63$ | $25.16^{a} \pm 6.78$ |
| Stomatocytes | $0.75^{a} \pm 0.21$ | $1.81^{a} \pm 0.74$ | $1.11^{a} \pm 0.30$ | $0.00^{a} \pm 0.00$ |

Values with different superscript (a,b,c) within a parameter different significantly ($p<0.05$)

The mean value of echinocytes was more in dogs showing leukocytosis, followed by normal TLC and no echinocytes were observed in leukopenia and leukemoid response. However, the mean value was 45.09 in normal TLC, 38.18 in leukopenia, 52.58 in leukocytosis and 18.5 in leukemoid response. The mean value of elliptocytes was more in dogs showing leukocytosis, followed by normal TLC, no elliptocytes were observed in leukemoid response. However, the mean value was 0.11 in normal TLC, 0.00 in leukopenia, 0.86 in leukocytosis and 0.00 in leukemoid response. The mean value of incomplete spherocytes was more in dogs showing leukopenia, followed by normal TLC and leukocytosis, no incomplete spherocytes were observed in leukemoid response. However, the mean value was 11.83 in normal TLC, 19.51 in leukopenia, 7.29 in leukocytosis and 0.00 in leukemoid response. The mean value of keratocytes was more in dogs showing leukopenia, followed by leukocytosis and normal TLC, no keratocytes were observed in cases showing leukemoid response. However, the mean value was 0.23 in normal TLC, 0.37 in leukopenia, 0.24 in leukocytosis and 0.00 in leukemoid response. The mean value of nucleated RBCs was more in dogs showing leukocytosis, followed by normal TLC and leukopenia, no neucleated RBCs were observed in cases having leukemoid response. However, the mean value was 0.15 in normal TLC, 0.07 in leukopenia, 0.20 in leukocytosis and 0.00 in leukemoid response. The mean value of polychromatophilic RBCs was more in dogs showing leukemoid response, followed by leukocytosis, normal TLC and leukopenia. However, the mean value was 1.65 in normal TLC, 0.40 in leukopenia, 4.01 in leukocytosis and 4.5 in leukemoid response. The mean value of q RBC was more in dogs showing leukemoid response, followed by leukocytosis, normal TLC and leukopenia. However, the mean value was 0.87 in normal TLC, 0.40 in leukopenia, 0.90 in leukocytosis and 1.5 in leukemoid response. The mean value of schistocytes was more in dogs showing leukemoid response, followed by leukocytosis, normal TLC and leukopenia. However, the mean value was 18.36 in normal TLC, 12.11 in leukopenia, 26.83 in leukocytosis and 28 in leukemoid response. The mean value of spherocytes was more in dogs showing normal TLC, followed by leukopenia, leukocytosis and leukemoid response. However, the mean value was 50.84 in normal TLC, 41.40 in leukopenia, 39.13 in leukocytosis and 25.16 in leukemoid response. The mean value of stomatocytes was more in dogs showing leukopenia, followed by leukocytosis, normal TLC respectively and no stomatocytes were observed in leukemoid response. However, the mean value was 0.75 in normal TLC, 1.81 in

leukopenia, 1.11 in leukocytosis and 0.00 in leukemoid response. The mean values of acanthocytes, blister cells, codocytes, dacrocytes, echinocytes, elliptocytes, incomplete spherocytes, keratocytes, neucleated RBCs, polychromatophilic RBCs, q RBCs, schistocytes, spherocytes and stomatocytes did not differ significantly in cases having normal TLC, leukopenia, leukocytosis and leukemoid response. Similarly, Benson *et al* (2015) reported that codocytes and leukemoid response was observed in dogs having renal carcinoma. Wong *et al* (2015) observed that echinocytes and leukocytosis was reported in dogs having hemangiosarcoma.

### 4.2.10 Correlation of serum chemistry findings with the leukogram findings

The table 28 depicts the correlation between serum chemistry finding with the findings of leukogram. The mean value of total protein was more in dogs showing leukopenia, followed by normal TLC, leukocytosis and leukemoid response. However, the mean value was 5.53 in normal TLC, 5.48 in leukopenia, 5.09 in leukocytosis and 4.58 in leukemoid response. The mean value of albumin was more in dogs showing normal TLC, followed by leukocytosis, leukemoid response and leukopenia. However, the mean value was 2.21 in normal TLC, 1.89 in leukopenia, 2.07 in leukocytosis and 1.98 in leukemoid response. The mean value of ALT was more in dogs showing leukocytosis, followed by normal TLC, leukopenia and leukemoid response. However, the mean value was 93.69 in normal TLC, 88.42 in leukopenia, 129.62 in leukocytosis and 66 in leukemoid response. The mean value of AST was more in dogs showing leukocytosis, followed by leukemoid response, leukopenia and normal TLC. However, the mean value was 75.85 in normal TLC, 102.13 in leukopenia, 105.51 in leukocytosis and 103.5 in leukemoid response. The mean value of GGT was more in dogs showing leukocytosis, followed by leukopenia, leukemoid response and normal TLC. However, the mean value was 11.30 in normal TLC, 21.13 in leukopenia, 21.72 in leukocytosis and 15.75 in leukemoid response. The mean value of total bilirubin was more in dogs showing leukemoid response, followed by leukocytosis, leukopenia and normal TLC. However, the mean value was 0.40 in normal TLC, 0.89 in leukopenia, 1.82 in leukocytosis and 8.15 in leukemoid response. The mean value of ALKP was more in dogs showing leukemoid response, followed by leukocytosis, leukopenia and normal TLC. However, the mean value was 242.17 in normal TLC, 270.29 in leukopenia, 348.61 in leukocytosis and 2800.17 in leukemoid response.

**Table 28: Correlation of serum chemistry findings with the leukogram findings**

| Parameter | Units | Normal TLC | Leukopenia | Leukocytosis | Leukemoid response |
|---|---|---|---|---|---|
| Total protein | (g/dL) | $5.53^{a} \pm 0.11$ | $5.48^{a} \pm 0.31$ | $5.09^{a} \pm 0.11$ | $4.58^{a} \pm 0.42$ |
| Albumin | (U/L) | $2.21^{a} \pm 0.05$ | $1.89^{a} \pm 0.08$ | $2.07^{a} \pm 0.05$ | $1.98^{a} \pm 0.45$ |
| Alanine aminotransferase (ALT) | (U/L) | $93.69^{a} \pm 10.07$ | $88.42^{a} \pm 33.56$ | $129.62^{a} \pm 15.68$ | $66^{a} \pm 18.30$ |
| Aspartate aminotransferase (AST) | (U/L) | $75.85^{a} \pm 7.63$ | $102.13^{a} \pm 58.03$ | $105.51^{a} \pm 12.36$ | $103.5^{a} \pm 43.95$ |
| Gamma-glutamyl transferase (GGT) | (U/L) | $11.30^{a} \pm 2.29$ | $21.13^{a} \pm 14.71$ | $21.72^{a} \pm 4.69$ | $15.75^{a} \pm 6.23$ |
| Total bilirubin | (mg/dL) | $0.40^{b} \pm 0.05$ | $0.89^{b} \pm 0.58$ | $1.82^{b} \pm 0.35$ | $8.15^{a} \pm 5.34$ |
| Alkaline phosphatase (ALKP) | (U/L) | $242.17^{b} \pm 24.98$ | $270.29^{b} \pm 73.94$ | $348.61^{b} \pm 36.25$ | $2800.17^{a} \pm 2409.03$ |
| Blood urea nitrogen (BUN) | (mg/dL) | $66.47^{a} \pm 10.62$ | $65.02^{a} \pm 10.62$ | $50.60^{a} \pm 4.10$ | $54.33^{a} \pm 21.31$ |
| Creatinine | (mg/dL) | $5.95^{a} \pm 0.49$ | $4.70^{a} \pm 1.11$ | $3.63^{a} \pm 0.34$ | $3.68^{a} \pm 1.47$ |

Values with different superscript (a,b,c) within parameter different significantly ($p<0.05$)

The mean value of BUN was more in dogs showing normal TLC, followed by leukopenia, leukemoid response and leukocytosis. However, the mean value was 66.47 in normal TLC, 65.02 in leukopenia, 50.60 in leukocytosis and 54.33 in leukemoid response. The mean value of creatinine was more in dogs showing normal TLC, followed by leukopenia, leukemoid response and leukocytosis. However, the mean value was 5.95 in normal TLC, 4.70 in leukopenia, 3.63 in leukocytosis and 3.68 in leukemoid response. The mean values of total protein, albumin, ALT, AST, GGT, total bilirubin, ALKP, BUN and creatinine did not differ significantly in cases having normal TLC, leukopenia, leukocytosis and leukemoid response. Similarly, Lachungpa *et al* (2019) reported that hypoalbuminemia, elevated ALT, ALKP levels and leukocytosis was observed in dogs having IMHA.

### 4.2.11 Correlation of hematological findings using hematological analyzer with the findings of the leukogram

The table 29 depicts the correlation of hematological findings using hematological analyzer with findings of the leukogram. The mean value of anisocytosis was more in dogs showing leukocytosis, followed by normal TLC, and no anisocytosis were observed in leukopenia and leukemoid response respectively. However, the mean value was 0.14 in normal TLC, 0.00 in leukopenia, 0.16 in leukocytosis and 0.00 in leukemoid response. The mean value of hypochromasia was more in dogs showing leukemoid response, followed by normal TLC and leukocytosis, and no hypochromasia were observed in leukopenia respectively. However, the mean value was 0.06 in normal TLC, 0.00 in leukopenia, 0.06 in leukocytosis and 0.25 in leukemoid response. The mean value of polychromasia was more in dogs showing leukemoid response, followed by normal TLC and leukocytosis, and no polychromasia were observed in leukopenia respectively. However, the mean value was 0.12 in normal TLC, 0.00 in leukopenia, 0.04 in leukocytosis and 0.25 in leukemoid response. The mean value of microcytosis was more in dogs showing normal TLC and leukopenia, followed by leukocytosis and no microcytosis were observed in leukemoid response. However, the mean value was 0.05 in normal TLC, 0.05 in leukopenia, 0.30 in leukocytosis and 0.00 in leukemoid response. The mean value of macrocytosis was more in dogs showing leukocytosis followed by normal TLC and no macrocytosis were observed in leukopenia and

leukemoid response. However, the mean value was 0.04 in normal TLC, 0.00 in leukopenia, 0.09 in leukocytosis and 0.00 in leukemoid response. The mean value of CH was more in dogs showing normal TLC, followed by leukemoid response, leukocytois and leukopenia. However, the mean value was 20.20 in normal TLC, 19.37 in leukopenia, 19.94 in leukocytosis and 19.5 in leukemoid response. The mean value of CHCM was more in dogs showing leukemoid response, followed by leukopenia, normal TLC and leukocytosis. However, the mean value was 32.49 in normal TLC, 33.0 in leukopenia, 32.08 in leukocytosis and 33.07 in leukemoid response. The mean value of HCT was more in dogs showing normal TLC, followed by leukocytosis, leukemoid response and leukopenia. However, the mean value was 31.68 in normal TLC, 26.35 in leukopenia, 29.44 in leukocytosis and 28.3 in leukemoid response. The mean value of HDW was more in dogs showing leukocytosis followed by leukemoid response, normal TLC and leukopenia. However, the mean value was 2.44 in normal TLC, 2.14 in leukopenia, 2.68 in leukocytosis and 2.66 in leukemoid response. The mean value of MCH was more in dogs showing normal TLC followed by leukocytosis, leukopenia and leukemoid response. However, the mean value was 19.17 in normal TLC, 16.38 in leukopenia, 18.90 in leukocytosis and 15.1 in leukemoid response. The mean value of MCHC was more in dogs showing normal TLC followed by leukocytosis, leukopenia and leukemoid response. However, the mean value was 31.04 in normal TLC, 27.9 in leukopenia, 29.98 in leukocytosis and 25.57 in leukemoid response. The mean value of MCV was more in dogs showing leukocytosis followed by normal TLC, leukopenia and leukemoid response. However, the mean value was 62.23 in normal TLC, 58.75 in leukopenia, 63.22 in leukocytosis and 45.85 in leukemoid response. The mean value of MPV was more in dogs showing leukocytosis followed by leukemoid response, normal TLC and leukopenia. However, the mean value was 13.47 in normal TLC, 13.3 in leukopenia, 14.07 in leukocytosis and 14.4 in leukemoid response. The mean value of RBC was more in dogs showing normal TLC followed by leukemoid response, leukocytosis and leukopenia. However, the mean value was 5.18 in normal TLC, 4.53 in leukopenia, 4.70 in leukocytosis and 4.82 in leukemoid response. The mean value of RDW was more in dogs showing leukemoid response followed by leukocytosis, normal TLC and leukopenia. However, the mean value was 15.01 in normal TLC, 14.72 in leukopenia, 16.35 in leukocytosis and 17.3 in leukemoid response. The mean value of WBC was

more in dogs showing leukemoid response followed by leukocytosis, normal TLC and leukopenia. However, the mean value was 12.74 in normal TLC, 4.37 in leukopenia, 29.07 in leukocytosis and 72.22 in leukemoid response. The mean value of WBCB was more in dogs showing leukemoid response followed by leukocytosis, normal TLC and leukopenia. However, the mean value was 11.28 in normal TLC, 4.37 in leukopenia, 29.21 in leukocytosis and 72.22 in leukemoid response. The mean values of anisocytosis, hypochromasia, polychromasia, normocytic RBC, microcytic RBC, macrocytic RBC, CH, CHCM, HCT, HDW, MCH, MCHC, MCV, MPV, RBC, RDW, WBC and WBCB did not differ significantly in cases having normal TLC, leukopenia, leukocytosis and leukemoid response. Joiner *et al* 1976 reported that hypochromasia, leukemoid response and codocytes were associated with chronic granulocytic leukemia in dogs. Holanda *et al* (2019) reported that low RBC count, leucopenia, thrombocytopenia was associated with Ehrlichia and Anaplasma infection in dogs. Ahuja *et al* (2019) reported that high WBC counts and leukemoid response was associated with dogs having pyometra.

In the present study, correlation between erythrocytic abnormality with leukogram finding was carried out ($p<0.05$) and there was significant correlation between normal TLC with the presence of spherocytes and polychromatophilic RBCs in the blood smears, between leukocytosis and the presence of codocytes, schistocytes, echinocytes and polychromatophilic RBCs in the blood smears. In addition, leukopenia was correlated with the presence of stomatocytes and polychromatophilic RBCs in blood smears of dogs. Benson *et al* (2015) reported that anisocytosis, polychromasia, codocytes and leukocytosis were observed in dogs having renal carcinoma.

In the present study, correlation between history and clinical signs with leukogram finding was also carried out ($p<0.05$) and there was significant correlation between leukocytosis with the presence of clinical signs like blood in vomit, leukopenia with the history of presence of ticks, icteric mucus membrane and distented abdomen. Puig *et al* (1995) reported that dog having renal failure showed clinical sign of blood in vomit along with leukocytosis. Abdullahi *et al* (1990) reported that leukopenia was observed in early cases of canine babesiosis having history of inappetence and ticks infestation.

**Table 29: Correlation of hematological findings using hematological analyzer with the findings of the leukogram (n=104)**

| Variables | Normal TLC | Leukopenia | Leukocytosis | Leukemoid response |
|---|---|---|---|---|
| Anisocytosis | $0.14^{a}\pm0.07$ | $0.00^{a}\pm0.00$ | $0.16^{a}\pm0.09$ | $0.00^{a}\pm0.00$ |
| Hypochromasia | $0.06^{a}\pm0.03$ | $0.00^{a}\pm0.00$ | $0.06^{a}\pm0.05$ | $0.25^{a}\pm0.25$ |
| Polychromasia | $0.12^{a}\pm0.04$ | $0.00^{a}\pm0.00$ | $0.04^{a}\pm0.03$ | $0.25^{a}\pm0.25$ |
| Normocytic | $0.00^{a}\pm0.00$ | $0.00^{a}\pm0.00$ | $0.00^{a}\pm0.00$ | $0.00^{a}\pm0.00$ |
| Microcytic | $0.5^{a}\pm0.12$ | $0.5^{a}\pm0.37$ | $0.30^{a}\pm0.11$ | $0.00^{a}\pm0.00$ |
| Macrocytic | $0.04^{a}\pm0.02$ | $0.00^{a}\pm0.00$ | $0.09^{a}\pm0.07$ | $0.00^{a}\pm0.00$ |
| CH | $20.20^{a}\pm0.37$ | $19.37^{a}\pm0.37$ | $19.94^{a}\pm0.40$ | $19.5^{a}\pm0.62$ |
| CHCM | $32.49^{a}\pm0.28$ | $33.0^{a}\pm0.73$ | $32.08^{a}\pm0.46$ | $33.07^{a}\pm1.12$ |
| HCT | $31.68^{a}\pm1.52$ | $26.35^{a}\pm2.54$ | $29.44^{a}\pm1.50$ | $28.3^{a}\pm4.16$ |
| HDW | $2.44^{a}\pm0.13$ | $2.14^{a}\pm0.16$ | $2.68^{a}\pm0.16$ | $2.66^{a}\pm0.31$ |
| MCH | $19.17^{a}\pm0.55$ | $16.38^{a}\pm1.49$ | $18.90^{a}\pm0.58$ | $15.1^{a}\pm2.34$ |
| MCHC | $31.04^{a}\pm0.89$ | $27.9^{a}\pm2.40$ | $29.98^{a}\pm1.03$ | $25.57^{a}\pm4.04$ |
| MCV | $62.23^{a}\pm1.04$ | $58.75^{a}\pm1.38$ | $63.22^{a}\pm1.26$ | $45.85^{b}\pm13.38$ |
| MPV | $13.47^{a}\pm0.87$ | $13.3^{a}\pm2.04$ | $14.07^{a}\pm0.95$ | $14.4^{a}\pm2.54$ |
| RBC | $5.18^{a}\pm0.26$ | $4.53^{a}\pm0.45$ | $4.70^{a}\pm0.23$ | $4.82^{a}\pm0.80$ |
| RDW | $15.01^{a}\pm0.38$ | $14.72^{a}\pm0.51$ | $16.35^{a}\pm0.58$ | $17.3^{a}\pm2.12$ |
| WBC | $12.74^{a}\pm1.45$ | $4.37^{a}\pm0.47$ | $29.07^{a}\pm1.94$ | $72.22^{a}\pm30.95$ |
| WBCB | $11.28^{c}\pm0.47$ | $4.37^{c}\pm0.47$ | $29.21^{b}\pm1.92$ | $72.22^{a}\pm30.95$ |

Values with different superscript (a,b,c) within a parameter differ significantly ($p<0.05$)

### 4.2.12 Comparison of serum chemistry findings in apparently healthy dogs and clinical cases

The table 30 shows the comparison of serum chemistry findings of apparently healthy animals and clinical cases. The mean value of total protein and albumin was on lower side, whereas, the mean values of ALT, AST, GGT, total bilirubin, ALKP, BUN and creatinine were higher when compared with apparently healthy animals.

