

# INTRODUCTION

Blood examination is a very important diagnostic tool that provides vital information about dog's health. A complete blood count (CBC) is a common blood test used in dogs to measure and evaluate cells that circulate in the blood. The CBC can determine the cause of an anemia, infection, drugs that affect the bone marrow, certain types of cancers, especially leukemia, may be evident on a blood smear. Blood parasites and some microorganisms are found by careful inspection of the blood cells during the CBC, (Testa, 2004). This study focused on the CBC examination of German shepherd dogs reared in the Sudan to underline the effect of Sudan climate on these dogs which can reflect on dogs health .This changes can be detected by complete blood test.

The partnership between dogs (*Canis lupus familiaris*) and humans is based on a human need for help with herding, hunting and guarding.

The history of dogs may involve three major stages including loosely engaged pre-domesticated scavengers, domesticated non-breed dogs with close human-dog interactions, and breed formation following intense human selection for diverse sets of traits. Dog history has been studied recently using mitochondrial DNA, which suggests that wolves and dogs split into different species around 10.000 years ago. (Boyko, 2009). Recent mitochondrial DNA analysis suggests that the origin and location of dog domestication be in Asia. A recent study reported that dogs appear to have a higher proportion of wolf haplotypes from grey wolves native to the middle East. So, many morphological changes similar to those found in dogs were noted in the domesticated fox population (Bridgett *et al* , 2010).

German shepherd dog is the one of America's most popular dog breeds originated in Germany since 1899. He is an intelligent and capable working dog; he is excelling at most anything he is trained to do. He is used for guiding and assisting the handicapped and police for herding search and rescue, drug detection, competitive obedience, as well as explosive detection. In late 19<sup>th</sup>-century Germany, the German Shepherd Dog was created in large part by a military officer named Max von Stephanitz. He took the less-than-uniform herding dogs of his country and molded them to develop a distinctive breed with the utility and intelligence to do police work. The American kennel club registered the first German Shepherd in 1912, and the German.

German shepherd dog breed standard height at the withers is 60-65cm for male and 55-60cm for female. The weight standard is 30-40kg for male, the female weight 22-32 kg. Litter size 4-9, the color most commonly tan with black saddle double coated. Jacquelyn *et al*, (2008)

Police center Sudan brought German shepherd dogs from Egypt when they were puppies in 1967, they brought 10 puppies with prepared meal higher in protein and enriched with vitamins, minerals and fats essential for growth, now a day they breed them from their origin locally and they use them for forensic evidences.

## **Rationale:**

Blood tests help prevent and treat dangerous diseases that have complicated diagnosis; blood test can give the pinpoint of diagnosis. Some medications can harm dog's kidney or liver. Blood chemistry tests can ensure that dog healthy enough to take the medication. Even in young and healthy dogs, lab testing gives a valuable baseline picture of good health for the dog.

German shepherd dogs were brought by the Police Center to the Sudan in 1967 for forensic purposes.

There is scarcity in studies regarding to the determination of the blood and biochemical parameters in dogs in the Sudan. Most of the studies focused on the farm animals .According to this, this study was planned to add to the data base of hematological and biochemical parameters of German shepherd dogs in the Sudan and the factors influencing these parameters, that are season, exercise and effect of sex on erythrocytes osmotic fragility.

## **Objectives:**

### **1- General objective:**

Hematological and biochemical measurements of German shepherd dogs in the Sudan.

### **2- Specific objective:**

1-To add to the data base of the hematological and biochemical parameters and the effect of the sex on CBC for the German shepherd dogs in the Sudan.

2- To investigate the seasonal variations on hematological, biochemical and minerals parameters of German shepherd dogs in the Sudan.

3- To investigate the effect of exercise on hematological parameters of German shepherd dogs in the Sudan.

4-To investigate the effect of sex on erythrocyte osmotic fragility of German shepherd dogs in the Sudan.

5-To investigate the effect of the time of the day on some clinical parameters of German shepherd dogs in the Sudan.

# CHAPTER ONE

## Literature Review

### 1-1: Complete blood count (CBC)

The complete blood count measures the number of different types of cells circulating in the blood stream. There are three major types of blood cells in circulation; red blood cells (RBC), white blood cells (WBC), and platelets (PLT). These cells exist suspended in the plasma. Plasma is yellow to colorless, depending on the quantity, the species of animal and the animal's diet. It contains 92% water, 8% blood plasma proteins, and trace amounts of other materials (Vinay *et al*, 2007). Plasma circulates dissolved nutrients, such as glucose, amino acids, and fatty acids (dissolved in the blood or bound to plasma proteins), and removes waste products, such as carbon dioxide, urea, and lactic acid (David , 2012).

Other important components of plasma include:

- Serum albumin
- Blood-clotting factors (to facilitate coagulation)
- Immunoglobulins (antibodies)
- lipoprotein particles
- Various other proteins
- Various electrolytes (mainly sodium and chloride)

The term serum refers to plasma from which the clotting proteins have been removed and does not contain white or red blood cells. Most of the proteins remaining are albumin and immunoglobulin. Serum includes all

the electrolytes, antibodies, antigens, hormones as well as exogenous substances such as drugs and microorganisms (David, 2012).

### **1-1-1: Red cells**

Red blood cells (RBCs), also called erythrocytes, are the most common type of blood cells. RBCs pick up oxygen brought into the body by the lungs, or gills and bring that oxygen to cells throughout the body through circulatory system. Red blood cells live in the blood stream for about 100 to 120 days although the actual time varies with the type of animal. Old red blood cells are removed from the blood stream by the spleen and liver. Red blood cell numbers can be decreased (anemia) if they are not produced in adequate numbers by the bone marrow, if their life span is shortened (a condition called hemolysis), or if they are lost due to bleeding. Increased red blood cell numbers is called polycythemia and is usually occurring when the marrow is overactive and produces more blood cells than the body needs. Or due to a high content of red blood cells builds up in response to low oxygen concentration in the air, because there's less oxygen in the blood, the body attempts to overcome the lack by making more red blood cells. Polycythemia also may occur in case of dehydration. (Melanie *et al*, 2006).