Further, on the basis of serum chemistry findings, all the 363 cases were further classified into liver damage (157), kidney damage (97) and concurrent liver and kidney damage (109).

**Table 30: Comparison of serum chemistry findings in apparently healthy dogs and clinical cases**

| Variable | Mean (Apparently healthy animals) | Mean value (n=363) | Normal refrence values |
|---|---|---|---|
| TP | 6.27 | 5.30 | 5.5-7.5 g/dL |
| Albumin | 2.98 | 2.12 | 2.6-4 g/dL |
| ALT | 39.18 | 112.72 | 8.2-57 U/L |
| AST | 57.00 | 90.94 | 8.9-49 U/L |
| GGT | 5.00 | 17.26 | 1.9-2.7 U/L |
| TB | 0.30 | 1.16 | 0.1-0.6 mg/dL |
| ALKP | 126.8 | 344.57 | 10.6-101 U/L |
| BUN | 16.15 | 59.12 | 8.8-25.9 mg/dL |
| CR | 1.04 | 4.81 | 0.5-1.6 mg/dL |

### 4.2.13 Correlation of history and clinical signs in dogs suffering from liver damage

A significant positive correlation ($p<0.05$) was observed between inappetence, vomition and black faeces in dogs suffering from liver damage (Table 31). Lester *et al* (2016) reported that dog having acute liver failure showed signs like anorexia, vomition, polydipsia and polyuria. Elhiblu *et al* (2015) showed that dogs suffering from liver cirrhosis manifested inappetence, halitosis, abdominal distension, weight loss, melena, icterus and anemia.

**Table 31: Correlation of history and clinical signs in dogs suffering from liver damage**

| Effect | Wald Chi-Square | Pr > ChiSq |
|---|---|---|
| **Inappetence** | 8.2727 | 0.0160 |
| **Vomition** | 9.3992 | 0.0022 |
| **Black faeces** | 5.0721 | 0.0243 |

### 4.2.14 Correlation of history and clinical signs in dogs suffering from kidney damage

A significant positive correlation ($p<0.05$) was observed between dogs passing black faeces and kidney damage. A marginal significant correlation ($p<0.1$) was observed between dogs showing halitosis and kidney damage. Whereas, high significant correlation ($p<0.001$) was observed between dogs showing inappetence and vomition and kidney damage (Table 32). Devipriya *et al* (2018) similarly reported that dogs having renal diseases exhibit clinical signs of, polyuria, polydipsia, anorexia, vomition, oral ulcers, halitosis, pale mucus membrane, weight loss and neurological complications.

**Table 32: Correlation of history and clinical signs in dogs suffering from kidney damage**

| Effect | Wald Chi-Square | Pr > ChiSq |
|---|---|---|
| **Inappetence** | 20.6743 | <.0001 |
| **Halitosis** | 3.0104 | 0.0827 |
| **Vomition** | 27.8550 | <.0001 |
| **Black faeces** | 4.6077 | 0.0318 |

### 4.2.15 Correlation of history and clinical signs in dogs suffering from concurrent liver and kidney damage

A significant positive correlation ($p<0.05$) was observed between occurance of inappetence and halitosis and dogs suffering from concurrent liver and kidney damage, whereas, high significant correlation ($p<0.001$) was observed between vomition and concurrent liver and kidney damage (Table 33). Brown *et al* 2007 studied clinicopathologic changes associated with renal failure in dogs and found that anorexia, vomition, lethargy, polyuria and azotemia were the main clinical signs**.**

**Table 33: Correlation of history and clinical signs in dogs suffering from concurrent liver and kidney damage**

| Effect | Wald Chi-Square | Pr > ChiSq |
|---|---|---|
| **Inappetence** | 12.4199 | 0.0020 |
| **Halitosis** | 4.3014 | 0.0381 |
| **Vomition** | 21.8219 | <.0001 |

### 4.2.16 Comparison of the serum chemistry findings of the apparently healthy animals with dogs suffering from liver and or kidney damage

The mean value of total protein (g/dL) in apparently healthy animals was 6.27, those suffering from liver damage was 4.99, those suffering from kidney damage was 6.08 and the mean value in dogs suffering from concurrent liver and kidney damage was 5.19g/dL. The mean value of total protein in dog suffering from liver damage and concurrent liver and kidney damage was lower than the apparently healthy animals (Table 34). The mean value of albumin (g/dL) in apparently healthy dogs was 2.98, those suffering from liver damage was 1.91, those suffering from kidney damage was 2.43 and the mean value in dogs suffering from concurrent liver and kidney damage was 2.21. The mean value of albumin was significantly lower in dogs suffering from liver damage as compared to apparently healthy dogs. The mean value of albumin differ significantly between dogs having liver damage alone and concurrent liver and kidney damage with apparently healthy animals. Comazzi *et al* (2004) reported that many anemias were associated with hypoalbuminaemia, this could suggest blood loss, or hepatic or renal failure. Kaneko *et al* (1971) reported that dogs suffering from chronic focal hepatitis had high ALT and hypoalbunimea, indicating severe hepatic injury.

The mean value of ALT (U/L) in apparently healthy dogs was 39.18, those suffering from liver damage was 144.03, those suffering from kidney damage was 38.34 and the mean value in dogs suffering from concurrent liver and kidney damage was 127.07. The mean value of ALT was significantly different in dogs suffering from liver damage and concurrent liver and kidney damage as compared to apparently healthy dogs. The mean value of ALT in liver damage was highest, followed by concurrent liver and kidney damage when compared to apparently healthy animals. Comazzi *et al* (2004) reported that ALT was found in high concentration in the cystosol of hepatocytes and increased activities of ALKP were observed. Leela-Arpon *et al* (2019) reported that dogs suffering from hepatocellular carcinoma had increased levels of ALT and ALKP along with hypercalcemia.

The mean value of AST (U/L) in apparently healthy dogs was 57.00, those suffering from liver damage was 99.73, those suffering from kidney damage was 67.70 and the mean value in dogs suffering from concurrent liver and kidney damage was 98.03. The mean value of AST among liver, kidney and concurrent liver and

kidney damage did not differ significantly when compared to healthy animals. Guilford *et al* (1996) reported that in hepatocellular injury, increase in activity of ALT was 4-8 times higher than the AST. On the contrary, Kohn *et al* (2010) and Claus (2008) reported elevated AST and ALP activities in dogs suffering from leptospirosis.

The mean value of GGT (U/L) in apparently healthy dogs was 5.00, those suffering from liver damage was 17.07, those suffering from kidney damage was 5.48 and the mean value in dogs suffering from concurrent liver and kidney damage was 27.84. The mean value of GGT were significantly higher in dogs suffering from concurrent liver and kidney damage as compared to dogs suffering from kidney damage. The serum GGT activity increased significantly in dogs following experimental bile duct obstruction (Noonam and Meyer 1979, Shull and Hornbuckle 1979). Shull *et al* 1979 reported that bile duct obstruction in dogs causes increase in serum levels of GGT as well as ALKP. Marked elevation in serum GGT has previously been reported in dogs suffering from renal carcinoma reported by (White-head *et al* 2012).

The mean value of total bilirubin (mg/dL) in apparently healthy dogs was 0.30, those suffering from liver damage was 0.9 mg/dL, those suffering from kidney damage was 0.41 mg/dL and the mean value in dogs suffering from concurrent liver and kidney damage was 2.28 mg/dL. The mean value of total bilirubin was significantly higher in dogs suffering from concurrent liver and kidney damage as compared to the apparently healthy dogs. Lester *et al* (2016) reported hyperbilirubinemia in dogs having acute liver failure, whereas, Leisewitz *et al* (2019) reported hypoglycemia, hyperlactemia, high urea, high creatinine, hyperbilirubinemia, hypercortisolemia and hypothyroxinaemia in dogs suffering from *Babesia rossi* infection.

The mean value of ALKP (U/L) in apparently healthy dogs was 126.8, those suffering from liver damage was 354.23, those suffering from kidney damage was 162.81 and the mean value in dogs suffering from concurrent liver and kidney damage was 474.59. The mean value of ALKP was significantly higher in dogs suffering from kidney damage as compared to apparently healthy dogs. Noonan and Meyer 1979 reported moderate increase in serum ALKP levels due to hepatic necrosis. Hoe and Jabara (1967), Abdelkader and Hauge (1986) and Center *et al* (1985) reported increase in serum ALKP levels in a variety of canine diseases associated with

cholestasis. Patel *et al* (2018) reported elevated SGPT, SGOT, BUN, creatinine and ALKP levels in dogs suffering from hepato renal damage due to leptospriosis.

The mean value of BUN (mg/dL) in apparently healthy dogs was 16.15, those suffering from liver damage was 16.12, those suffering from kidney damage was 84.67 and the mean value in dogs suffering from concurrent liver and kidney damage was 97.62. The mean value of BUN was significantly higher in dogs suffering from concurrent liver and kidney damage as compared to apparently healthy dogs. Similarly, the mean values of dogs suffering from liver damage differ significantly from the apparently healthy dogs. Balint *et al* 1975, Balint and Sturcz 1959, Balint and Fekete 1960, Balint and Forgacs 1965, reported that dehydration caused reduced cardiac output, markedly reduced GFR and the auto regulatory capacity of the kidney was also lost which resulted in increased BUN levels. Singh *et al* (2006) reported that pyometra was one of the most common cause of kidney damage in bitches and they reported high values of BUN and hyperprotienemia in the affected dogs.

**Table 34: Comparison of the serum chemistry findings of the apparently healthy animals with dogs suffering from liver and or kidney damage**

| Variable | Mean ± SE (Apparently healthy animals) | Liver damage | Kidney damage | Concurrent liver and kidney damage |
|---|---|---|---|---|
| TP | $6.27^{a} \pm 0.29$ | $4.99^{b} \pm 0.11$ | $6.08^{a} \pm 0.11$ | $5.19^{b} \pm 0.13$ |
| Albumin | $2.98^{a} \pm 0.14$ | $1.91^{c} \pm 0.04$ | $2.43^{a} \pm 0.07$ | $2.21^{b} \pm 0.06$ |
| ALT | $39.18^{b} \pm 2.60$ | $144.03^{a} \pm 16.54$ | $38.35^{b} \pm 1.69$ | $127.07^{a} \pm 16.32$ |
| AST | $57.00^{a} \pm 11.52$ | $99.73^{a} \pm 13.61$ | $67.70^{a} \pm 8.68$ | $98.03^{a} \pm 10.75$ |
| GGT | $5.00^{a} \pm 0$ | $17.08^{ab} \pm 3.07$ | $5.48^{b} \pm 0.22$ | $27.84^{a} \pm 7.54$ |
| TB | $0.30^{b} \pm 0.08$ | $0.90^{ab} \pm 0.22$ | $0.41^{b} \pm 0.10$ | $2.28^{a} \pm 0.50$ |
| ALKP | $126.8^{a} \pm 18.50$ | $354.2^{ab} \pm 34.60$ | $162.8^{b} \pm 22.41$ | $474.6^{a} \pm 156.58$ |
| BUN | $16.15^{b} \pm 1.93$ | $16.12^{c} \pm 1.43$ | $84.67^{b} \pm 5.19$ | $97.62^{a} \pm 6.32$ |
| CR | $1.04^{b} \pm 0.05$ | $0.85^{c} \pm 0.02$ | $6.99^{b} \pm 0.53$ | $8.26^{a} \pm 0.60$ |

Values with different superscript (a,b,c) within a parameter differ significantly ($p<0.05$)

The mean value of creatinine (mg/dL) in apparently healthy dogs was 1.04, those suffering from liver damage was 0.85, those suffering from kidney damage was 6.99 and the mean value in dogs suffering from concurrent liver and kidney damage was 8.26. The mean value of creatinine was significantly higher in dogs suffering from concurrent liver and kidney damage when compared to the apparently healthy dogs. King *et al* (1992) reported that there was a direct correlation between degree of anemia and extent of chronic renal failure as assessed by serum creatinine concentration. Mortier *et al* (2012) reported that lymphoma affected dogs had increase in liver and renal function enzymes which could be due to hepatic and renal metastasis. Elhiblu *et al* (2015) reported that dogs suffering from liver cirrhosis had higher creatinine, ALT, AST and ALKP.

### 4.2.17 Comparison of hematological parameters with serum chemistry findings of apparently healthy animals and dogs suffering from liver and or kidney damage

The mean value of hemoglobin in apparently healthy dogs was 12.72, those suffering from liver damage was 9.23, those suffering from kidney damage was 9.12 and the mean value in dogs suffering from concurrent liver and kidney damage was 9.94 (Table 35). The mean value of hemoglobin was lower in dogs suffering from liver, kidney and concurrent liver and kidney damage. Naigamwalla *et al* (2012) reported that one of the most common cause of anemia in dogs is iron deficiency anemia.

The mean value of total leucocyte count in apparently healthy dogs was 1551, those suffering from liver damage was 20875.83, those suffering from kidney damage was 17077.63 and the mean value in dogs suffering from concurrent liver and kidney damage was 24485.46. The mean value of TLC was significantly higher in dogs suffering from concurrent liver and kidney damage as compared to dogs suffering from kidney damage alone. Sumit *et al* (2018) reported that dogs suffering from chronic kidney disease showed higher mean TLC count. Elhiblu *et al* (2015) reported that dogs suffering from liver cirrhosis showed elevated total leucocyte count, neutrophils and MCH concentration. On the contrary, Bhagat (2017) revealed decrease in hemoglobin, PCV, TEC, TLC and platlet count in dogs suffering from canine distemper.

The mean value of neutrophil counts in apparently healthy dogs was 13360.52, those suffering from liver damage was 18227, those suffering from kidney damage was 15120 and the mean value in dogs suffering from concurrent liver and kidney damage was 22297. The mean value of neutrophil counts were significantly higher in dogs suffering from concurrent liver and kidney damage as compared to dogs suffering from liver and kidney damage alone. Jackobson and Clark (1994) reported that increase in neutrophil count could be due to either secondary bacterial infection or severe tissue hypoxia and wide spread inflammation. Singh *et al* (2006) reported that pyometra was one of the most common cause of kidney damage in bitches and it also resulted in leukocytosis with increased neutrophil count. On the contrary, Cook *et al* (2016) reported that neutropenia could be due to non bacterial inflammatory disease, increased demand due to marked inflammation, drug-association, bone marrow disease, immune-mediated due to its physiologic and miscellaneous reasons.

The mean value of eosinophils counts in apparently healthy dogs was 312.58, those suffering from liver damage was 135.6, those suffering from kidney damage was 98.35 and the mean value in dogs suffering from concurrent liver and kidney damage was 123.88. The mean value of lymphocytes counts in apparently healthy dogs was 1822.25, those suffering from liver damage was 2324.9, those suffering from kidney damage was 1850.5 and the mean value in dogs suffering from concurrent liver and kidney damage was 1768.5. The mean value of monocytes counts in apparently healthy dogs was 24, those suffering from liver damage was 4.51, those suffering from kidney damage was 4.52 and the mean value in dogs suffering from concurrent liver and kidney damage was 4.16.

In the present study, the mean value of hemoglobin, eosinophils, lymphocytes and monocytes in dogs suffering from liver, kidney damage alone or those suffering from concurrent liver and kidney damage did not differ significantly when compared to the apparently healthy animals. Whereas, Karunanithy *et al* (2019) reported that eosinophilia was observed in dogs having renal failure. Sharun *et al* (2019) reported that lymphocytosis, elevated BUN and creatinine were observed in dog having leptospira infection.

**Table 35: Comparison of hematological parameters with serum chemistry findings of apparently healthy animals and dogs suffering from liver and or kidney damage**

| Parameter | Mean ± SE (Apparently healthy animals) | Liver damage | Kidney damage | Concurrent liver-kidney |
|---|---|---|---|---|
| Hemoglobin (g/dl) | $12.72^{a} \pm 0.74$ | $9.23^{a} \pm 0.30$ | $9.12^{a} \pm 0.37$ | $9.94^{a} \pm 0.79$ |
| TLC ($\times 10^{3}$ µl) | $1551^{ab} \pm 1987.44$ | $20876^{ab} \pm 1497.93$ | $17078^{b} \pm 1520.76$ | $24485^{a} \pm 2162.57$ |
| Neutrophils (µL) | $13360.52^{a} \pm 1682.01$ | $18227^{ab} \pm 1396.01$ | $15120^{a} \pm 1458.33$ | $22297^{a} \pm 2106.46$ |
| Eosinophils (µL) | $312.58^{a} \pm 148.0$ | $135.6^{a} \pm 34.40$ | $98.35^{a} \pm 25.12$ | $123.88^{a} \pm 32.30$ |
| Lymphocytes (µL) | $1822.25^{ab} \pm 530.67$ | $2324.9^{a} \pm 231.92$ | $1850.5^{a} \pm 157.02$ | $1768.5^{a} \pm 151.60$ |
| Monocytes (µL) | $24^{a} \pm 16.69$ | $4.51^{a} \pm 2.76$ | $4.52^{a} \pm 3.86$ | $4.16^{a} \pm 2.97$ |

Values with different superscript (a,b,c) within a parameter differ significantly ($p<0.05$)

### 4.2.18 Comparison of morphological alterations of RBCs with serum chemistry findings

The table 36 shows the comparision of morphological alterations of RBCs with serum chemistry findings. The mean value of spherocytes in dogs suffering from liver damage was 40.17, those suffering from kidney damage was 54.75 and the mean value in dogs suffering from concurrent liver and kidney damage was 42.02. The mean value of spherocytes were significantly higher in dogs suffering from kidney damage as compared to dogs suffering from liver and concurrent liver and kidney damage. However, the mean value of spherocytes in dogs suffering from concurrent liver and kidney damage did not differ significantly from dogs suffering from liver damage alone. Olhovich *et al* (2013) reported that hypochromic regenerative anemia in the presence of spherocytes and agglutination due to immune-mediated hemolytic anemia in dogs suffering from acute pancreatitis and multiorganic complications. Moraes *et al* (2017) reported that spherocytosis was observed in dogs suffering from IMHA. The mean value of stomatocytes in dogs suffering from liver damage was 1.41, those suffering from kidney damage was 0.82 and the mean value in dogs suffering from concurrent liver and kidney damage was 0.45. The mean value of stomatocytes were significantly higher in dogs suffering from liver damage as compared to dogs suffering from concurrent liver and kidney damage. However, the mean value of stomatocytes in dogs suffering from kidney damage did not differ significantly from dogs suffering from liver and concurrent liver and kidney damage. Slappendel *et al* 1991 observed that in familial stomatocytosis- hypertrophic gastritis (FSHG), stomatocytosis, hemolytic anemia, increased liver enzymes and serum bilirubin was observed. Feldman (1996) reported that stomatocytes were observed in renal vascular thrombosis based on several radiographic findings in dog.

The mean value of polychromatophilic RBCs in dogs suffering from liver damage was 4.03, those suffering from kidney damage was 1.21 and the mean value in dogs suffering from concurrent liver and kidney damage was 1.80. The mean value of polychromatophilic RBCs were significantly higher in dogs suffering from liver damage as compared to dogs suffering from concurrent liver and kidney damage and kidney damage alone. However, the mean value of polychromatophilic RBCs in dogs suffering from kidney damage did not differ significantly from dogs suffering from

concurrent liver and kidney damage. Seneviratna (1965) reported that dog having *Babesia gibsoni* reveals polychromatophilic macrocytic anemia with anisocytosis and enlargement of liver and spleen. Hodges and Christopher (2011) reported that polychromasia with or without high RDW was a more accurate indicator of regenerative anemia than other erythrocyte indices.

The mean value of dacrocytes dogs suffering from liver damage was 0.66, those suffering from kidney damage was 1.91 and the mean value in dogs suffering from concurrent liver and kidney damage was 0.98. The mean value of dacrocytes were significantly higher in dogs suffering from kidney damage as compared to dogs suffering from liver damage. However, the mean value of dacrocytes in dogs suffering from concurrent liver and kidney damage did not differ significantly from dogs suffering from liver and kidney damage alone. In humans, Rojas-Maya *et al* (2016) similarly reported that dacrocytosis may be seen in very low number in various systemic diseases. Dunn *et al* (1986) reported dacrocytes and marked anisocytosis in dogs having myeloid metaplasia in the liver and spleen.

The mean value of echinocytes dogs suffering from liver damage was 42.47, those suffering from kidney damage was 47.47 and the mean value in dogs suffering from concurrent liver and kidney damage was 53.83. The mean value of echinocytes were significantly higher in dogs suffering from concurrent liver and kidney damage, followed by kidney damage and liver damage. Mandal *et al* (1991) reported that echinocytes are poorly deformable, impart high viscocity to the blood and may hinder reperfusion by increasing resistance to renal capillary blood flow. Weiss *et al* 1990 reported that nonartifactual echinocytosis occurs in the dogs that may be associated with specific diseases. Wong (1999) reported that echinocytosis in disc sphere transformation are induced by changes in lipid layer and integral membrane protiens.

In the present study, other erythrocytic abnormalities viz. acanthocytes, blister cells, codocytes, eccentrocytes, elliptocytes, incomplete spherocytes, keratocytes, nucleated RBCs, q RBCs and schistocytes did not differ significantly in dogs suffering from liver and or kidney damage alone and concurrent liver and kidney damage. In human studies, Adewoyin *et al* (2019) reported that codocytes are observed in sickle hemoglobinopathies, iron deficiency and post splenectomy stage, whereas, dacrocytes formation results from abnormal spleen or bone marrow pathology.

**Table 36: Comparison of morphological alterations of RBCs with liver and kidney damage**

| Morphological alteration of erythrocyte | Liver damage | Kidney damage | Concurrent liver and kidney damage |
|---|---|---|---|
| Spherocytes | $40.17^{b} \pm 3.58$ | $54.75^{a} \pm 5.40$ | $42.02^{b} \pm 3.79$ |
| Stomatocytes | $1.41^{a} \pm 0.33$ | $0.82^{ab} \pm 0.27$ | $0.45^{b} \pm 0.19$ |
| Polychromatophilic RBC | $4.03^{a} \pm 0.78$ | $1.21^{b} \pm 0.34$ | $1.80^{b} \pm 0.49$ |
| Dacrocytes | $0.66^{b} \pm 0.22$ | $1.91^{a} \pm 0.56$ | $0.98^{ab} \pm 0.34$ |
| Echinocytes | $42.47^{a} \pm 5.72$ | $47.47^{a} \pm 6.34$ | $53.83^{a} \pm 6.63$ |
| Acanthocytes | $10.05^{a} \pm 2.07$ | $6.15^{a} \pm 1.62$ | $5.07^{a} \pm 1.27$ |
| Blister cells | $0.34^{a} \pm 0.01$ | $0.90^{a} \pm 0.35$ | $0.33^{a} \pm 0.16$ |
| Codocytes | $35.12^{a} \pm 2.93$ | $27.54^{a} \pm 3.74$ | $36.11^{a} \pm 4.44$ |
| Eccentrocytes | $1.74^{a} \pm 5.44$ | $0.83^{a} \pm 2.74$ | $1.10^{a} \pm 3.58$ |
| Elliptocytes | $0.12^{a} \pm 0.12$ | $1.39^{a} \pm 1.38$ | $0.00^{a} \pm 0.00$ |
| Incomplete spherocytes | $11.96^{a} \pm 3.02$ | $8.08^{a} \pm 1.76$ | $9.53^{a} \pm 2.03$ |
| Keratocytes | $0.28^{a} \pm 0.12$ | $0.20^{a} \pm 0.08$ | $0.22^{a} \pm 0.09$ |
| Nucleated RBCs | $0.27^{a} \pm 0.10$ | $0.05^{a} \pm 0.02$ | $0.11^{a} \pm 0.08$ |
| Q RBCs | $0.75^{a} \pm 0.15$ | $1.04^{a} \pm 0.25$ | $0.87^{a} \pm 0.23$ |
| Schistocytes | $23.11^{a} \pm 2.67$ | $17.95^{a} \pm 3.29$ | $22.88^{a} \pm 3.31$ |

Values with different superscript (a,b,c) within a parameter differ significantly ($p<0.05$)

### 4.2.19 Hematological parameters in dogs suffering from liver damage using ADVIA hematology system

In the present study, in the 104 cases analyzed by automated cell counter, the values of different hematological parameters along with their range and their median values in dogs suffering from liver damage are presented in the table 37. The median value of CH ranged from 11.4 to 25.4 pg with a median value of 20.20 pg, which was less than the normal range. The median value of CHCM ranged from 23.3 to 37.5 g/dL with a median value was 32.40, which was within the normal range. The values of RBC count ranged from 1.17 to 8.84 × $10^{6}$ cells/µL with a median value was 4.79 × $10^{6}$ cells/µL, which was below the normal range. Matur *et al* (2019) reported that dogs having chronic liver disease showed hemostatic dearrangements and low RBC count.

**Table 37: Hematological parameters in dogs suffering from liver damage in prospective cases using ADVIA hematology system**

| S. No. | Hematological parameter | Units | Range in clinical cases | Median value | Normal range |
|---|---|---|---|---|---|
| 1 | Mean hemoglobin content (CH) | (pg) | 11.4-25.4 | 20.20 | 24.0-35.0 |
| 2 | Hemoglobin concentration mean (CHCM) | (g/dL) | 23.3-37.5 | 32.40 | 29.0-34.0 |
| 3 | Red blood cell count (RBC) | ($\times 10^6$ cells/µL) | 1.17-8.84 | 4.79 | 5.5-8.5 |
| 4 | Hematocrit (HCT) | (%) | 10.3-53.3 | 30.30 | 37.0-55.0 |
| 5 | Mean corpuscular volume (MCV) | (fL) | 37.7-102.1 | 62.00 | 60.0-77.0 |
| 6 | Mean corpuscular hemoglobin (MCH) | (pg) | 9.5-24.2 | 19.50 | 19.5-24.5 |
| 7 | Mean corpuscular hemoglobin concentration (MCHC) | (g/dL) | 13.9-41.9 | 32.70 | 31.0-34.0 (Wintrobe) |
| 8 | Hemoglobin concentration distribution width (HDW) | (g/dL) | 1.63-5.65 | 2.53 | 1.9-3.0 |
| 9 | Red cell volume distribution width (RDW) | (%) | 12.4-24.2 | 15.30 | 12.0-15.0 |
| 10 | White blood cell count (WBC) | ($\times 10^3$ cells/µL) | 1.73-50.06 | 15.28 | 6-17 |
| 11 | Mean platlet volume (MPV) | (fL) | 3.5-26.8 | 11.10 | 6-11 |

The values of HCT ranged from 10.3 to 53.3 % with a median value was 30.30%., which is below the normal range. The values of MCV ranged from 37.7 to 102.1 fL with a median value of 62.20 fL, which was within the normal range. The values of MCH ranged from 9.5 to 24.2 pg, with a median value of 19.50 pg, which was within the normal range. The values of MCHC ranged from 13.9 to 41.9 g/dL, with a median value was 32.70 g/dL, which was within the normal range. On the contrary, Briggs *et al* (2017) reported that CHCM is more directly measured equivalent of the MCHC and showed that MCHC and CHCM decreases as hypochromia develops. The values of HDW ranged from 1.63 to 5.65 g/dL with a median value of 2.53 g/dL, which was within the normal range. The values of RDW ranged from 12.4 to 24.2 % with a median value of 15.30 %, which was above the normal range. Kim *et al* (2013) reported that increased RDW was associated with advanced fibrosis in non alcoholic fatty liver disease in human patients. The WBC count ranged from 1.73 to 50.06 × $10^3$ cells/µL with a median value of 15.28, which was within the normal range. The values of MPV ranged from 3.5 to 26.8 fL with a median value of 11.10 fL, which was within the normal range.

### 4.2.20 Hematological parameters in dogs suffering from renal failure using ADVIA hematology system

In the present study the values of different haematological parameters in dogs suffering from renal damage alone, along with their range and median values are presented in the table 38. The values of mean CH ranged from 17.1 to 22.5 pg with a median value of 20.25 pg, which was less than the normal range. The values of CHCM ranged from 29.9 to 36.5 g/dL with a median value of 32.10, which was within the normal range. The values of RBC count ranged from 2.17 to 8.54 × $10^6$ cells/µL with a median value of 5.15 × $10^6$ cells/µL, which was below the normal range. The values of HCT ranged from 13.2 to 49.7 % with a median value of 31.10 %., which was below the normal range. The values of MCV ranged from 6.7 to 73.1 fL with a median value of 61.65 fL, which was within the normal range. The values of MCH ranged from 11.1 to 24.8 pg, with a median value of 19.95 pg, which was within the normal range. The values of MCHC ranged from 17.7 to 42.5 g/dL, with a median value of 31.65 g/dL, which was within the normal range.

**Table 38: Hematological parameters in dogs suffering from renal failure in prospective cases using ADVIA hematology system**

| S. No. | Hematological parameters | Units | Range in clinical cases | Median value | Normal range |
|---|---|---|---|---|---|
| 1 | Mean hemoglobin content (CH) | (pg) | 17.1-22.5 | 20.25 | 24.0-35.0 |
| 2 | Hemoglobin concentration mean (CHCM) | (g/dL) | 29.9-36.5 | 32.10 | 29.0-34.0 |
| 3 | Red blood cell count (RBC) | ($\times 10^6$ cells/µL) | 2.17-8.54 | 5.15 | 5.5-8.5 |
| 4 | Hematocrit (HCT) | (%) | 13.2-49.7 | 31.10 | 37.0-55.0 |
| 5 | Mean corpuscular volume (MCV) | (fL) | 6.7-73.1 | 61.65 | 60.0-77.0 |
| 6 | Mean corpuscular hemoglobin (MCH) | (pg) | 11.1-24.8 | 19.95 | 19.5-24.5 |
| 7 | Mean corpuscular hemoglobin concentration (MCHC) | (g/dL) | 17.7-42.5 | 31.65 | 31.0-34.0 (Wintrobe) |
| 8 | Hemoglobin concentration distribution width (HDW) | (g/dL) | 1.54-4.84 | 2.02 | 1.9-3.0 |
| 9 | Red cell volume distribution width (RDW) | (%) | 11.4-23.2 | 14.70 | 12.0-15.0 |
| 10 | White blood cell count (WBC) | ($\times 10^3$ cells/µL) | 4.5-124.5 | 14.42 | 6-17 |
| 11 | Mean platlet volume (MPV) | (fL) | 6.7-31.2 | 11.40 | 6-11 |

The values of HDW ranged from 1.54 to 4.84 g/dL with a median value of 2.02 g/dL, which was within the normal range. The values of RDW ranged from 11.4 to 23.2 % with a median value of 14.70 %., which was within the normal range. The values of WBC count ranged from 4.5 to 124.5 × $10^3$ cells/µL with a median value of 14.42, which was within the normal range. The values of MPV ranged from 6.7 to 31.2 fL with a median value of 11.40 fL, which was within the normal range.

Munoz and Alessi (2008) reported that low CH was associated with chronic kidney disease in dogs, Similarly, Ramesh *et al* (2018) reported that low RBC count was observed in dogs having chronic kidney disease due to lack of erythropoietin production. McBride *et al* (2019) reported that low HCT and high MPV was observed in dogs having acute kidney disease. Buttarello and Plebani (2008) observed that high RDW was associated with iron deficieny in human patients.

### 4.2.21 Hematological parameters in dogs suffering from concurrent liver and kidney damage using ADVIA hematology system

In the present study the values of different hematological parameters in dogs suffering from concurrent liver and kidney damage along with their range and median values are presented in the table 39. The values of CH ranged from 11.7 to 24.3 pg with a median value of 20.00 pg, which was less than the normal range. Similarly, Sumit *et al* (2018) reported that low CH was associated with the failure of erythropoietin secretion in dogs having renal imparement. Niwetpathomwat *et al* (2006) reported that dogs infected with ehrlichiosis had decreased red cell count and hemoglobin. The values of CHCM ranged from 27.1 to 36.6 g/dL with a median value of 32.30, which was within the normal range. The values of RBC count ranged from 1.39 to 7.95 × $10^6$ cells/µL with a median value of 4.63 × $10^6$ cells/µL, which was below the normal range. The values of HCT ranged from 9.6 to 56.9 % with a median value of 26.70 %., which was below the normal range. Similarly, Harrison *et al* (2018) reported that low HCT and elevated liver enzymes were observed in dogs suffering from cholangiohepatitis. The values of MCV ranged from 40.6 to 81.1 fL with a median value of 62.70 fL, which was within the normal range. The values of MCH ranged from 6.3 to 23.5 pg, with a median value of 19.80 pg, which was within the normal range. The values of MCHC ranged from 15.5 to 42.4 g/dL, with a median value of 32.00 g/dL, which was within the normal range.