The cytoplasm of erythrocytes is rich in hemoglobin, an iron-containing bio molecule that can bind oxygen and is responsible for the red color of the cell. The cell membrane is composed of proteins and lipids, and this structure provides properties essential for physiological cell function such as deformability and stability while traversing the circulatory system and specifically the capillary network.

Mammalian erythrocytes are unique among the vertebrates as they are non-nucleated cells in their mature form (Uzoigwe, 2006).

Mammalian erythrocytes are typically shaped as biconcave disks: flattened and depressed in the center, with a dumbbell-shaped cross section, and a torus-shaped rim on the edge of the disk (Dukes, 2005). This distinctive biconcave shape optimizes the flow properties of blood in the large vessels, such as maximization of laminar flow and minimization of platelet scatter, which suppresses their atherogenic activity in those large vessels (Gregory, 2001). However, there are some exceptions concerning shape in the artiodactyls order (even-toed ungulates including cattle, deer, and their relatives), which displays a wide variety of bizarre erythrocyte morphologies: small and highly ovaloid cells in llamas and camels (family *Camelidae*), tiny spherical cells in mouse deer (family *Tragulidae*), and cells which assume fusiform, lanceolate, crescentic, and irregularly polygonal and other angular forms in red deer and wapiti (family *Cervidae*). Members of this order have clearly evolved a mode of red blood cell development substantially different from the mammalian norm (Gregory, 2001). Overall, mammalian erythrocytes are remarkably flexible and deformable so as to squeeze through tiny capillaries, as well as to maximize their apposing surface by assuming a cigar shape, where they efficiently release their oxygen load (Goodman *et al*, 2007).

Erythrocytes vary in diameter and thickness according to the species and nutritional status of the animal. The dog erythrocyte is markedly biconcave, cat and horse erythrocytes are slightly biconcave, goat erythrocytes show little biconcavity (Dukes, 2005). The circulating life span

of dog erythrocytes are similar to those of human erythrocytes approximately of 110-115 days and 7µm in diameter and much longer than those of rodents(40-50 days) (Robert and Nancy 2003; Khan *et al* ,2011). The morphometric parameters of red blood cells are biggest in dogs followed by horses, cattle, and sheep, while goats have the lowest ones (Adili and Melizi ,2014).

Total red blood cells:is the number of red cells given as an absolute number per liter( David ,2012).

### **1-1-1-1: Erythropoiesis**

Erythropoiesis is the process which produces red blood cells (RBC). It is stimulated by decreased O<sub>2</sub> in circulation, which is detected by the kidneys, which then secrete the hormone erythropoietin(Sherwood *et al* ,2005). This hormone stimulates proliferation and differentiation of red cell precursors, which activates increased erythropoiesis in the hemopoietic tissues, ultimately producing red blood cells.

In postnatal birds and mammals (including humans), this usually occurs within the red bone marrow .In the early fetus, erythropoiesis takes place in the mesodermal cells of the yolk sac(Sherwood *et al* ,2005). By the third or fourth month, erythropoiesis moves to the liver. After seven months, erythropoiesis occurs in the bone marrow. Increased level of physical activity can cause an increase in erythropoiesis(Le Tao *et al*,2010).

The differentiation and stages of erythrocytes development occurs within the bone marrow from the stem cell hemocytoblast this becomes erythroblast then turn to poly chromatophilic or intermediate normoblast, then polychromatic erythroblast then late normoblast. At this stage the



nucleus is expelled before the cell becomes reticulocyte. The cell is released from the bone marrow after the stage of normoblast, and so in newly circulating red blood cells there are about 1% reticulocytes. The reticulocytes, go on to lose all other cellular organelles such as their mitochondria, Golgi apparatus and endoplasmic reticulum (Uzoigwe, 2006).

As a result of not containing mitochondria, these cells use none of the oxygen they transport; instead they produce the energy carrier ATP by the glycolysis of glucose and lactic acid fermentation on the resulting pyruvate (Rich, 2003).

After one to two days, these ultimately become erythrocytes or mature red blood cells. (Le Tao *et al*, 2010). The characterizations of erythrocyte maturation are cell size reduction, disappearance of the nucleus and the condensation of the chromatin material. The color of the cytoplasm changes from blue to a pinkish red as a result of the increasing expression of hemoglobin as the cell develops. (Jain, 2007).

The nutritional requirements for the maturation of red blood cells are Vitamin B<sub>12</sub> (Cobalamin) and Vitamin B<sub>9</sub> (Folic acid). Lack of one of these Vitamin causes maturation failure in the process of erythropoiesis, which clinically reflect as reticulocytopenia, an abnormally low count of reticulocytes. Because vitamin B generally affecting DNA synthesis, fatty acid and amino acid metabolism (Miller *et al*, 2005).

### **1-1-2: Hemoglobin**

Hemoglobin is the principal determinant of the color of the blood in vertebrates. It is the actual substance in the red blood cell that carries oxygen. Each molecule has four heme groups, and their interaction with various molecules alters the exact color (Costanzo,2007). In vertebrates and other hemoglobin-using creatures, arterial blood and capillary blood are bright red, as oxygen imparts a strong red color to the heme group. Deoxygenated blood is a darker shade of red; this is present in veins.

The amount of hemoglobin in the blood, is expressed in grams per deciliter (Weber and Vinogradov, 2001). A low level of hemoglobin is a sign of anemia.

The sex difference in adult hemoglobin levels is conserved throughout Mammalia a higher adult male hemoglobin level occurs in almost all mammal species studied to date, including non-menstruating and non-placental species: dogs (Michaelson *et al*,1966) rhesus macaques( Buchl and,Jand,1997), baboons( Harewood *et al* ,1999), chimpanzees (Howell *et al* 2003), marsupials ( Clark ,2004), capuchin monkeys (Wirz *et al* ,2008), and rodents( Kane *et al* ,2012).

### **1-1-3: Hematocrit**

Hematocrit (HCT) is the fraction of whole blood volume that consists of red blood cell volume percentage. Can become a point of reference of its capability of delivering oxygen because RBCs carries oxygen from lungs to the tissues. (David, 2012).