**Table 39: Hematological parameters in dogs suffering from concurrent liver and kidney damage in prospective cases using ADVIA hematology system**

| S.No. | Hematological parameter | Units | Range in clinical cases | Median value | Normal range |
|---|---|---|---|---|---|
| 1 | Mean hemoglobin content (CH) | (pg) | 11.7-24.3 | 20.00 | 24.0-35.0 |
| 2 | Hemoglobin concentration mean (CHCM) | (g/dL) | 27.1-36.6 | 32.30 | 29.0-34.0 |
| 3 | Red blood cell count (RBC) | ($\times 10^6$ cells/µL) | 1.39-7.95 | 4.63 | 5.5-8.5 |
| 4 | Hematocrit (HCT) | (%) | 9.6-56.9 | 26.70 | 37.0-55.0 |
| 5 | Mean corpuscular volume (MCV) | (fL) | 40.6-81.1 | 62.70 | 60.0-77.0 |
| 6 | Mean corpuscular hemoglobin (MCH) | (pg) | 6.3-23.5 | 19.80 | 19.5-24.5 |
| 7 | Mean corpuscular hemoglobin concentration (MCHC) | (g/dL) | 15.5-42.4 | 32.00 | 31.0-34.0 (wintrobe) |
| 8 | Hemoglobin concentration distribution width (HDW) | (g/dL) | 1.55-5.86 | 2.20 | 1.9-3.0 |
| 9 | Red cell volume distribution width (RDW) | (%) | 10.8-20.5 | 14.30 | 12.0-15.0 |
| 10 | White blood cell count (WBC) | ($\times 10^3$ cells/µL) | 3.31-74.81 | 17.92 | 6-17 |
| 11 | Mean platlet volume (MPV) | (fL) | 5.5-30.4 | 13.10 | 6-11 |

The values of HDW ranged from 1.55 to 5.86 g/dL with a median value of 2.20 g/dL, which was within the normal range. The values of RDW ranged from 10.8 to 20.5 % with a median value of 14.30 %., which was within the normal range. The values of WBC count ranged from 3.31 to 74.81 × $10^3$ cells/µL with a median value of 17.92, which was above the normal range. The values of median of MPV ranged from 5.5 to 30.4 fL with a median value of 13.10 fL, which was within the normal range. On the contrary, Wilkinson (2019) reported that dogs having chronic liver disease showed decreased platelets functions which resulted in hemostatic abnormalities.

### 4.2.22 Erythrocytic indices in dogs suffering from liver and kidney damage alone or concurrent liver and kidney damage using ADVIA hematology system

The table 40 depicts the erythrocytic indices observed in dogs suffering from liver and kidney damage alone or concurrent liver and kidney damage. The mean value of hemoglobin in dogs suffering from liver damage was 10.41, those suffering from kidney damage was 10.28 and the mean value in dogs suffering from concurrent liver and kidney damage was 9.74. The mean value of hemoglobin in dogs suffering from liver, kidney and concurrent liver and kidney damage did not differ significantly. The mean value of CH in dogs suffering from liver damage was 19.86, those suffering from kidney damage was 20.18 and the mean value in dogs suffering from concurrent liver and kidney damage was 19.81. The mean value of CH in dogs suffering from liver, kidney and concurrent liver and kidney damage did not differ significantly. The mean value of HCT in dogs suffering from liver damage was 31.54, those suffering from kidney damage was 30.86 and the mean value in dogs suffering from concurrent liver and kidney damage was 28.05. The mean value of hematocrit in dogs suffering from liver, kidney and concurrent liver and kidney damage did not differ significantly. The mean value of RBC count in dogs suffering from liver damage was 5.19, those suffering from kidney damage was 4.99 and the mean value in dogs suffering from concurrent liver and kidney damage was 4.58. The mean value of RBC count in dogs suffering from liver, kidney and concurrent liver and kidney damage did not differ significantly. The mean value of MCV in dogs suffering from liver damage was 61.91, those suffering from kidney damage was 60.17 and the mean value in dogs suffering from concurrent liver and kidney damage was 62.18.

**Table 40: Erythrocytic indices in dogs suffering from liver and kidney damage alone or concurrent liver and kidney damage in prospective cases using ADVIA hematology system**

| Erythrocytic indices | Liver damage | Kidney damage | Concurrent liver and kidney damage |
|---|---|---|---|
| Hemoglobin (Hgb) | $10.41^{a}$±0.58 | $10.28^{a}$±0.62 | $9.74^{a}$±0.57 |
| Mean hemoglobin content (CH) | $19.86^{a}$±0.15 | $20.18^{a}$±0.14 | $19.81^{a}$±0.17 |
| Hematocrit (HCT) | $31.54^{a}$±1.62 | $30.86^{a}$±4.70 | $28.05^{a}$±3.08 |
| Red blood cell count (RBC) | $5.19^{a}$±0.28 | $4.99^{a}$±0.38 | $4.58^{a}$±0.26 |
| Mean corpuscular volume (MCV) | $61.91^{a}$±1.70 | $60.17^{a}$±1.77 | $62.18^{a}$±1.56 |
| Mean corpuscular hemoglobin (MCH) | $19.03^{a}$±1.53 | $18.78^{a}$±2.14 | $17.91^{a}$±1.28 |
| Mean corpuscular hemoglobin concentration (MCHC) | $30.77^{a}$±0.57 | $30.32^{a}$±0.72 | $28.95^{a}$±0.79 |
| Hemoglobin concentration mean (CHCM) | $32.30^{a}$±0.48 | $32.61^{a}$±0.27 | $32.26^{a}$±0.45 |
| Hemoglobin concentration distribution width (HDW) | $2.80^{a}$±0.95 | $2.22^{b}$±1.15 | $2.45^{ab}$±1.30 |
| Red cell volume distribution width (RDW) | $16.48^{a}$±0.63 | $15.41^{a}$±0.57 | $15.01^{a}$±0.38 |

Values with different superscripts (a,b,c) within a parameter differ significantly ($p<0.05$)

The mean value of MCV in dogs suffering from liver, kidney and concurrent liver and kidney damage did not differ significantly. The mean value of MCH in dogs suffering from liver damage was 19.03, those suffering from kidney damage was 18.78 and the mean value in dogs suffering from concurrent liver and kidney damage was 17.91. The mean value of MCH in dogs suffering from liver, kidney and concurrent liver and kidney damage did not differ significantly. The mean value of MCHC in dogs suffering from liver damage was 30.77, those suffering from kidney damage was 30.32 and the mean value in dogs suffering from concurrent liver and kidney damage was 28.95. The mean value of MCHC in dogs suffering from liver, kidney and concurrent liver and kidney damage did not differ significantly. The mean value of CHCM in dogs suffering from liver damage was 32.30, those suffering from kidney damage was 32.61 and the mean value in dogs suffering from concurrent liver and kidney damage was 32.26. The mean value of CHCM in dogs suffering from

liver, kidney and concurrent liver and kidney damage did not differ significantly. The mean value of HDW in dogs suffering from liver damage was 2.80, those suffering from kidney damage was 2.22 and the mean value in dogs suffering from concurrent liver and kidney damage was 2.45. The mean value of HDW were significantly higher in dogs suffering from liver damage as compared to dogs suffering from kidney damage alone. However, the mean value of HDW in dogs suffering from concurrent liver and kidney damage did not differ significantly from dogs suffering from liver and or kidney damage alone. The mean value of RDW in dogs suffering from liver damage was 16.48, those suffering from kidney damage was 15.41 and the mean value in dogs suffering from concurrent liver and kidney damage was 15.01. The mean value of RDW in dogs suffering from liver, kidney and concurrent liver and kidney damage did not differ significantly.

Brugnara (2000) reported that low levels of CH, CHCM, MCV and MCH were associated with iron deficiency in dogs. Cengiz *et al* (2013) reported that high RDW was associated with liver disease in human patients and Xu *et al* (2015) reported that high HDW, RDW was associated with liver fibrosis in human patients.

### 4.2.23 Correlation of erythrocytic indices on the basis of colour and size analyzed by automated analyzer with serum chemistry findings

The table 41 depicts the erythrocytic indices (on the basis of colour and size) observed in dogs suffering from liver and kidney damage alone and concurrent liver and kidney damage. The mean value of hypochromasia in dogs suffering from liver damage was 0.12, those suffering from kidney damage was 0.03 and the mean value in dogs suffering from concurrent liver and kidney damage was 0.02. However, the mean value of hypochromasia in dogs suffering from liver, kidney and concurrent liver and kidney damage did not differ significantly. However, Hall and German (2016) reported that hypochromasia, slight anisocytosis and codocytes were observed in hepatic diseases of dogs. The mean value of polychromasia in dogs suffering from liver damage was 0.12, those suffering from kidney damage was 0.10 and the mean value in dogs suffering from concurrent liver and kidney damage was 0.02. However, the mean value of polychromasia in dogs suffering from liver, kidney and concurrent liver and kidney damage did not differ significantly. Whereas, Padula and Leister (2017) reported that polychromasia and elevated liver enzymes were observed in dogs having liver damage.

**Table 41: Correlation of erythrocytic indices on the basis of colour and size analyzed by automated analyzer with serum chemistry findings**

| Erythrocytic indices | Liver damage | Kidney damage | Concurrent liver and kidney damage |
|---|---|---|---|
| Hypochromasia | $0.12^{a} \pm 0.06$ | $0.03^{a} \pm 0.03$ | $0.02^{a} \pm 0.02$ |
| Polychromasia | $0.12^{a} \pm 0.05$ | $0.10^{a} \pm 0.05$ | $0.02^{a} \pm 0.02$ |
| Anisocytosis | $0.20^{a} \pm 0.11$ | $0.17^{a} \pm 0.11$ | $0.02^{a} \pm 0.03$ |
| Microcytic | $0.58^{a} \pm 0.16$ | $0.42^{ab} \pm 0.14$ | $0.16^{b} \pm 0.09$ |
| Macrocytic | $0.12^{a} \pm 0.08$ | $0.03^{a} \pm 0.03$ | $0.00^{a} \pm 0.00$ |

Values with different superscripts (a,b,c) within a parameter differ significantly ($p<0.05$)

The mean value of anisocytosis in dogs suffering from liver damage was 0.20, those suffering from kidney damage was 0.17 and the mean value in dogs suffering from concurrent liver and kidney damage was 0.02. However, the mean value of anisocytosis in dogs suffering from liver, kidney and concurrent liver and kidney damage did not differ significantly. However, Omobowale (2017) reported that in the presence of babesiosis, the most common morphological abnormality observed was anisocytosis.

The mean value of microcytic RBCs in dogs suffering from liver damage was 0.58, those suffering from kidney damage was 0.42 and the mean value in dogs suffering from concurrent liver and kidney damage was 0.16. The mean value of microcytic RBCs were significantly higher in dogs suffering from liver damage as compared to dogs suffering from concurrent liver and kidney damage. However, the mean value of microcytic RBCs in dogs suffering from kidney damage did not differ significantly from dogs suffering from liver and or concurrent liver and kidney damage. Schaefer and Stokol (2015) reported that microcytosis was associated with the iron deficiency in dogs.

The mean value of macrocytic RBCs in dogs suffering from liver damage was 0.12, those suffering from kidney damage was 0.03 and the mean value in dogs suffering from concurrent liver and kidney damage was 0.00. However, the mean value of macrocytic RBCs in dogs suffering from liver, kidney and concurrent liver and kidney damage did not differ significantly. Gavazza *et al* (2012) reported that macrocytosis have been reported in many inflammatory diseases in dogs including gastro-intestinal, respiratory and skin diseases.

### 4.2.24 Correlation of morphological alterations of RBCs in dogs suffering from liver damage

The table 42 shows a significant positive correlation ($p<0.05$) between the occurrance of polychromatophils and liver damage, whereas, marginal correlation ($p<0.1$) was observed between the occurrence of dacrocytes in blood smears of dogs suffering from liver damage.

**Table 42: Correlation of morphological alterations of RBCs in dogs suffering from liver damage**

| Parameter | Estimates ± SE | Wald Chi-Square | Pr > ChiSq | Odds ratio |
|---|---|---|---|---|
| **Polychromatophils** | -0.28 ±0.13 | 4.2373 | 0.0395 | 0.567 |
| **Dacrocytes** | 0.31 ± 0.17 | 3.0000 | 0.0833 | 1.860 |

### 4.2.25 Correlation of morphological alterations of RBCs in dogs suffering from kidney damage on the basis of counts

The table 43 shows the morphological alterations seen in dogs suffering from kidney damage. A significant positive correlation ($p < 0.05$) was observed between occurrance of acanthocytes, stomatocytes and polychromatophils in blood smears of dogs suffering from kidney damage. Thrall (2006) reported that Drentse Patrijshond dogs showing stomatocytosis suffering from hypertrophic gastritis, retarted growth, diarrhea, renal cysts and polyneuropathy.

**Table 43: Correlation of morphological alterations of RBCs in dogs suffering from kidney damage on the basis of counts**

| Parameter | Estimate ± SE | Wald Chi-Square | Pr > ChiSq | Odds ratio |
|---|---|---|---|---|
| **Acanthocytes** | -0.01 ± 0.00 | 4.2782 | 0.0386 | 0.988 |
| **Stomatocytes** | -0.07 ± 0.03 | 4.6314 | 0.0314 | 0.925 |
| **Polychromatophils** | -0.05 ± 0.02 | 7.3109 | 0.0069 | 0.946 |

### 4.2.26 Correlation of morphological alterations of RBC in dogs suffering from concurrent liver and kidney damage

The table 44 shows the correlation of morphological alterations in RBC of dogs with concurrent liver and kidney damage. Significant positive correlation (p<

0.05) was observed between occurrance of echinocytes and polychromatophils in blood smears of dogs suffering from concurrent liver and kidney damage whereas, marginal correlation ($p<0.1$) was observed between the occurrence of acanthocytes and stomatocytes in blood smears of dogs suffering from concurrent liver and kidney damage. Slappendel *et al* (1991) reported stomatocytosis, increased liver enzymes and serum bilirubin in Drenste Patrijshond breed suffering from familial stomatocytosis-hypertrophic gastritis. Walton *et al* (1997) observed echinocytes in dogs suffering from glomerulonephritis.

**Table 44: Correlation of morphological alterations of RBC in dogs suffering from concurrent liver and kidney damage in prospective cases**

| Parameter | Estimates ± SE | Wald Chi-Square | Pr > ChiSq | Odds ratio (estimates) |
|---|---|---|---|---|
| **Echinocytes** | -0.21 ± 0.11 | 3.8711 | 0.0491 | 0.647 |
| **Acanthocytes** | 0.18 ± 0.10 | 2.9602 | 0.0853 | 1.447 |
| **Stomatocytes** | 0.27 ± 0.16 | 2.8840 | 0.0895 | 1.734 |
| **Polychromatophils** | 0.26 ± 0.10 | 5.8335 | 0.0157 | 1.693 |

In the present study**,** correlation between serum chemistry findings and hematological parameters in dogs suffering from liver damage was carried out at ($p<0.05$) and there was high correlation (0.80) between levels of potassium and presence of acanthocytes in blood smear of dogs, whereas, moderate correlation (0.30) was observed between the levels of total protein and presence of spherocytes in blood smears of dogs, between the levels of GGT and presence of dacrocytes in the blood smears of dogs (0.32). Low correlation (0.18) was observed between the levels of ALKP with the presence of schistocytes and dacrocytes in the blood smears of dogs, levels of albumin with stomatocytes (0.17) and with polychromatophils in the blood smears of dogs (0.21). In addition, high negative correlation (-0.35) was observed between the levels of albumin and presence of echinocytes in the blood smears of dogs and hemoglobin value and presence of nucleated RBCs in the blood smears of dogs ($p<0.001$).

In the present study**,** correlation between serum chemistry findings and hematological parameters in dogs suffering from kidney damage was carried out at ($p<0.05$) and there was moderate correlation between the levels of ALKP with

presence of polychromatophilic RBCs (0.31) and dacrocytes (0.26) in the blood smears of dogs and between the levels of total protein with presence of spherocytes in the blood smears of dogs (0.26) and between the levels of GGT and presence of eccentrocytes in blood smears of dogs (0.33). There was low correlation (0.18) between the levels of AST with the presence of stomatocytes and eccentrocytes in the blood smears of dogs, levels of albumin and polychromatophils in the blood smears (0.21), levels of BUN with keratocytes in the blood smears (0.21), levels of creatinine and spherocytes in the blood smears (0.22) and levels of sodium with stomatocytes in the blood smear of dogs (0.19). In addition, negative correlation (-0.35) was observed between the levels of total bilirubin and presence of nucleated RBCs in the blood smears (-0.27), between the levels of albumin with echinocytes and blister cells in the blood smears (-0.22), between the levels of BUN with codocytes (-0.21) and nucleated RBCs in the blood smears (-0.20) and between the levels of creatinine with presence of nucleated RBCs (-0.21) and codocytes in the blood smears (-0.20). In addition, high correlation (0.46) was observed between the levels of total protein and dacrocytes in the blood smears of dogs ($p<0.001$)

In the present study**,** correlation between serum chemistry findings and hematological parameters in dogs suffering from concurrent liver and kidney damage was carried out at ($p<0.05$) and there was high correlation (0.64) between the levels of potassium with presence of polychromatophilic RBCs in the blood smears of dogs and moderate correlation (0.36) between the levels of AST and the presence of polychromatophils in the blood smears, low correlation between the levels of ALKP and presence of codocytes in the blood smears, levels of albumin and blister cells in the blood smears and levels of GGT and acanthocytes in the blood smears (0.24), levels of AST with eccentrocytes in the blood smears and levels of ALT with dacrocytes in the blood smears (0.23), levels of total protein and stomatocytes in the blood smears (0.22) and levels of BUN with acanthocytes in the blood smears (0.19). In addition, negative correlation was observed between the levels of ALT and presence of echinocytes (-0.20) and q RBCs (-0.19) in the blood smears, levels of ALKP with spherocytes in the blood smears (-0.26), levels of albumin with presence of echinocytes in the blood smears (-0.20), levels of GGT with presence of spherocytes in the blood smears (-0.28), levels of creatinine with presence of polychromatophils (-0.27) in the blood smears.

In the present study**,** correlation of ADVIA parameters in dogs suffering from liver damage was carried out at ($p<0.05$) and there was moderate correlation between hypochromasia with presence of stomatocytes and eccentrocytes in the blood smears, polychromasia with presence of blister cells in the blood smears, anisocytosis with presence of echinocytes, polychromatophilic RBCs and blister cells in the blood smears, RBC count with presence of echinocytes in the blood smears, WBC counts with presence of acanthocytes and polychromatophilic RBCs in the blood smears, HCT counts with echinocytes in the blood smears, MCV with presence of polychromatophilic RBCs in the blood smears, MCH value with polychromatophilic RBCs and schistocytes in the blood smears. WBCB counts with presence of acanthocytes, polychromatophilic RBCs and eccentrocytes in the blood smears, CHCM value with presence of q RBCs in the blood smears, RDW with presence of polychromatophilic RBCs in the blood smears and HDW with nucleated RBCs and polychromatophilic RBCs in the blood smears. In addition, negative correlation was observed between RBC count and presence of polychromatophilic RBCs in the blood smears, WBC counts with presence of incomplete spherocytes in the blood smears, MCV with presence of schistocytes in the blood smears, value of MCH and CH with presence of schistocytes in the blood smears and values of WBCB with presence of incomplete spherocytes in blood smears.

In the present study**,** correlation of ADVIA parameters in dogs suffering from kidney damage was carried out at ($p<0.05$) and there was moderate correlation between the values of CHCM and presence of echinocytes in the blood smears, anisocytosis and presence of polychromatophilic RBCs in the blood smears, MCV and presence of incomplete spherocytes in the blood smears and WBCB with presence of q RBCs in the blood smears. In addition, negative correlation was observed between values of MPV and presence of echinocytes in the blood smears, MCV and presence of eccentrocytes in the blood smears and CH value with the presence of polychromatophilic RBCs and eccentrocytes in the blood smears.

In the present study**,** correlation of ADVIA parameters in dogs suffering from concurrent liver and kidney damage was carried out at ($p<0.05$) and there was moderate correlation between MCV with the presence of incomplete spherocytes and

dacrocytes in the blood smears, values of RDW with presence of codocytes and polychromatophilic RBCs in the blood smears, HDW value and anisocytosis with presence of polychromatophilic RBCs in the blood smears, MCH value with presence of dacrocytes in the blood smears, MCHC value with presence of polychromatophilic RBCs in the blood smears and CH value with presence of dacrocytes in the blood smears and negative correlation was observed between value of HCT and presence of acanthocytes and dacrocytes in the blood smears, HDW value with presence of spherocytes in the blood smears. In addition, high significant correlation was observed between hypochromasia and presence of dacrocytes in the blood smears ($p<0.001$).

### 4.2.27 Serum chemistry, ultrasound findings and erythrocytic abnormalities in dogs suffering from liver and kidney damage

The table 45 depicts that codocytes were observed in the case of a dog having a mass of around 1.6 X 2.1 cm in the right kidney, suspected to be a tumor (Fig 24), codocytes were also observed in another dog having mass of heterogenous ecogenicity seen in cranial part of left kidney, echinocytes observed in dog showing reduced cortical thickness in both kidney and echinocytes observed in dog having anechoic free fluid in abdominal cavity (Fig 25). Gong *et al* (2019) reported that ultrasonographic examination showed hybrid echogenic mass in bilateral kidney in dogs having collecting duct carcinoma with high serum levels of creatinine. Jana *et al* (2019) reported that low total protein and albumin levels in serum suggested ascites of hepatic origin which was confirmed by ultrasonographic examination which revealed anechoic free fluid in the abdominal cavity.

However, no special case in which radiographic findings could be correlated with erythrocytic abnormalities.

**Table 45: Serum chemistry, ultrasound findings and erythrocytic abnormalities in dogs suffering from liver and kidney damage**

| Organ involved | Serum chemistry | Ultrasound findings | Erythrocytic abnormality |
|---|---|---|---|
| Kidney | Total bilirubin-0.1<br>AST- 29<br>ALKP- 155<br>ALT-21<br>TP-4.8<br>Albumin- 1.3<br>GGT- <5<br>BUN-4<br>Creatinine-0.7 | Right kidney showing mass of 1.6 X 2.1 cm, suspected to be a tumor | Codocytes |
| Kidney | Total bilirubin- 0.3<br>ALT- 48<br>ALKP-149<br>TP- 5.8<br>Albumin- 1.5<br>BUN >140<br>Creatinine- 4.2 | Mass of heterogenous ecogenicity seen in cranial part of left kidney | Codocytes |
| Kidney | BUN- 60<br>Creatinine- 4.3 | Reduced cortical thickness in both kidney | Echinocytes |
| Liver | AST- 42<br>ALT-18<br>TP-4.8<br>Albumin-1.4<br>GGT< 5<br>BUN- 7<br>Creatinine-1 | Anechoic free fluid in abdominal cavity | Echinocytes |

### 4.2.28 Correlation of erythrocytic abnormalities with cytology findings

The table 46 showed that spherocytes and q RBCs were found in TVT cases (Fig 26) and echinocytes and schistocytes were found in lymphoma cases (Fig 27), acanthocytes were found in malignant melanoma (Fig 28) whereas, acanthocytes and schistocytes were observed in malignant histiocytoma (Fig 29). The codocytes was observed in chronic active inflammation (Fig 30).

Parachini *et al* (2019) reported that acanthocytes and schistocytes were observed in blood smear of dog having lymphoma. Caldin *et al* (2005) reported that dog having malignant melanoma showed acanthocytes and codocytes in the blood smears. Melendez *et al* (2018) reported that codocytes and acanthocytes were seen in blood smear of dogs suffering from chronic kidney diseases.

**Table 46: Correlation of erythrocytic abnormalities with cytology findings**

| **A. Neoplastic conditions** | | |
|---|---|---|
| **S. No.** | **Cytological diagnosis** | **Erythrocytic abnormality observed** |
| 1 | TVT | Spherocytes and q RBCs |
| 2 | Lymphoma | Acanthocytes, echinocytes and shistocytes |
| 3 | Malignant melanoma | Acanthocytes |
| 4 | Malignant histiocytoma | Acanthocytes and schistocytes |
| **B. Non- neoplastic conditions** | | |
| 1 | Chronic active inflammation | Codocytes |

### 4.3 Necropsy and Histopathological examination

In the present study, one post mortem examination could be was carried out in one case only. Important gross lesions recorded were congestion in lungs, liver and intestines. Histopathological examination revealed congestion in myocardium, depletion of lymphocytes in spleen, severe congestion and mild degenerative changes in hepatocytes (Fig 31), severe congestion and haemorrhage in kidney (Fig 32), severe congestion, emphysema and thickening of interstitium (Fig 33) in lungs and necrotic enteritis (Fig 34).

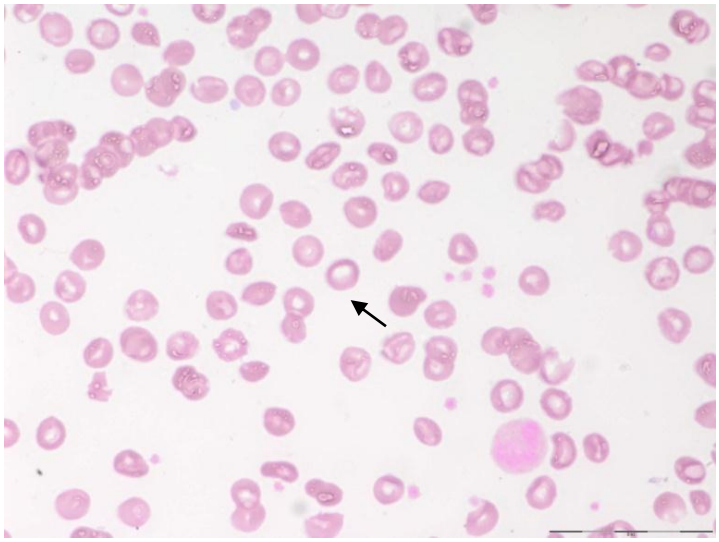

**Fig. 1. Peripheral blood smear revealing hypochromasia. Leishman stain x 100X (Bar=2µm)**

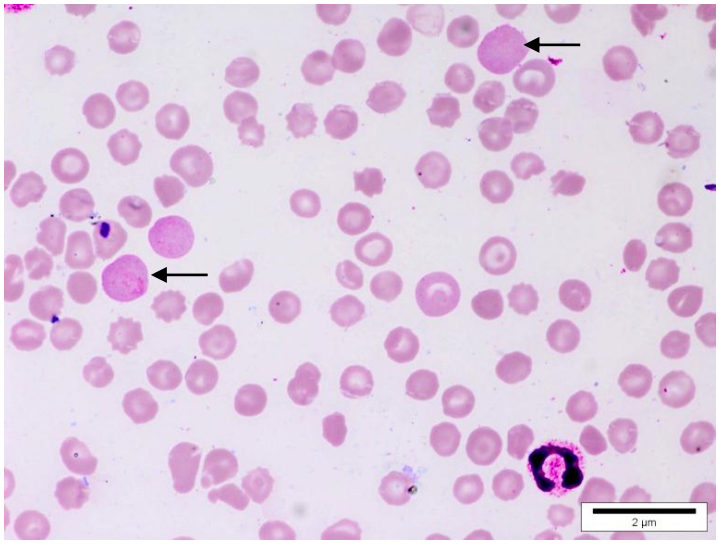


**Fig. 2.Peripheral blood smear revealing polychromasia. Leishman stain x 100X (Bar=2µm)**

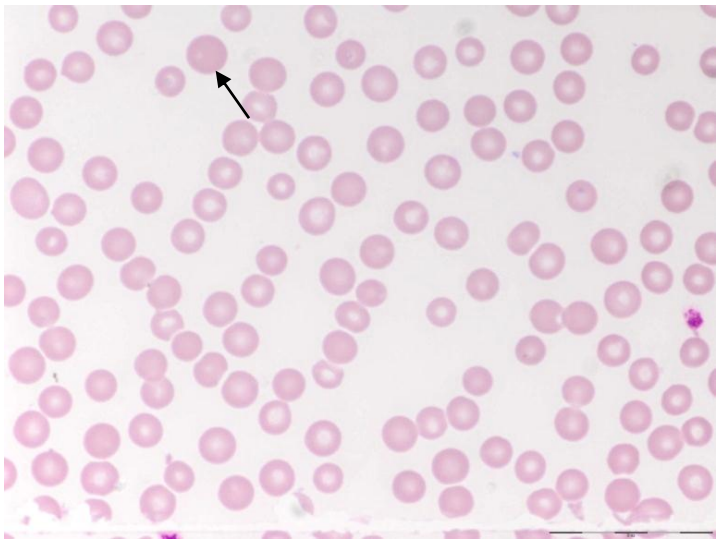

**Fig. 3. Peripheral blood smear revealing macrocytosis. Leishman stain x 100X (Bar=2µm)**

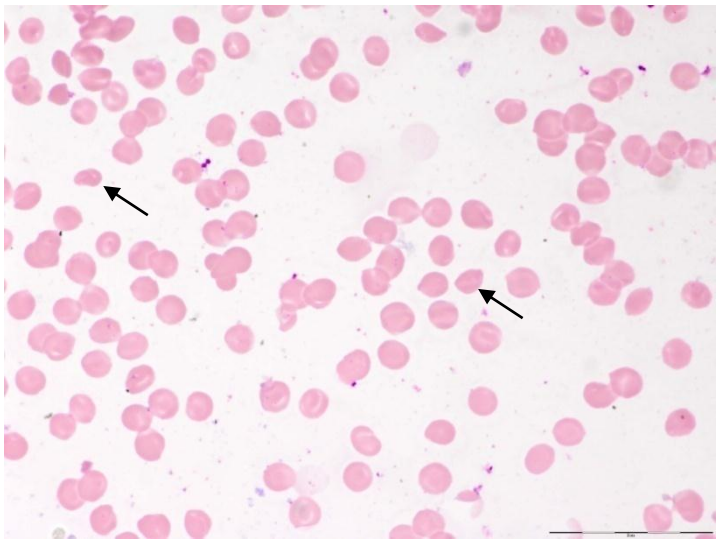

**Fig. 4. Peripheral blood smear revealing microcytosis. Leishman stain x 100X (Bar=2µm)**

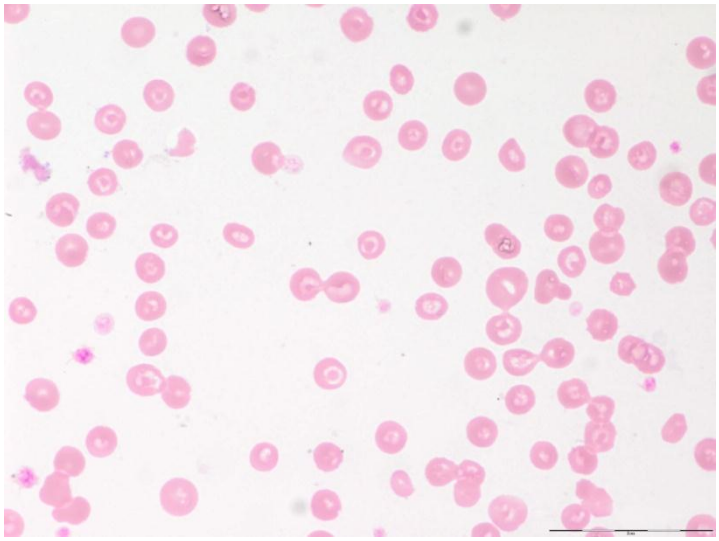

**Fig. 5. Peripheral blood smear revealing anisocytosis. Leishman stain x 100X(Bar=2µm)**

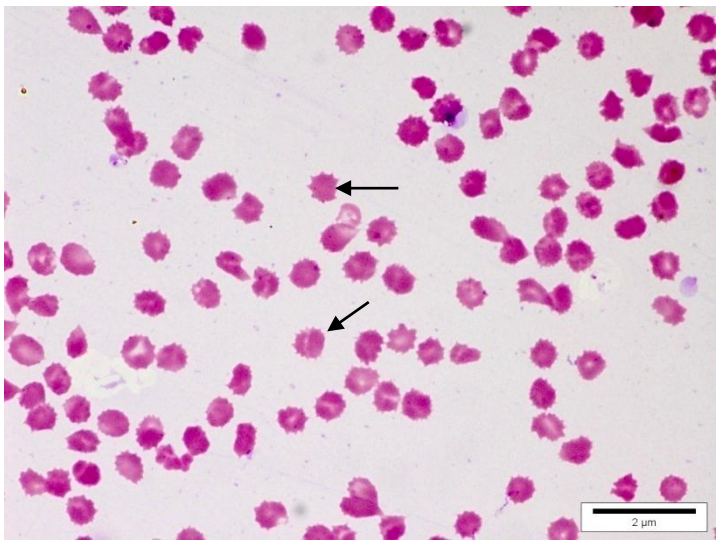


**Fig. 6. Peripheral blood smear revealing echinocytes. Leishman stain x 100X (Bar=2µm)**

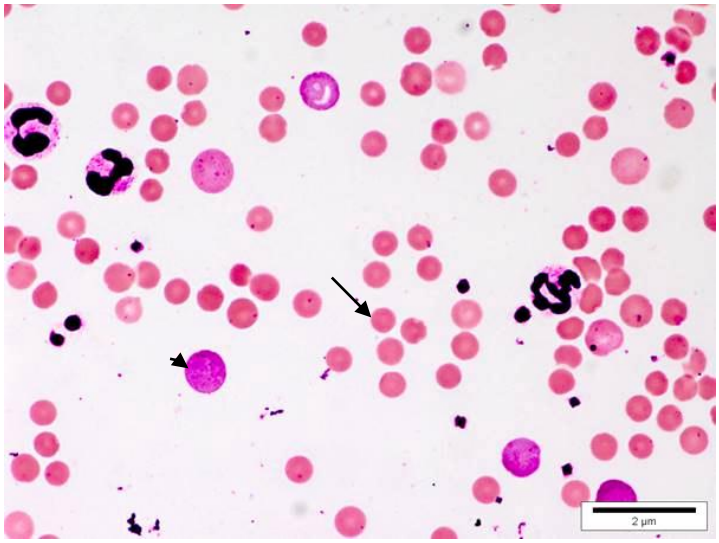


**Fig. 7. Peripheral blood smear revealing spherocytes (arrow) and polychromatic cells (arrow head). Leishman stain x 100X (Bar=2µm)**