In recent years, the hematocrit is calculated by an automated analyzer and not directly measured. It is determined by multiplying the red cell count by

the mean cell volume. The hematocrit is slightly more accurate as the PCV includes small amounts of blood plasma trapped between the red cells. An estimated hematocrit as a percentage may be derived by tripling the hemoglobin concentration in g/dL and dropping the units (Wintrob, 2009).

The packed cell volume (PCV) can be determined by centrifuging heparinized blood in a capillary tube (also known as a microhematocrit tube) at 10,000 RPM for five minutes. This separates the blood into layers. The volume of packed red blood cells divided by the total volume of the blood sample gives the PCV. Since a tube is used, this can be calculated by measuring the lengths of the layers (David, 2012).

An abnormally low hematocrit may suggest anemia, a decrease in the total amount of red blood cells, while an abnormally high hematocrit is called polycythemia (David, 2012).

#### **1-1-4: Red blood cell indices**

Red cell indices were first introduced by Wintrobe in 1929 to define the size mean cell volume(MCV) and hemoglobin content ,mean cell hemoglobin and mean cell hemoglobin concentration (MCH,MCHC) of red blood cells these values are useful in the etiology of anemia( Bunn,2011).

### **1-1-4-1: Mean corpuscular volume determination (MCV)**

The average volume of the red cells, measured in femtolitres (fl) or cubic microns ( $\mu\text{m}^3$ ). Anemia is classified as microcytic or macrocytic if the MCV value is above or below the expected normal range; anemias are classified as normocytic if the MCV is within the expected range. Other conditions that can affect MCV include thalassemia( inherited blood disorders characterized by abnormal hemoglobin production) ,reticulocytosis (is an increase in reticulocytes, immature red blood cells) , vitamin B<sub>12</sub> deficiency, and folic acid deficiency ( Bunn,2011).

MCV= volume of packed cells/1000ml of blood

Red blood cell count in millions/ml

### **1-1-4-2: Mean corpuscular hemoglobin (MCH)**

The average amount of hemoglobin per red blood cell, in picograms (pg). MCH value is diminished in hypochromic anemias.(Bunn,2011)

MCH=Hemoglobin in g/1000 ml of blood

Red blood cell count in millions/ml

### **1-1-4-3: Mean corpuscular hemoglobin concentration (MCHC)**

The average concentration of hemoglobin in the cells it expressed as g/dl of red blood cells or as a percentage value ( Bunn,2011).

MCHC= Hemoglobin in g/100 ml of blood  $\times 100$

Volume of packed cells/100ml of blood

A low MCHC can be interpreted as identifying decreased production of hemoglobin. MCHC can be normal even when hemoglobin production is decreased (such as in iron deficiency) due to a calculation artifact (Bunn, 2011).

#### **1-1-4-4: Red blood cell distribution width (RDW)**

Is a measure of the variation in cellular volume of the RBC population that is reported as part of a standard complete blood count. The standard size of red blood cells are about 6-8  $\mu\text{m}$  in diameter. Higher RDW values indicate greater variation in size (this is observed in anemia). RDW test results are often used together with mean corpuscular volume (MCV) results to determine the possible causes of the anemia. It is mainly used to differentiate an anemia of mixed causes from an anemia of a single cause (Bunn, 2011)

The red cell distribution width (RDW) is used as a measurement of anisocytosis (unequal size of red cells, this is commonly found in anemia and other blood conditions, it is a characteristic feature of bovine blood.) and is calculated as a coefficient of variation of the distribution of RBC volumes divided by the mean corpuscular volume (MCV) (Barbara, 2006)

Red cell indices are now automatically measured in all blood count determination (Ravi, 1990).

#### **1-1-5: Leukocytes**

White blood cells (WBCs), also called leukocytes or leucocytes, are the cells of the blood that are produced and derived from multipotent cells in the bone marrow known as hematopoietic stem cells. Leukocytes are found throughout the body, including the blood and lymphatic system (Maton *et al*, 1997).

All white blood cells have nuclei, which distinguishes them from the other blood cells, the anucleated red blood cells (RBCs) and platelets (PLT).

White blood cells are classified in two categories, either by structure (granulocytes and granulocytes) or by cell division lineage (myeloid cells or lymphoid cells). These categories can be further divided into the five main types: neutrophils, eosinophils, basophils, lymphocytes and monocytes.

Among lymphocytes, there are B cells T cells and natural killer (NK) cells (LaFleur, 2008).

A group of granulocyte leukocytes contain granules which store a mixture of cytotoxic molecules, including many enzymes and antimicrobial peptides, histamine and heparin that are released by a process called degranulation following activation of the granulocyte by an immune stimulus (John, *et al*, 2008).

The number of leukocytes in the blood is often an indicator of disease, and thus the WBC count is an important subset of the complete blood count. The normal white cell count of dog is usually between  $9 \times 10^9/L$  and  $13 \times 10^9/L$  (Bruce *et al*, 2002).

### **1-1-5-1: Leukopoiesis**

Leukopoiesis is the process of formation of leukocytes (WBC) from stem cells in hematopoietic organs. Leukocytes develop from either multipotential myeloid stem cells or multipotential lymphoid stem cells.

Leukocytes developing from multipotential myeloid are granulocytes (neutrophils, basophils and eosinophils) or monocytes. Leukocytes developing from multipotential lymphoid stem cells are lymphocytes, T and B cells, dendritic and NK cells (Saladin, 2012).

### **1-1-5-2: Neutrophil**

Neutrophils are the most abundant white blood cell, constituting 60-70% of the circulating leukocytes (Bruce *et al*, 2002). They defend against bacterial or fungal infection. They are usually first responders to microbial infection; their activity and death in large numbers forms pus. They are commonly referred to as polymorph nuclear (PMN) leukocytes, although, in the technical sense, PMN refers to all granulocytes. They have a multi-lobed nucleus, which consists of three to five lobes connected by slender strands (Saladin, 2012). This gives the neutrophils the appearance of having multiple nuclei. The cytoplasm may look transparent because of fine granules that are pale lilac when stained. Neutrophils are active in phagocytosing bacteria and are present in large amount in the pus of wounds. These cells are not able to renew their lysosomes (used in digesting microbes) and die after having phagocytosed a few pathogens (Wheater and Stevens, 2002). Neutrophils are the most common cell type seen in the early stages of acute inflammation. The life span of a circulating neutrophil is about 4- 5 days (Pillay, 2010).

### **1-1-5-3: Eosinophil**

Eosinophils compose about 2-4% of the total of WBC. This count fluctuates throughout the day, and seasonally. It rises in response to allergies, parasitic infections, collagen diseases, and disease of the spleen and central nervous system. They are rare in the blood, but numerous in the mucous membranes of the respiratory, digestive, and lower urinary tracts (Saladin, 2012). They primarily deal with parasitic infections. Eosinophils are also the predominant inflammatory cells in allergic reactions. The most important causes of eosinophilia include allergies such as asthma, hay fever, and hives; and also parasitic infections. They secrete chemicals that destroy the large parasites, such as hook worms and tape worms that are too big for any other WBC to phagocytize. In general, their nucleus is bi-lobed. The lobes are connected by a thin strand (Saladin, 2012). The cytoplasm is full of granules that assume a characteristic pink-orange color with eosin staining.

### **1-1-5-4: Basophil**

Basophils are the rarest of the white blood cells (less than 0.5% of the total count) and share physicochemical properties with other blood cells. They can be recognized by several coarse, dark violet granules, giving them a blue hue. The nucleus is bi- or tri-lobed, but it is hard to see because of the number of coarse granules that hide it (Falcone *et al*, 2000).

They excrete two chemicals that aid in the body's defenses: histamine and heparin. Histamine causes blood vessels dilatation and increase the flow of blood to injured tissue. It also makes blood vessels more permeable, so neutrophils and clotting proteins can get into connective tissue more easily. Heparin is an anticoagulant that promotes the movement



of white blood cells into an area and inhibits blood clotting. Basophils can also attract eosinophils and neutrophils to an infection site by releasing chemical signals (Saladin, 2012).

### **1-1-5-5: Lymphocyte**

Lymphocytes are much more common in the lymphatic system than in blood. Lymphocytes are distinguished by having a deeply staining nucleus that may be eccentric in location, and a relatively small amount of cytoplasm. Lymphocytes are divided into:

B cells which make antibodies that can bind to pathogens, block pathogen invasion, activate the complement system, and enhance pathogen destruction.

T cells: which are categorized into:

**CD4+ helper T cells:** T cells displaying co-receptor CD4 (cluster of differentiation 4) is a glycoprotein found on the surface of immune cells such as T helper cells, monocytes, macrophages, and dendritic cells are known as CD4+ T cells (Ansari *et al*, 1996). These cells have T-cell receptors and CD4 molecules that, in combination, bind antigenic peptides presented on major histocompatibility complex (MHC) class II molecules on antigen-presenting cells. Helper T cells make cytokines and perform other functions that help coordinate the immune response.

**CD8+ cytotoxic T cells:** T cells displaying co-receptor CD8 are known as CD8+ T cells. These cells bind antigens presented on MHC I complex of virus-infected or tumour cells and kill them. Nearly all nucleated cells display MHC I.

$\gamma\delta$  T cells possess an alternative T cell receptor (different from the  $\alpha\beta$  TCR found on conventional CD4<sup>+</sup> and CD8<sup>+</sup> T cells). Found in tissue more commonly than in blood,  $\gamma\delta$  T cells share characteristics of helper T cells, cytotoxic T cells, and natural killer cells (Abaas *et al*, 2003).

Natural killer cells are able to kill cells of the body that do not display MHC class I molecules, or display stress markers such as MHC class I polypeptide-related sequence A (MIC-A). Decreased expression of MHC class I and up-regulation of MIC-A can happen when cells are infected by a virus or become cancerous (Janeway *et al*, 2001).

### **1-1-5-6: Monocyte**

Monocytes, the largest type of WBCs, share neutrophils their function, the phagocytosis, but are much longer lived as they have an extra role: they present pieces of pathogens to T cells so that the pathogens may be recognized again and killed. This causes an antibody response to be mounted. Monocytes eventually leave the bloodstream and become tissue macrophages, which remove dead cell debris as well as attack microorganisms. Neither dead cell debris nor attacking microorganisms can be dealt with effectively by the neutrophils. Unlike neutrophils, monocytes are able to replace their lysosomal contents and are thought to have a much longer active life. (Swirski *et al*, 2009). They have the kidney shaped nucleus and are typically agranulated. They also possess abundant cytoplasm.

Once monocytes move from the bloodstream out into the body tissues, they differentiate and are then known as macrophages allowing phagocytosis (Dukes, 2005).

### **1-1-5-7: Total leukocyte count**

Total leukocytes count is affected by various factors such as time of the day, a meal, pregnancy, exercise, epinephrine (exogenous or endogenous), anesthesia and other stress conditions. (Kenneth, 2010).

Neutrophilia (a high number of neutrophil granulocytes in the blood) occurs following exercise. Epinephrine causes neutrophilia and increase in lymphocytes in some species of animals. Digestive leukocytosis occurs in some animals with continuous grazing and eating habits (Dukes, 2015).

In the postnatal period marked changes take place in the number of circulating leukocytes. In the new born calf the number of WBC is similar to that found in the adult. WBC count in the dog would be between 6,000 and 17,000/ $\mu$  l, and in the cat 4,900-20,000/ $\mu$  l. (Dukes, 2015). The mean life span of granulocytes including the time of development in the bone marrow is approximately nine days. When granulocytes enter the blood stream they are about six days old (Dukes, 2015).

### **1-1-6: Platelets**

Platelets (Thrombocytes) are small circular or oval plates with diameter of 1-4 micro meters; originate from cells in the bone marrow called Megakaryocytes. Megakaryocytes are fragmented and become platelets either in the bone marrow or immediately after entering the blood. Platelets are a part of the blood that helps the blood clot (Guyton and Hall, 2006). Platelet count is a lab test that measure how many platelets animals or humans have in their blood. Platelets and proteins (factors of coagulation and fibrinolysis) are involved in hemostasis responding to any vascular injury (Pernille *et al*, 2014). Platelet numbers have to drop dramatically before

you see spontaneous bruising – including pinpoint petechiation and bleeding (Furie and Furie, 2008). A blood smear will be examined microscopically as confirmation of the cell numbers, as well as abnormal cell shapes and arrangements. Platelets may clump in samples giving false low readings and the blood smear will determine whether an adequate number is indeed present (Rita *et al*, 2015).