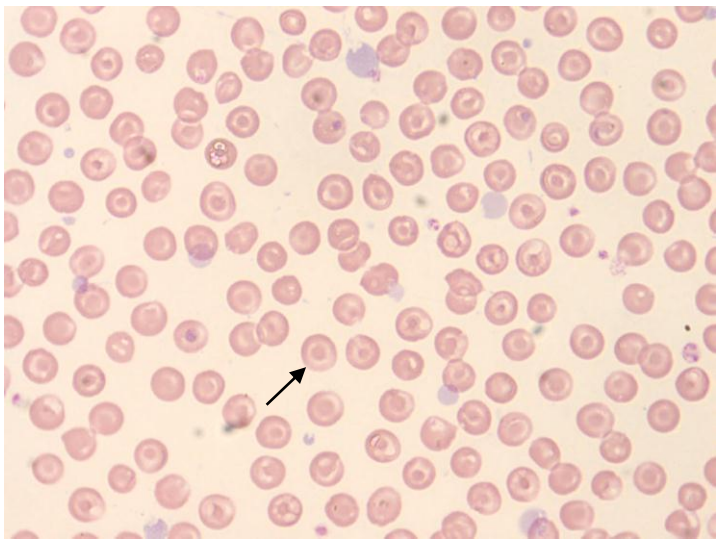

**Fig. 8. Peripheral blood smear revealing codocytes. Leishman stain x 100X**

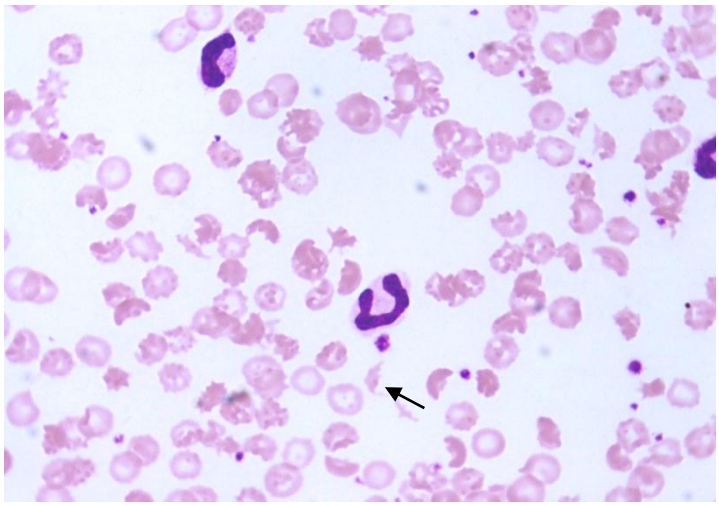

**Fig. 9. Peripheral blood smear revealing schistocytes. Leishman stain x 100X**

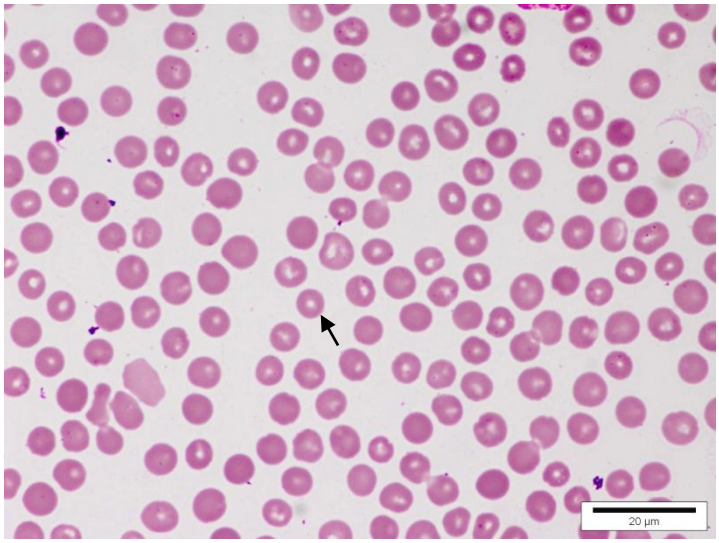


**Fig. 10. Peripheral blood smear revealing incomplete spherocytes. Leishman stain x 100X (Bar=2µm)**

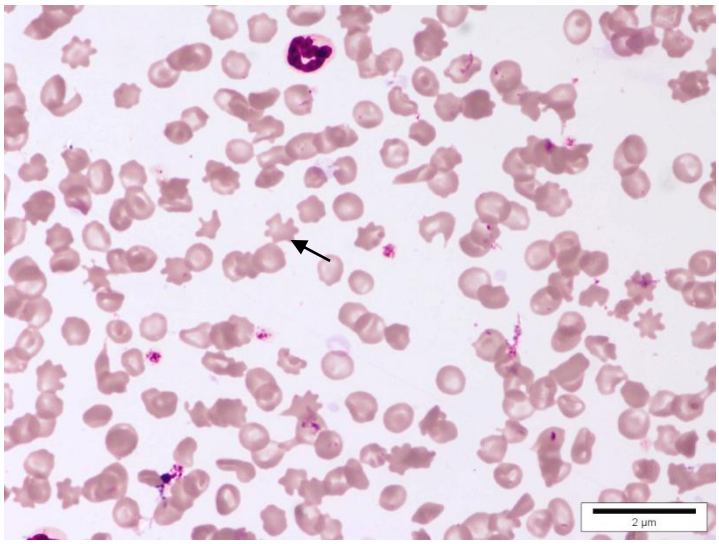


**Fig. 11. Peripheral blood smear revealing acanthocytes. Leishman stain x 100X (Bar=2µm)**

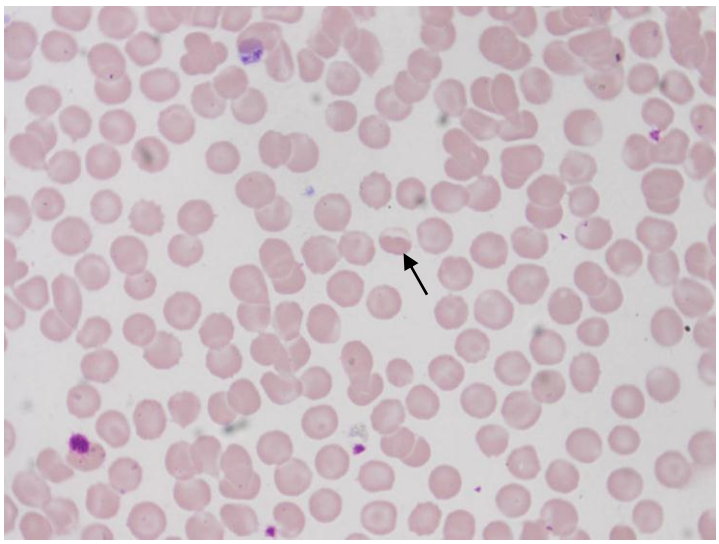

**Fig. 12. Peripheral blood smear revealing eccentrocytes. Leishman stain x 100X**

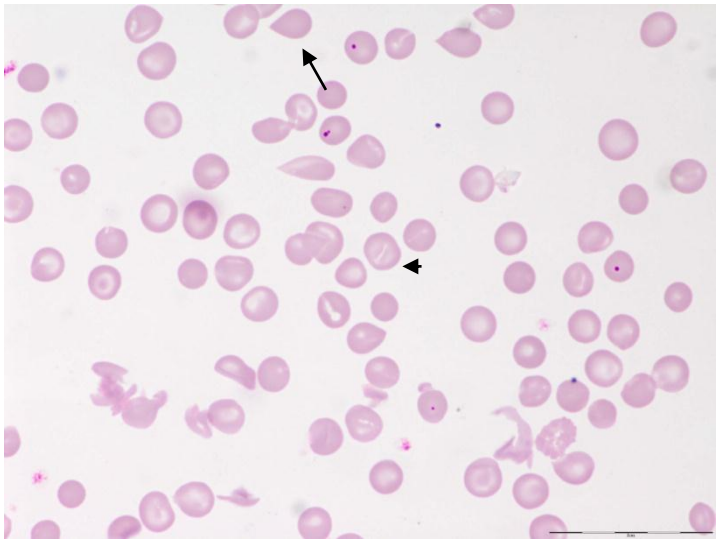

**Fig. 13 . Peripheral blood smear revealing dacrocytes (arrow) and stomatocytes (arrow head). Leishman stain x 100X (Bar=2µm)**

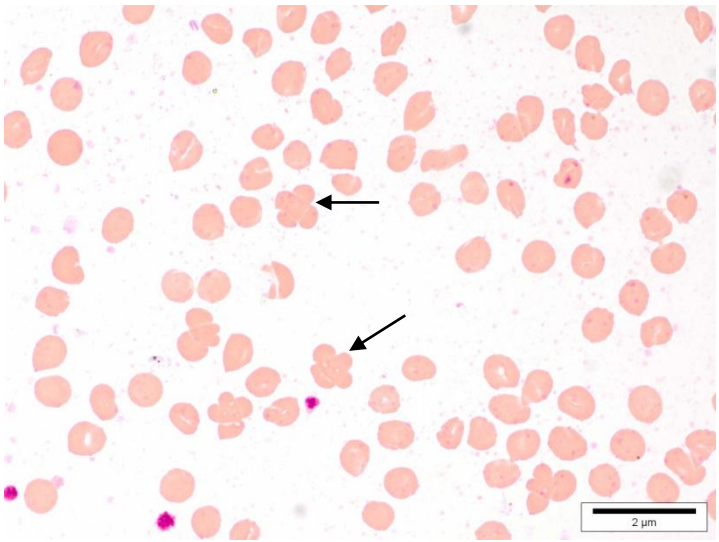


**Fig. 14. Peripheral blood smear revealing quatrefoil RBCs (q RBCs). Leishman stain x 100X (Bar=2µm)**

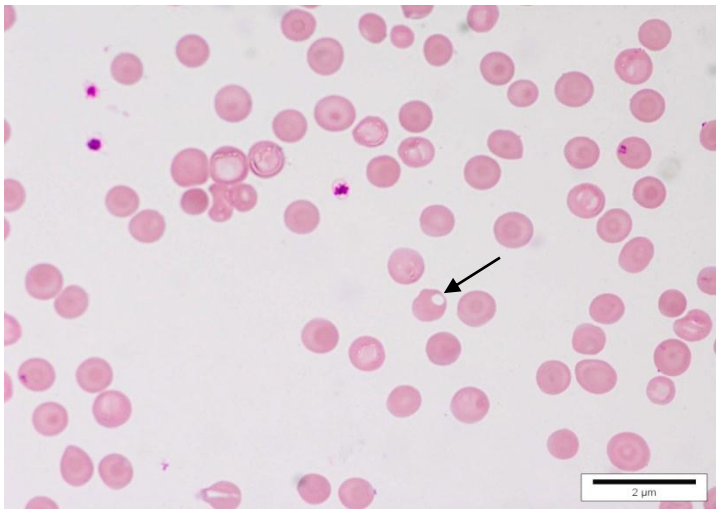


**Fig. 15. Peripheral blood smear revealing blister cells. Leishman stain x 100X (Bar=2µm)**

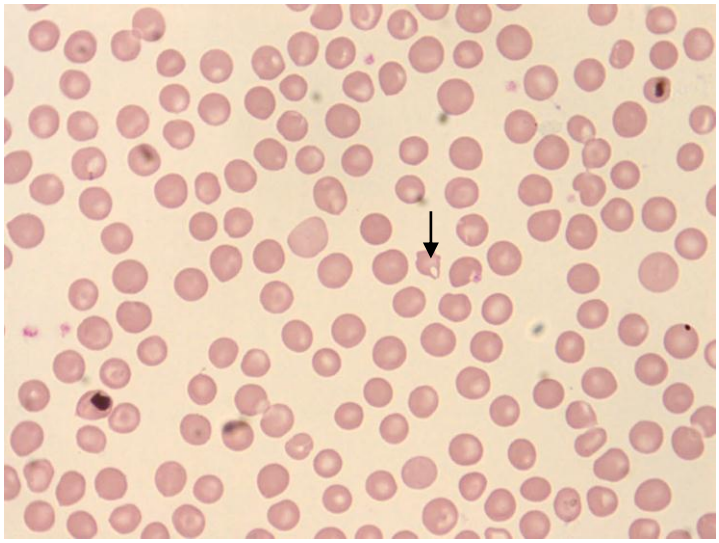

**Fig. 16. Peripheral blood smear revealing keratocytes. Leishman stain x 100X**

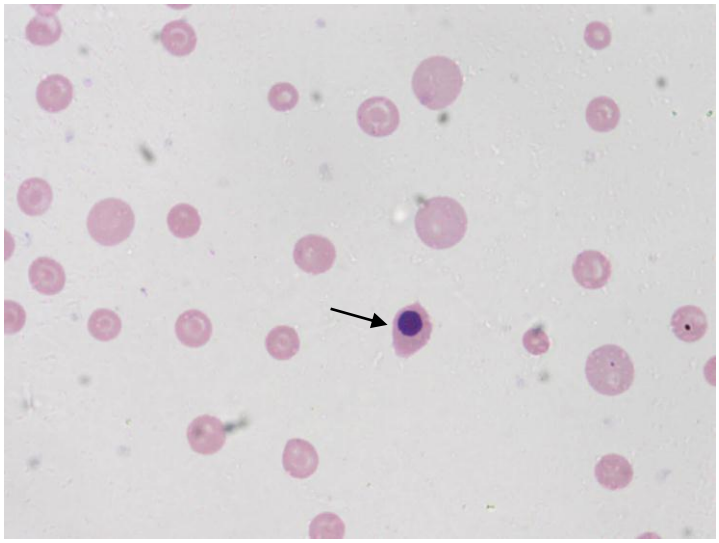

**Fig. 17. Peripheral blood smear revealing nucleated RBCs. Leishman stain x 100X**

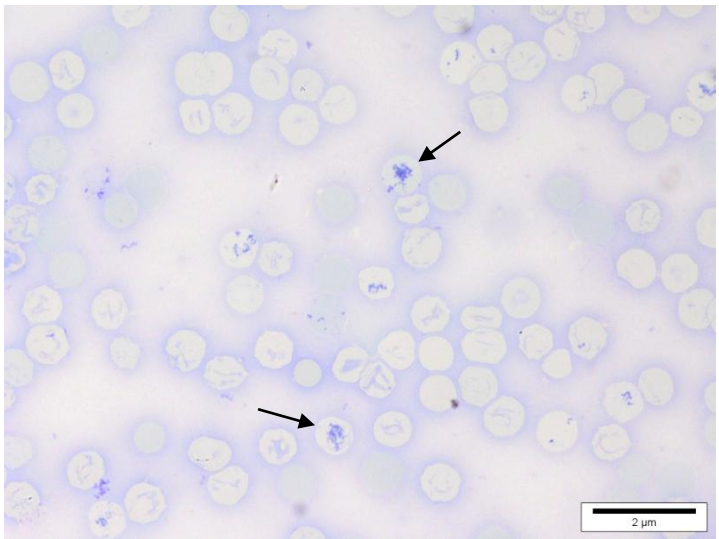


**Fig. 18. Peripheral blood smear revealing reticulocytes. New Methylene Blue x 100X (Bar=2µm)**

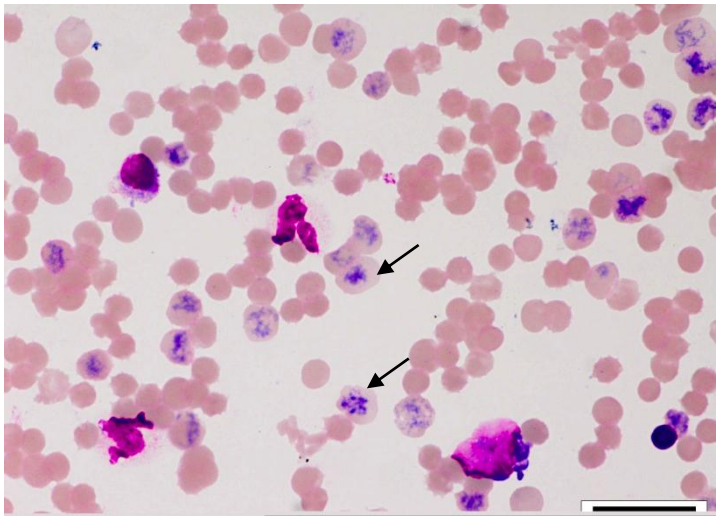

**Fig. 19. Peripheral blood smear revealing reticulocytes, New Methylene Blue stained with Leishman stain x 100X (Bar=2µm)**