Increased platelets count higher than the reference interval for the species is a reactive thrombocytosis and not of direct pathologic importance (Heit, 2007). Decreased number of platelet count (thrombocytopenia) may be due to decreased production of platelet in the marrow, consumption of platelets in coagulation, or destructions of platelets by macrophages (Lewis *et al*, 2006).

### **1-1-6-1: Platelets indices**

Platelets ,Platelet volume(MPV) and Platelet crit( PCT) are values may be used as valuable parameters for diagnosis and probably for monitorization and prognosis in infected dogs with Ehrlichiosis and/or Anaplasmosis( Funda and Kerem,2014). Platelet count may be a reliable screening test for dog granulocytic ehrlichiosis (Bexfield,*et al*,2005, Mazepa,*et al*, 2010), and dog monocytic ehrlichiosis (Bulla *et al* ,2004). The effect of breed and age should be considered in clinical interpretation of dog platelet variables (Lysann and Reinhard, 2016).

(MPV) and (PCT) are indices used in evaluating immune mediated thrombocytopenia (IMT) in dogs and humans with congenital macro thrombocytopenia (David *et al*, 2008). (MPV) is a measurement of the average size of platelets in blood (Lewis *et al*, 2006).The high number of

(MPV) is an indicator of a large platelets size. The platelets distribution width (PDW) reflects the variability in the platelets size (Amin *et al* ,2004). Schwartz *et al*, (2014) studied platelet volume and plateletcrit in dogs with presumed primary immune-mediated thrombocytopenia. They recorded PLT, PCT, MPV and PDW from CBCs of 49 dogs. They found that platelet volume (MPV) was higher in immune mediated thrombocytopenia (IMT) in dogs (17.3fl) than the reference population (10.5 fl). No significant deference in (PDW). The median time for (PCT) to reach threshold in confirmed responders was faster (3days) compared with PLT (4days).They suggested that (PCT) maybe useful platelet parameter for monitoring dogs with (IMT).

The Platelets indices including platelet count (PLT),mean platelet volume(MPV),platelet crit(PCT),and platelet distribution width(PDW) along parallel red blood cell parameter including red blood cell count(RBC), mean cell volume(MCV) ,hematocrit(HCT)and red cell distribution width of camel had been studied by Hussen *et al*,(2010).They found a high significant correlation  $p \leq 0.001$  between PLT and PCT in males ,females and all camels and a low significant  $p \leq 0.05$  correlation between MPV and PWD in males and all camels. No significant correlation was found between RBC and PLT,PCT and HCT or MPV and MCV.However a highly significant correlation was recorded between PCT and RDW in all camels.

Some factors affecting platelet yield using continuous flow cell separator such as donation and pre donation, Punnet *et al*, (2014) stated that there was a significant decrease ( $P \leq 0.001$ ) in platelets count, and hemoglobin

concentration increased significantly pre and post donation in human when they used this procedure.

Botma *et al*,(2012) investigated reference range for platelet indices and gender differences using Sysmex XE2100 for human. Their result obtained a significant difference  $P \leq 0.05$  between genders for platelet count and PCT, while other indices did not show significant differences.

Reference value of platelets count and indices in Sudanese using Sysmex KX-21 exhibited significant difference  $P \leq 0.001$  between males, females and age below/over 35 years for platelet count and PCT. The other indices did not show significant difference by Awad-Elkareem *et al*,(2015). They stressed in the importance of determination of local reference values because reagent, instrument and population may differ from published reference values.

### **1-1-7: Red blood cells and biochemical parameters in relation to the sex and age**

A sex difference in red blood cell count has been observed many years ago, the count in general, has been found to be higher in the male than that in the female

animals, human and birds that in turn reflects on hemoglobin concentration (Wlodzimierz *et al*,1984). These results lead to research on the effects of castration, and of sex hormones on red blood cell counts of various animals. It is generally agreed that androgen stimulates erythropoiesis and estrogen produce anemia by an inhibiting effect on erythropoiesis ( Ganji and Kafai, 2009).

and Shadia, (2009) studied the effect of sex variations on some normal values of serological parameters of German shepherd dogs in Sudan. Her study covered serum concentration of total protein, albumin, glucose, cholesterol, triglycerides, creatinine urea, uric acid, and the activity of the enzymes ALT, ALP and AST. The result exhibited that sex had no significant effect on the studied parameters except the activity of the enzyme AST.

Shiela *et al*, (2007) studied the hematologic values in young pre training healthy Greyhounds dogs. The study assessed hematologic values of young Greyhounds at age of 5-13 months; mean hematologic results for different age groups and correlation with age and sex were determined and compared with adult canine. The result showed higher HCT, Hb and RBC count compared with adult canine reference values. Younger Greyhounds (5-6 months) had values comparable with reference values. Total WBC, neutrophil, lymphocyte and platelet counts were below the reference values intervals. Hemoglobin, HCT and RBC counts were correlated positively with age, and platelet count was correlated negatively with age. Sex had no correlation with the hematological values.

Ozkan *et al*, (2015) determined the changes in hematology and serum biochemistry in Turkish Angora cats (*Felis catus*) during growth period. Blood samples were collected from 32 Angora cats (16 adults and 16 kittens) and were analyzed for complete blood count (CBC), biochemical profile and some liver enzymes. They found that monocyte level higher and alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatine kinase (CK) activities and Pi levels were lower in adult cats compared with kittens. Mean corpuscular volume (MCV) was lower in adult kittens than the young kittens. And gamma glutamyl transferase (GGT) and aspartate amino transferase (AST) activities and glucose level were higher in kittens

of 1.5-3 months old than kittens of less than 3 months. Concentration of total cholesterol and Mg were higher in kittens at age 1.5-3 month than in adult cats.

## **1-2: Effect of the season on CBC and biochemical parameters**

The hematological profile of an individual, to large extent, reflects its general health( Jain 1993; peinado *et al* ,1999) ,and could reflect the correct diagnosis in different pathological states, particularly the complete blood count is an important diagnostic tool as a component of database for stress and welfare indicators (Anderson *et al*, 1999, Aengwanich *et al*, 2009). Variations in certain components of the hematological profile such as hemoglobin(Hb) and hematocrit (HCT)were included in metabolic profile test among other biochemical constituents(Kumar and Pachauri ,2000).The adaptability to adverse environmental condition had been indicated by total red blood cell(RBC),white blood cell(WBC),mean corpuscular volume (MCV), mean corpuscular hemoglobin(MCH) and mean corpuscular hemoglobin concentration (MCHC) (Kumar and Pachauri2000;Koubkova *et al* ,2002).The blood maintain the physiological equilibrium in the body but physiological and environmental conditions may alter this equilibrium. So many of the hematological parameters are influenced by factors like breed,sex,age ,nutrition ,pregnancy and mainly by seasonal changes (Aengwanich 2002;Al-Shami ,2007;Mohammed *et al* ,2007).

The seasonal changes on the blood parameters and biochemical profile of stray dogs was evaluated by Elenica *et al*,(2013).Their results showed that the exposure of stray dogs to extreme environmental temperatures have negative effects on physiological function and blood parameters



.Hematological values including RBC count,HCT and Hb concentration were significantly decreased during summer, the plasma total protein, glucose and cholesterol were significantly increased during summer .

Stefania *et al*,(2013) measured the red blood cells, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume, platelets and platelet crit in different time of the year of dairy cow .They observed a significant effect on RBC,Hb,HCT,PLT and PCT.Autumn decreased significantly RBC,Hb,HCT,while PLT and PCT significantly decreased during summer.

Effect of season on blood constituents of one- humped camel (*Camelus dromedaries*) had been investigated in Southern Darfur, Sudan during summer and autumn seasons by Alia *et al* ,(2007) .Their data revealed that, the red blood count, lymphocytes and basophiles percentages increased significantly during summer, while MCV, MCH and neutrophils percentages recorded high values during autumn. The serum total protein, globulins and triglyceride increased significantly in summer, but plasma glucose, serum urea, creatinine, phosphorous and calcium increased significantly during autumn.

### **1-3: Exercise**

In response to a stressor, physiological changes are set into motion to help individual or animal to cope with the stressor. Chronic activation of these stress responses, which include the hypothalamic- pituitary-adrenal axis(HPA)and the sympathetic-adrenal-medullary axis(SAM),results in chronic production of glucocorticoid hormones and catecholamines( David *et al*,2003).

Lymphocytes, monocytes or macrophages and granulocytes exhibit receptors for many neuroendocrine products of the HPA and SAM axis (McGregor,2016) such as cortisol and catecholamines, which can cause changes in cellular trafficking proliferation, cytokine secretion, antibody production and cytolytic activity( Cannon,2000). The study of serum chemistry of Alaskan sled dogs during prolonged exercise was carried out by Erica *et al*, (2007) .The dogs ran 160 km/day for 5 consecutive days and the serum was obtained before exercise and immediately after each exercise run. Their results indicated that serum globulin concentration was low before exercise and progressively decreased as exercise continued. Exercise increased serum chloride, urea nitrogen and cardiac troponin-1 and progressively decreased serum potassium, total protein and albumin concentrations. While in stray dogs Elenica *et al*,(2013) recorded marginal lymphopenia and mild leukocytosis responding to short term exercise .

Rovira, (2008), studied the effect of exercise in a normal session of searching and rescue training of 20 minutes, on physiological, blood and endocrine parameters in searching and rescue –trained dogs. The blood samples were collected at rest, immediately after exercise and at 5, 15, 30

minutes after exercise. RBC, Hb and HCT were not affected immediately after exercise, whereas WBC increased immediately after exercise. In conclusion, they found that significant changes in physiological and laboratorial parameters were induced by searching and rescue exercise, with values outside the reference range for healthy dogs.

In human Gina *et al*, (2008) studied the effect of exercise on hematological indices circulating side population (SP) cells and cytokines, specifically hemoglobin, hematocrit, white blood cell and platelets counts, plasma vascular endothelial growth factor (VEGF) and interleukin 6 (IL-6). They recorded significant increase in hemoglobin, hematocrit, platelets, (SP) cell number and (IL-6) in both trained and untrained individuals of both gender. On the other hand they found no significant changes in WBC count. Then they stated that exercise have physiological impact by mobilizing stem cells and thereby enhancing tissue repair mechanisms.

#### **1-4: Erythrocyte osmotic fragility**

Erythrocyte osmotic fragility (EOF) refers to the degree or proportion of hemolysis that occurs when samples of red blood cells are subjected to osmotic stress by placing them into a hypotonic solution.

The method for determining the susceptibility of erythrocytes to hemolysis in hypotonic salt solution has been of interest and developed since 1883 (Perk *et al* , 1964). The degree of EOF has been used as a measure of the red cells viability and also clinically, as a diagnostic characterization (Figueiredo *et al* ,2012). It measures the capacity of erythrocytes to resist hemoglobin leakage in solutions of decreasing NaCl concentration (Parpart *et al*, 1947). The fragility of the erythrocytes to hemolysis may be increased

or decreased in diseases (Perk *et al*, 1964; Binder and Mathois, 1986) and it is influenced by several factors: pH, temperature, oxygenation, cell size, cell membrane, age of animal, species, breed, lipemia, (Rodak2007;Fischbach *et al*,2008 and Greer,2008). EOF also measures the capacity of erythrocytes to resist hemoglobin leakage in solutions of decreasing NaCl concentration. It is a good tool to estimate erythrocyte deformability in blood stream, and detect increased erythrocyte fragility associated with hemolysis (Ariel *et al*, 2017).

Canine erythrocytes are unique compared with other mammalian erythrocytes: they show an increased fragility at alkaline pH *in vivo* and *in vitro* and intracellular Na<sup>+</sup> and K<sup>+</sup> concentrations are similar to plasma (Giber *et al*, 1985).