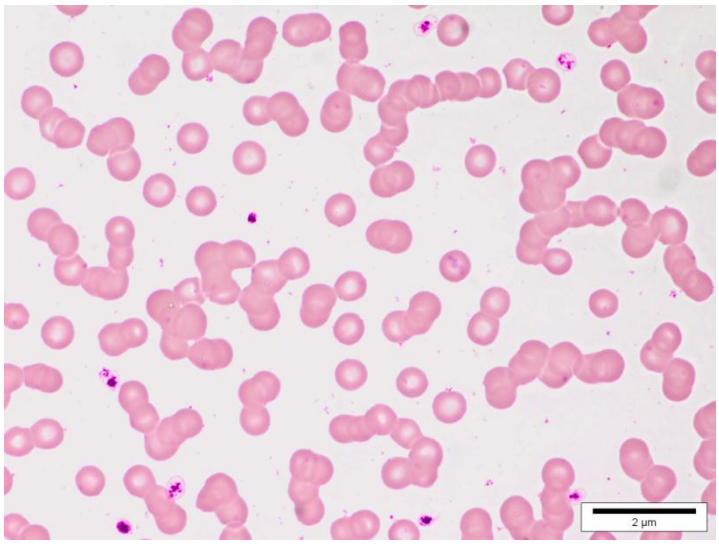


**Fig. 20. Peripheral blood smear revealing agglutination. Leishman stain x 100X (Bar=2µm)**

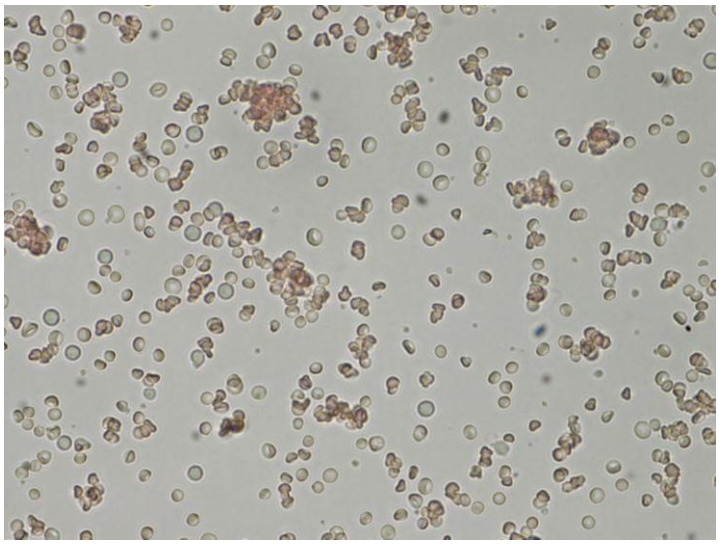

**Fig. 21. Saline agglutination test revealing agglutination x 40X**

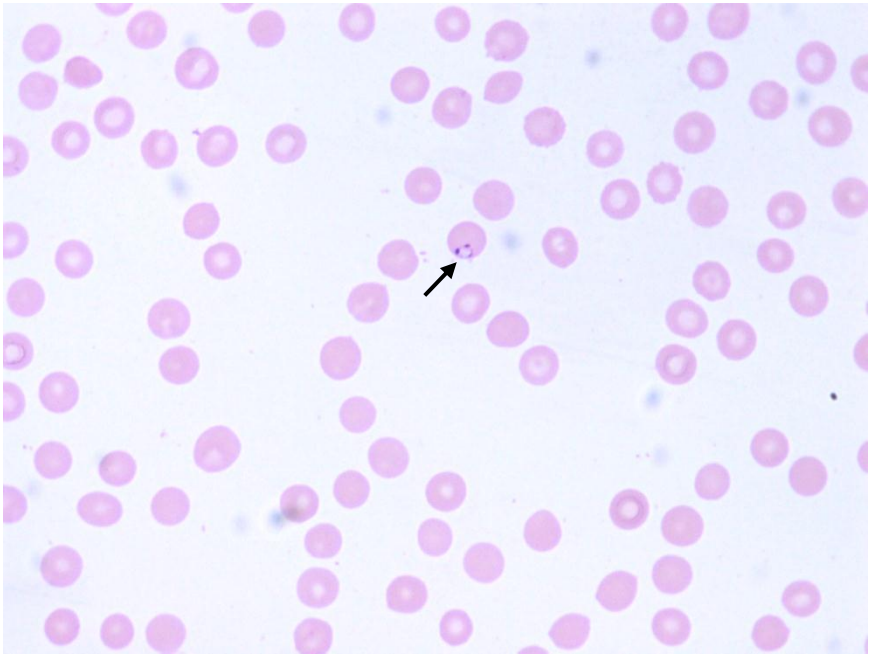

**Fig. 22. Peripheral blood smear revealing *Babesia gibsoni*. Leishman stain x 100X**

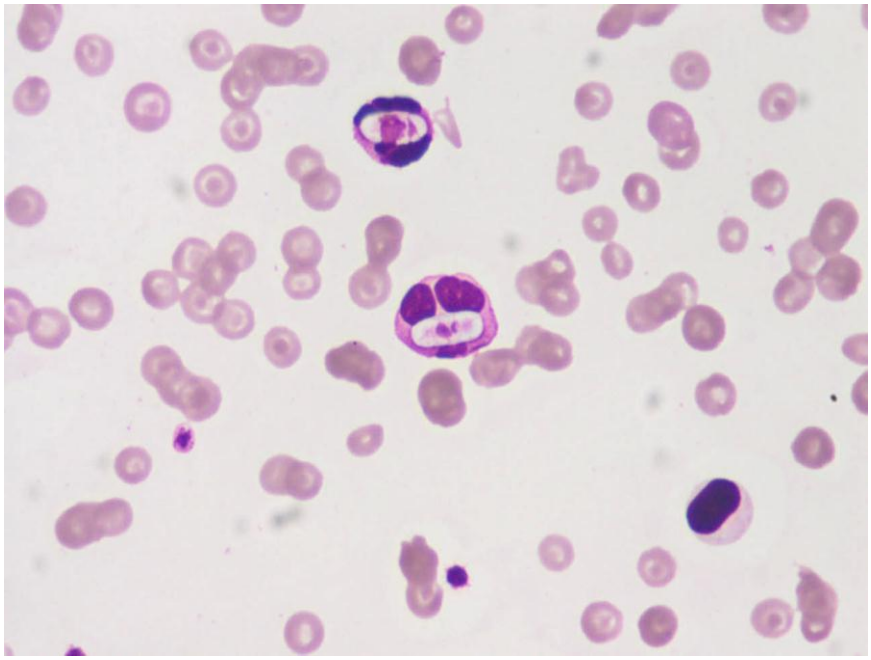

**Fig. 23. Peripheral blood smear revealing *Hepatozoon canis*. Leishman stain x 100X**

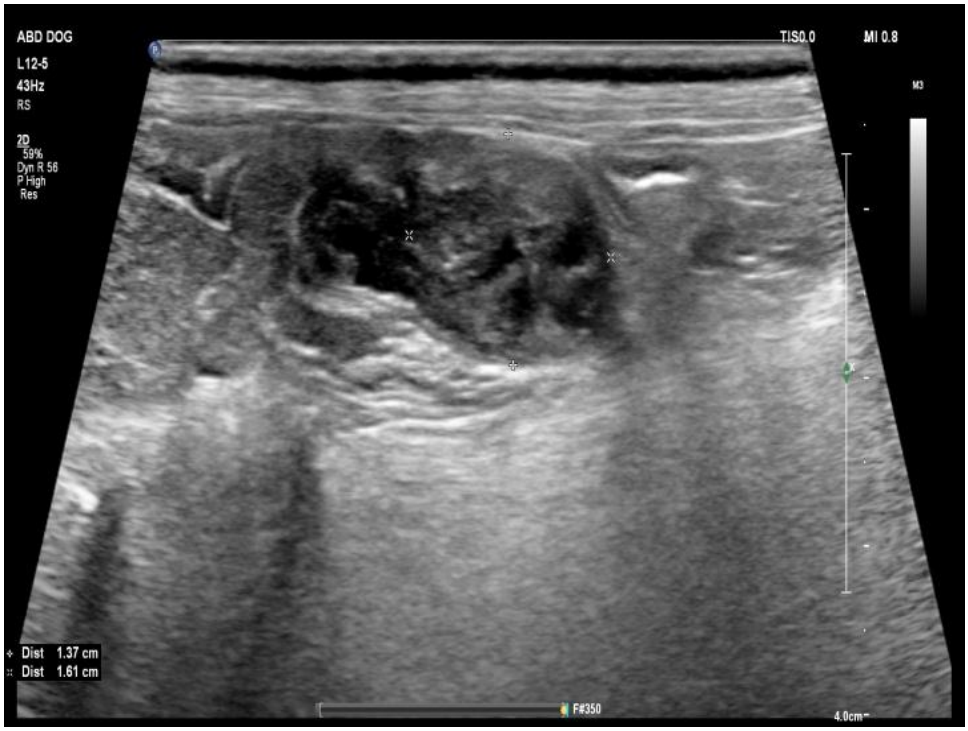


**Fig. 24. Ultrasonographic image of right kidney showing mass of 1.6X 2.1cm, suspected to be a tumor.**

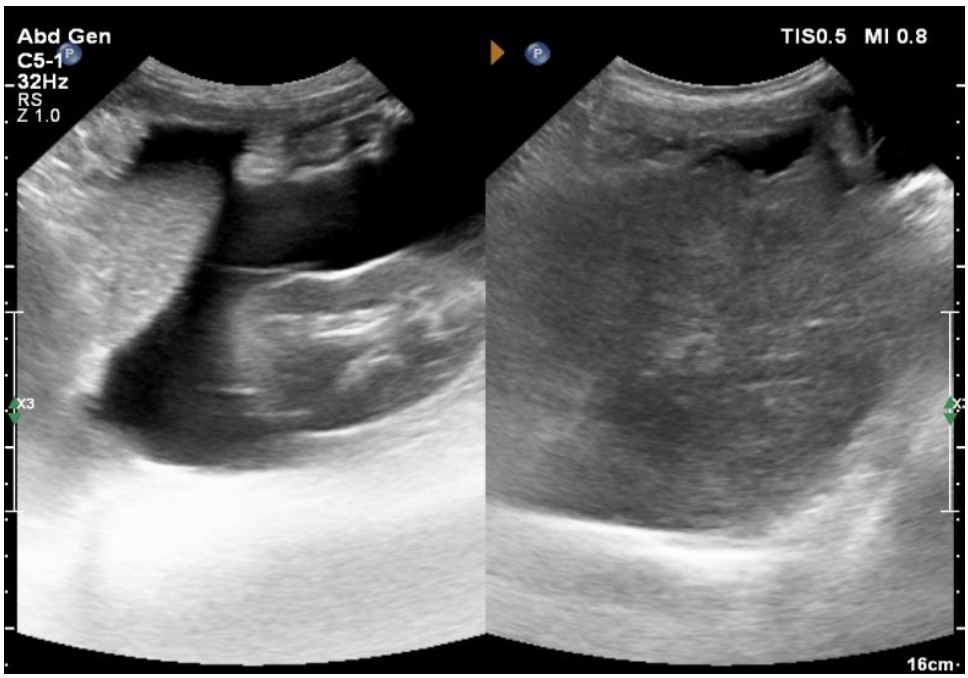


**Fig. 25. Ultrasonographic image of anechoic free fluid in the abdominal cavity**

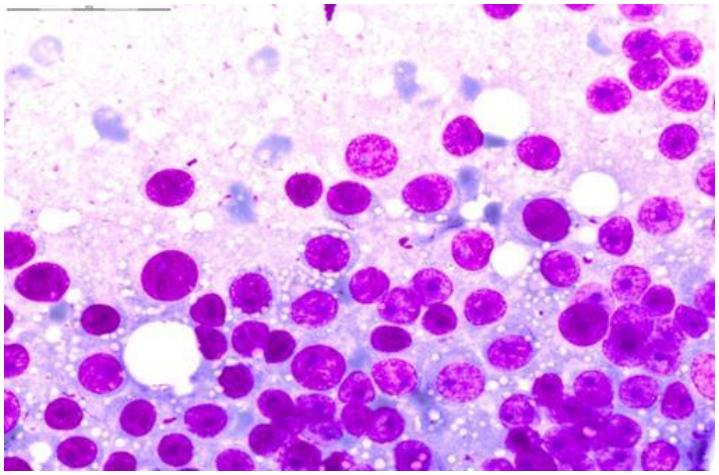

**Fig. 26. Impression smear from tumor growth showing round, slightly pleomorphic cells having vacuolated cytoplasm, suggestive of TVT. Leishman stain x 100X**

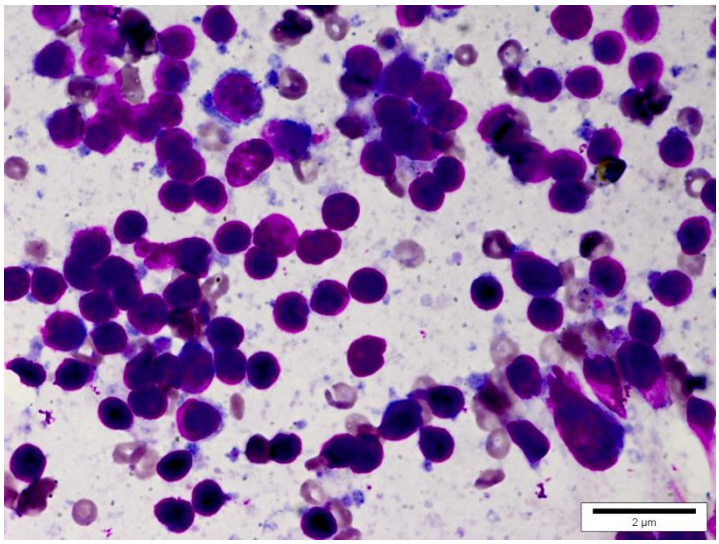


**Fig. 27. FNAB from lymph node showing pleomorphic lymphocytes, suggestive of lymphoma. Leishman stain x 100X (Bar=2µm)**

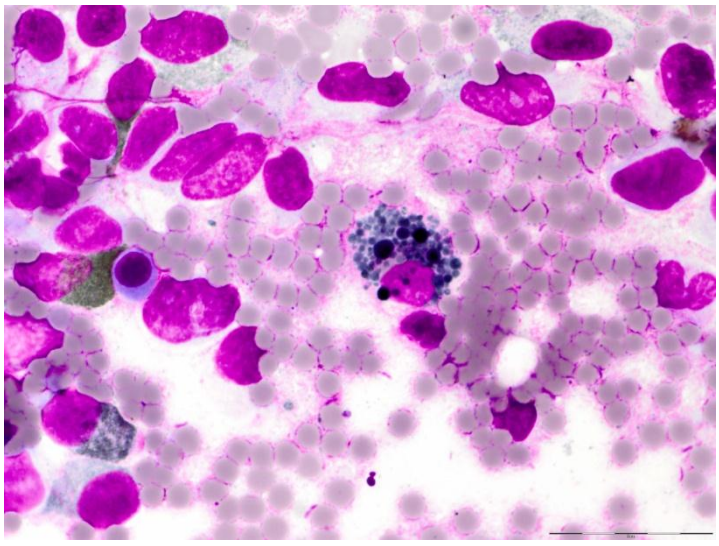

**Fig 28. FNAB showing pleomorphic cells having black coloured pigment, suggestive of malignant melanoma. Leishman stain x 100X (Bar=2µm)**

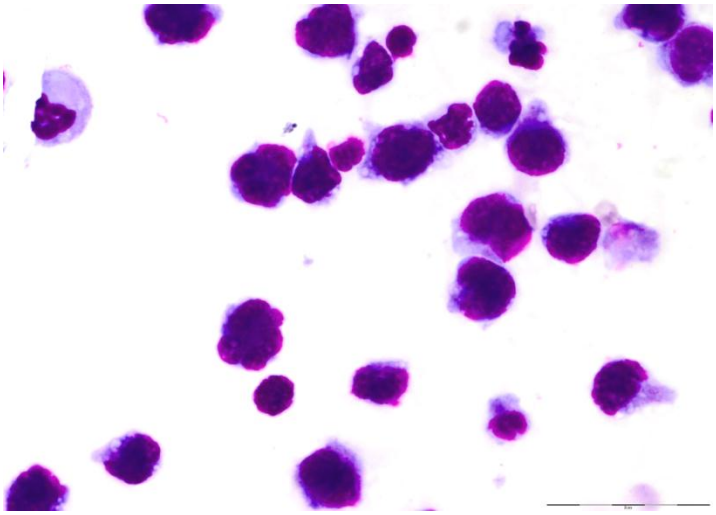

**Fig 29. FNAB showing pleomorphic cells showing vacuolated cytoplasm and greyish blue tinge of cytoplasm with vacuoles. The nuclei of cells are round to oval, suggestive of malignant histiocytoma. Leishman stain x 100X (Bar=2µm)**