Studies of dog's erythrocytes osmotic fragility are not well published but many studies were carried out to estimate the EOF of different animals.

Alia *et al*,(2007), found that camels erythrocytes did not show any sort of hemolysis when subjected to hypotonic NaCl solution up to 0.5% when blood samples collected in both summer and autumn seasons, but hemolysis started at 0.4% NaCl concentration during summer, and at 0.3% NaCl concentration during autumn ,that means camels' erythrocyte are more fragile during summer .

Oyewale *et al*,(2011) examined the changes in erythrocyte fragility caused by variations in temperature, sex, pH and blood storage of camels and donkeys. They found that fragility did not differ between sexes in both species. Camels' erythrocytes were more resistant than donkeys' erythrocytes. At lower temperature and constant PH7.4, the fragility

decreased in camel but increased in donkey. In addition to that the blood storage for 14 hours at 4<sup>0</sup>C increased the EOF of Camels' but donkeys' erythrocytes did not change. The EOF of both species increased after 48-72 hours of blood storage at 4<sup>0</sup>C. The authors suggested that variation in temperature and PH of the erythrocyte environment and duration of blood storage cause significant variation in erythrocyte osmotic fragility of camels and donkeys.

# CHAPTER TWO

## Materials and Methods

### 2-1: Study area

The study was carried out at Police Directorate for Dogs, Unit of the Forensic Evidences Khartoum State (latit15<sup>0</sup> 360N, longitudes 32<sup>0</sup> and 32<sup>0</sup> E and altitude 380m) during 2014-2015.

### 2-2: Study design

This is an analytical study conducted to find CBC reference values for German shepherd dogs reared in the Sudan, and to evaluate the effects of the sex on the correlations between platelets count and platelets indices and between platelets and parallel red cells indices, effect of seasons on some blood parameters and serum biochemical and metabolites values, erythrocyte osmotic fragility, effect of exercise on some blood parameters as well as some clinical parameters at different time of a day of the year 2014-2015.

### 2-3: Metrological data

The monthly mean, maximum and minimum of ambient temperatures, relative humidity, and rain fall were obtained from Khartoum Meteorological Unit for the years 2014-2015 (table 1).

## **2-4: Animals**

Thirty three imported German shepherd dogs (14 males and 19 females), aged 2-4 years were used in this study, all the dogs were apparently healthy.

All the females were non pregnant and non lactating. The animals were housed individually in Kennels and were fed chicken soup with noodles. No food additives or minerals were offered.

All the dogs were vaccinated against Rabies, Parvo, Canine distemper and Para Influenza viruses.

They categorized into:

- Explosive dogs
- Drugs detectors dogs
- Cognition dogs
- Dogs for crowd and riots break up

Usually all the dogs are taken into walk for about one to three hours daily from 8 to 11 am. It is important to mention that riots dogs are more aggressive, so the sampling was more difficult.

## **2-5: Blood collection**

The blood sample (10 ml) was collected from the cephalic vein from each dog using vacutainer tubes divided into two groups, 5ml containing ethylene diamine tetra acetic acid ( EDTA) as anti coagulant each tube was gently inverted 3-4 times to insure mixing of sample and immediately transported to the laboratory using an automated hematology analyzer

Sysmex KX2 IN to determine the CBC parameters The other 5ml were allowed to clot for 2hrs at room temperature; the sera were separated by centrifugation at 5000 rpm for 10 minutes and frozen at-20<sup>0</sup>C pending analysis.

An automated biochemical analyzer Mindray BS-2000 was used for the sera biochemical and minerals test.

## **2-6: Hematological examinations**

The whole blood was analyzed to find out the reference range of the complete blood count (CBC) in both sexes of the German shepherd dogs, correlation between Platelets indices and parallel RBC indices in addition to that find the effect of the season on these parameters. That are total count of red blood cell (RBC), hemoglobin concentration (Hb) , hematocrit (HCT), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), red cell distribution width (RDW), total count of White blood cells (WBC), White blood cells differential count , Neutrophil, Lymphocyte and Mix cells, as well as Platelets count(PLT), Platelets crit(PCT), mean Platelets volume(MPV) and Platelets distribution width (PDW ).

## **2-7: Effect of the season**

This study started in March 2014 and ended in February 2015. The blood samples were collected from each animal every 30 days at the same time (11 am) for one year. To evaluate the seasonal changes on the CBC. The sera were used to determine the effect of the season on the total protein, Albumin, Globulin, Triglyceride, Urea and Cholesterol in addition to serum Calcium and Phosphorus.



**Table (1): Variation in maximum (max) and minimum (min) of ambient temperature relative humidity and total rain fall for the 12 months of the experimental period**

Month	Mean temperature C <sup>0</sup>		Relative humidity%	Total Rain fall MM
	Max	Min		
March	37.4	23.1	14	0.2
April	40.9	27.4	16	TR
May	41	28.4	17	4.6
June	42	25	21	0.6
July	36.9	26.1	45	73.6
August	34.7	25.5	54	52.3
September	37.2	26.3	45	29.4
October	38	26.5	27	5.7
November	34.7	22.1	21	0
December	33.3	19.3	29	0
January	30.2	15.8	25	0
February	36.1	20.4	16	0

TR: trace

## **2-8: Effect of exercise on CBC**

This study was carried out to examine the effect of exercise on the CBC parameters. The blood samples were collected at three stages as follows.

### **2-8-1: Before exercise**

Five ml blood with anti coagulant (k3 EDTA) was collected from the resting dogs the samples were kept into ice till transported to laboratory for analysis. The same dogs were subjected to one hour walking exercise.

### **2-8-2: Immediately after exercise**

Five ml blood were collected using the same procedure from the previous dogs immediately after exercise.

### **2-8-3: One hour post exercise**

The previous process was repeated after one hour post exercise and the three groups of blood samples were transported to the laboratory for complete blood count (CBC) analysis.