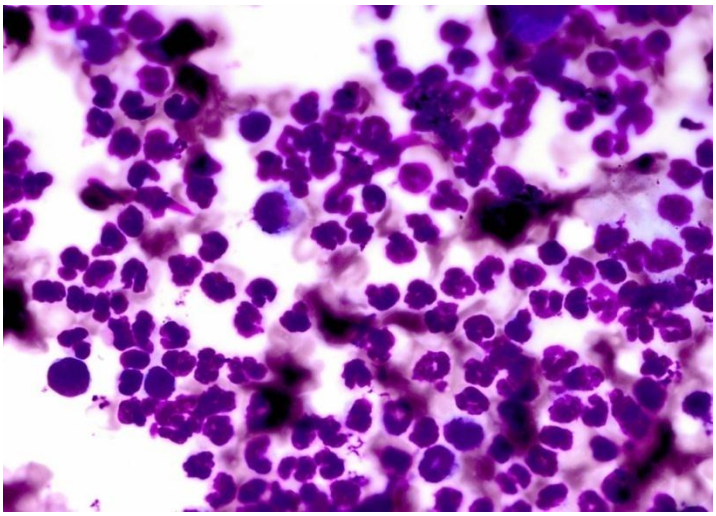

**Fig. 30. FNAB showing large number of neutrophils and few macrophages, suggestive of chronic active inflammation. Leishman stain x 100X**

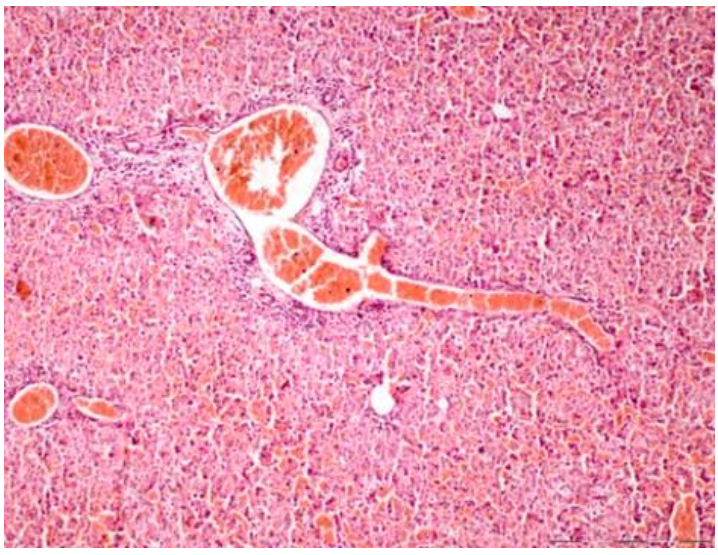

**Fig. 31. Microphotograph of liver showing severe congestion. H & E x 20X (Bar=10µm)**

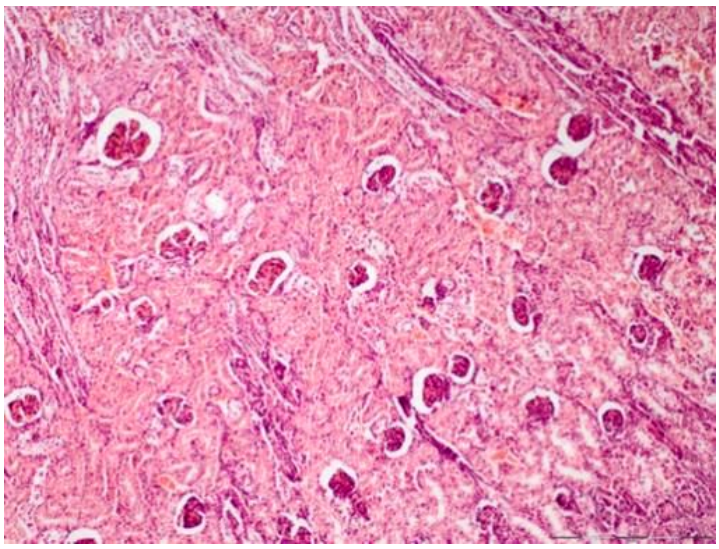

**Fig. 32. Microphotograph of kidney showing severe congestion. H & E x 20X (Bar=10µm)**

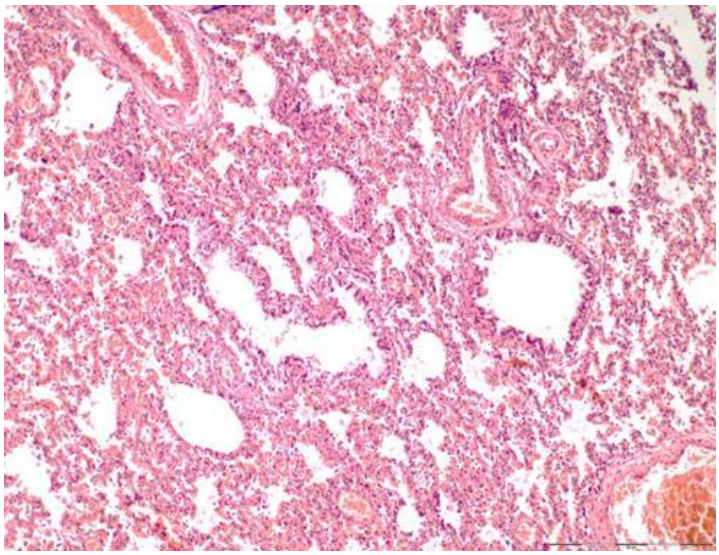

**Fig. 33. Microphotograph of lung showing severe congestion. . H & E x 20X (Bar=10µm)**

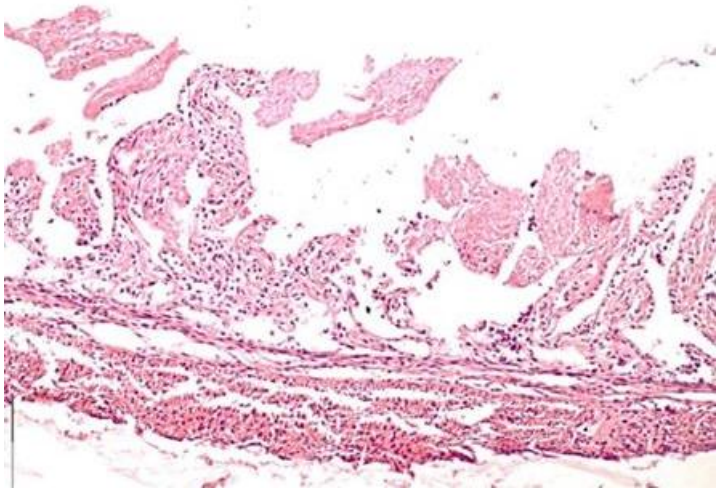

**Fig. 34. Microphotograph of intestine showing necrotic enteritis. H & E x 20X**

# CHAPTER V

## SUMMARY AND CONCLUSIONS

The present study was undertaken to correlate hematological abnormalities observed in retrospective and prospective cases with different disease conditions in dogs. For retrospective studies, a total of 7,375 cases of dogs were analyzed. In the retrospective cases, the mean value of hemoglobin was lower than the normal range. The hemoglobin concentration was normal in 3202 cases, whereas, in 124 cases hemoconcentration was observed. On the basis of hemoglobin concentration anemia was classified into mild (2191), moderate (735) and severe anemia (731). PCV was within the normal range and TEC was slightly lower than the mean value. The erythrocytic abnormalities were classified on the basis of size and staining intensity of RBC. The erythrocytic abnormalities were further classified into normocytic normochromic (187), normocytic hypochromic (183), macrocytic hypochromic (137), microcytic hypochromic (118) and microcytic normochromic in (2). In addition, hypochromasia was observed in 60, anisocytosis was observed in 28 cases and polychromasia in two cases were observed. On the basis of abnormal shapes of RBCs, spherocytes were observed in 16, schistocytes in 11, acanthocytes in 6, leptocytes in 5 and dacrocytes in one case respectively. The different disease conditions recorded in retrospective cases included, gastro-intestinal affections (107), reproductive tract affections (98), urinary tract affection (83), tumors (50), miscellaneous affections (67), parasitic affections (63), ear and eye affections (21), respiratory affections (14), cutaneous affections (12), musculoskeletal disorders (11) and cardiac affections (9). In the retrospective cases, the most common erythrocytic abnormality associated with kidney failure was normocytic normochromic anemia, in gastritis, it was non-regenerative anemia, in ascites microcytic hypochromic anemia with target cells, in hemoprotozoan infections normocytic hypochromic anemia, and in pyometra normocytic hypochromic anemia was observed, whereas, anemia was as a consistent finding in other conditions including viral diseases, cutaneous affections and tumors.

For prospective studies hematological findings of 31 cases of apparently healthy animals were correlated with the hematologic findings of 363 clinical cases. Out of the 363 cases, 259 were analyzed manually, whereas, 104 cases were analyzed using ADVIA 2120 Hematology System. The hematological parameters analyzed

manually included Hb, TLC and DLC. The hemoglobin was found to be below the normal range, TLC was above the normal range, and absolute counts of neutrophils were above the normal range, whereas, the absolute count of lymphocytes and eosinophils were within the normal range.

The parameters analyzed using ADVIA 2120 Hematology System included CH, CHCM, RBC, HCT, MCV, MCH, MCHC, HDW, RDW, WBC and MPV. The value of CH varied from 11.4 to 25.4 pg and the median value was 20.20 pg, which was less than the normal range. The value of CHCM varied from 23.3 to 37.5 g/dL with a median value of 32.40, which was within the normal range. The median value of RBC varied from 1.17 to 8.84 × 106 cells/µL and the median value was 4.74× 106 cells/µL, which was below the normal range. The median value of HCT varied from 9.6 to 53.3 % and the median value was 30.25%, which was below the normal range. The median value of MCV varied from 6.7 to 102.1 fL with a median value was 62.20 fL, which was within the normal range. The median value of MCH varied from 6.3 to 24.8 pg, with a median value of 19.75 pg, which was within the normal range. The median value of MCHC varied from 15.5 to 42.5 g/dL, with a median value of 32.10 g/dL, which was within the normal range. The median value of HDW varied from 1.55 to 5.86 g/dL with a median value of 2.27 g/dL, which was within the normal range. The median value of RDW varied from 10.8 to 25 % and the median value was 14.70 %., which was within the normal range. The WBC varied from 1.73 to 124.5 × $10^3$ cells/µL with a median value was 16.00, which was above the normal range. The median of MPV varied from 1.73 to 124.5 fL with a median value of 15.54 fL, with was above the normal range.

In cases analyzed manually hypochromasia was observed in 157 cases, polychromasia in 40 and microcytosis in 24 cases. In addition, in the cases analyzed using ADVIA system, hypochromasia was further classified into grade (+) and grade (++), a total of 6 cases of hypochromasia were detected which consisted of 5 cases of grade + and 1 case was of grade ++. A total of four cases of macrocytic RBC were observed which consisted of three cases of grade + and one case of grade +++. Similarly, microcytic RBCs were present in 24 cases, in which 13 were of grade +, 5 were of grade ++ and 6 were of grade +++.

On the basis of abnormal shape of the RBCs, the most predominant erythrocyte abnormality observed was spherocytes (111), followed by echinocytes

(95), codocytes (88), schistocytes (39), acanthocytes (14), incomplete spherocytes (12), polychromatophils (3) and elliptocyte in one case. Most common erythrocytic abnormality on the basis of occurrence was echinocytes followed by spherocytes, codocytes, schistocytes, incomplete spherocytes, acanthocytes, polychromatophils, eccentrocytes, dacrocytes, stomatocytes, q RBCs, blister cells, elliptocytes, keratocytes and nucleated RBCs.

Comparison of serum chemistry findings in apparently healthy dogs and clinical cases was done. The mean value of total protein and albumin was on lower side, whereas, the mean values of ALT, AST, GGT, total bilirubin, ALKP, BUN and creatinine were higher when compared with apparently healthy animals. On the basis of serum biochemical changes, 157 cases of liver damage, 97 cases of kidney damage and 109 cases of concurrent liver and kidney damage were observed. The mean value of total protein was significantly lower in dogs suffering from liver damage and concurrent liver and kidney damage as compared to dogs apparently healthy dogs. The mean value of albumin was significantly lower in dogs suffering from liver damage as compared to apparently healthy dogs. The mean value of ALT was significantly higher in dogs suffering from liver damage, followed by concurrent liver and kidney damage as compared to apparently healthy dogs. The mean value of GGT was significantly higher in dogs suffering from concurrent liver and kidney damage as compared to apparently healthy dogs. The mean value of total bilirubin was significantly higher in dogs suffering from concurrent liver and kidney damage as compared to apparently healthy dogs. The mean value of ALKP was significantly higher in dogs suffering from kidney damage as compared to apparently healthy dogs. The mean value of BUN was significantly higher in dogs suffering from concurrent liver and kidney damage as compared to apparently healthy dogs. The mean value of creatinine was significantly higher in dogs suffering from concurrent liver and kidney damage as compared to apparently healthy dogs.

A significant positive correlation ($p<0.05$) was observed between occurrence of polychromatophils in dogs suffering from liver damage, between occurrence of acanthocytes, stomatocytes and polychromatophils in dogs suffering from kidney damage and between echinocytes and polychromatophils in dogs suffering from concurrent liver and kidney damage.

Correlation of hematological parameters analyzed by hematology analyzer with serum chemistry analysis revealed that the mean value of HDW was significantly higher in dogs suffering from liver damage as compared to dogs suffering from kidney damage alone. The mean value of microcytic RBCs was significantly higher in dogs suffering from liver damage as compared to dogs suffering from concurrent liver and kidney damage. The mean value of WBCB were significantly higher in dogs suffering from concurrent liver and kidney damage as compared to dogs suffering from liver damage. A high positive correlation was observed between the presence of, hypochromasia and stomatocytes and anisocytosis and RDW with polychromatophilic RBCs. Moderate correlation was observed between hypochromasia with stomatocytes and eccentrocytes, polychromasia with blister cells, anisocytosis with echinocytes, polychromatophilic RBCs and blister cells, WBC with acanthocytes and polychromatophilic RBCs, HCT with echinocytes, MCV with polychromatophilic RBCs, MCH with polychromatophilic RBCs and schistocytes. WBCB with acanthocytes, polychromatophilic RBCs and eccentrocytes, CHCM with q RBCs, RDW with polychromatophilic RBCs and HDW with nucleated RBCs and polychromatophilic RBCs in case of liver damage.

In clinical cases, a leukogram analysis was done and the leukogram findings were classified into normal TLC (174), leukopenia (27), leukocytosis (155) and leukemoid response (6). The mean value of total leucocyte counts were significantly higher in dogs suffering from concurrent liver and kidney damage as compared to dogs suffering from kidney damage alone. The mean value of neutrophil counts were significantly higher in dogs suffering from concurrent liver and kidney damage as compared to dogs suffering from liver and kidney damage alone.

Correlation between hematological findings and leukogram findings was carried out. Spherocytes and polychromatophilic RBCs were significantly correlated ($p<0.05$) with normal TLC, codocytes, schistocytes, echinocytes, polychromatophilic RBCs with leukocytosis and stomatocytes and polychromatophilic RBCs with leukopenia. Whereas, marginally significant ($p<0.1$) correlation was observed between occurrence of schistocytes and leukopenia.

In cases analyzed by ultrasonography, codocytes were observed in the cases of dogs having suspected neoplastic lesions in kidney, whereas, echinocytes observed in

dog showing reduced cortical thickness in kidney. Echinocytes were also observed in dog having anechoic free fluid in abdominal cavity.

Correlation of cytological finding revealed the presence of spherocytes and q RBCs in cases of TVT, echinocytes and schistocytes in cases of lymphoma, acanthocytes in malignant melanoma, whereas, acanthocytes and schistocytes were predominant in malignant histiocytoma cases.

From the present study, following conclusions could be drawn:

## CONCLUSIONS

### In retrospective cases

- Anemia was the most common findings, and there were 2,191 cases of mild, 735 cases of moderate and 731 cases of severe anemia.
- On the basis of size and staining intensity of RBC, normocytic normochromic RBC's were most common observed (187 cases) followed by normocytic hypochromic (181), macrocytic hypochromic (135), microcytic hypochromic (117) and microcytic normochromic (2 cases).
- On the basis of shape of RBC, codocyte were most predominat (76 cases) followed by spherocytes (16), schistocytes (11), acanthocytes (6), leptocytes (5) and dacrocytes (1).
- Normocytic normochromic anemia was observed in cases of kidney failure, microcytic hypochromic anemia in cases of hepatitis, whereas, normocytic hypochromic anemia was observed in cases of pyometra.

### In prospective cases

- Hypochromasia was observed in 157 cases, polychromasia in 40 and microcytosis was observed in 24 cases.
- Spherocytes were observed in 111 cases followed by echinocytes in 95 and codocytes in 88 cases.
- The most common erythrocytic abnormalities associated with liver damage were polychromatophilic RBCs. Acanthocytes, stomatocytes and polychromatophilic RBCs were more common in cases of kidney damage, whereas, polychromatophils and echinocytes were commonly observed in cases having concurrent liver and kidney damage.

- In cases analyzed by hematology analyzer, significant association was observed between occurrence of microcytes and acanthocytes with liver damage.
- The mean HDW values were significantly higher in dogs suffering from liver damage as compared to those suffering from kidney damage.
- Acanthocytes, echinocytes and schistocytes were the most common erythrocytic abnormalites observed in cases of lymphoma.

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