## **2-9: Erythrocyte Osmotic fragility**

This experiment was conducted to study the erythrocyte Osmotic fragility (EOF) of German shepherd dogs. Five ml blood were collected from the cephalic vein of each dog into EDTA-k3 vacutainer ,each tube was gently inverted 3-4 times to insure mixing of sample and immediately transported to the laboratory for analysis. A stock solution of Phosphate buffered sodium chloride was prepared as follows: 180gNacl, 27.31g NaHPO<sub>4</sub> and3.74gNaH<sub>2</sub>PO<sub>4</sub> made up to 2 liters of distilled water.

For each blood sample to be tested five ml stock solution was diluted with distilled water to prepare 1.0% Nacl solution ( Parpart *et al* 1947;Schalm *et al*,1975) .

Erythrocyte osmotic fragility was performed as described by Jain (1986a,b) for which briefly 20 $\mu$ L of blood was aspirated into 16 tubes containing 5mL of varying buffered concentrations of Nacl solution as exhibited in Table (2).

The suspensions were mixed by inversion and incubated at room temperature for 30 minutes and then all the tubes were centrifuged at 2000rpm for 10 minutes. A SpectrophotometerAVI -574was used to measure the absorbance of supernatant (hemoglobin concentration) at 540 nm and distilled water was used as blank. The hemolysis in tube 16(zero %) was regarded as being 100% of hemolysis. The percentage of hemolysis at certain Nacl concentration was =

$$\frac{\text{Optical density of un known}}{\text{Optical density of tube conc 0.0\%}} \times 100$$

**Table (2): Concentrations of NaCl solution used to perform EOF,  
according Jain (1986)**

<b>Tube number</b>	<b>mL of 1% NaCl</b>	<b>mL of distilled water</b>	<b>%NaCl solution</b>
1	4.25	0.75	0.85
2	4.00	1.00	0.80
3	3.75	1.25	0.75
4	3.50	1.50	0.70
5	3.25	1.75	0.65
6	3.00	2.00	0.60
7	2.75	2.25	0.55
8	2.50	2.50	0.50
9	2.25	2.75	0.45
10	2.00	3.00	0.40
11	1.75	3.25	0.35
12	1.50	3.50	0.30
13	1.25	3.75	0.25
14	1.00	4.00	0.20
15	0.50	4.50	0.10
16	0.00	5.00	0.00

## **2-10: Clinical parameters**

Respiratory rate, rectal temperature, pulse rate and heart rate were measured at three times of the day that is 8:30 am, 12:pm and 3:pm. The rectal temperature was measured using digital thermometer, the values by degree centigrade, respiratory rate and heart rate were measured using stethoscope. The pulse rate was measured from the femoral artery.

## **2-11: Statistical analysis**

The results were statistically analyzed using the IBM SPSS version 20 for windows. The data were presented as means  $\pm$  standard deviation. The differences for mean values between groups were detected by student's t test as described by Gomez and Gomez, (1984). Overall means and range were calculated. The nonparametric test was used to determine the normality and Spearman correlations were used to determine correlations among platelets indices and between the platelets and parallel red cell parameters.

The analysis of variance (ANOVA) test was used to evaluate the effect of the season on blood constituents, the effect of exercise on blood constituents, at addition to determine the diurnal variation on rectal temperature, pulse rate, heart rate and respiratory rate in different time of the day. Significant differences among means were then determined using Least Significant Differences (LSD) according to Gomez and Gomez (1984).

The descriptive means were used to determine the erythrocyte osmotic fragility for all animals and between the males and females.

## CHAPTER THREE

### Results

#### 3-1: Effect of the sex on CBC

Table (1) shows the effect of sex on CBC. Females showed significantly ( $p \leq 0.02$ ) higher values for RDW than males, whereas, males showed numerical high values red cell count and hemoglobin concentration (Hb). Females showed significantly ( $p \leq 0.02$ ) higher values platelets count (PLT) and platelet crit(PCT) than males. Males exhibited higher numerical values for white blood cells count (WBCs), neutrophil and Mix cells than females (Table 2).

#### 3-2: Correlation between Platelets count and Platelets indices

A highly positive significant correlation ( $P \leq 0.01$ ) was found between PLT and PCT in females and all the dogs, on the contrary to the males who exhibited low positive significant ( $P \leq 0.05$ ) correlation between PLT and PCT (Table3). No significant correlation was found between PLT and MPV, PDW in all the dogs.

#### 3-3: Correlation between Platelet indices and parallel RBC

No significant correlation was observed between RBCs and PLT, PCT, HCT in all the dogs Table (4). However a highly significant correlation ( $P \leq 0.01$ ) was found between PDW and RBCs, MCV, MCH and RDW in all the dogs, a negative correlation between PDW and RBC, RDW, positive correlation between PDW and MCV, MCH, MCHC.

Low negative significant correlations ( $p \leq 0.05$ ) between MPV and RDW in all the dogs and females. PDW was highly correlate ( $P \leq 0.01$ ) positively with MCV, MCH and negatively with RDW in females, while the similar significant between PDW and MCH was found in males. The high positive significant correlation ( $P \leq 0.01$ ) between PDW and MCH and low significant correlation ( $P \leq 0.05$ ) between PDW and MCV, MCHC was found in males, further more females were showed high positive significant correlation ( $P \leq 0.01$ ) between PDW, MCV, MCH and negative between PDW and RDW. Low significant correlation ( $P \leq 0.05$ ) between PCT and MCHC, the similar significant but negative between PLT and MCV in males. The females showed a low negative significant correlation between PWD and RBC.

**Table (1): Red Cells Parameters and Platelets count and Indices in Male and Female German Shepherd Dogs in the Sudan (Mean±Sd)**

Parameter	Males	Females	Overall
<b>Red Cells parameters</b>			
RBC( $\times 10^{12}$ )	6.60± 0.81	6.35± 0.74	6.46± 0.77
Hb(gm/dl)	18.25± 2.26	17.09± 3.71	17.58± 3.19
HCT (%)	46.57± 4.93	44.73± 5.16	45.5± 5.07
MCV(fL)	70.79± 2.49	70.56± 4.47	70.66± 3.71
MCH(pg)	27.65± 1.58	27.53± 2.15	27.58 ±1.89
MCHC(%)	39.15± 1.96	39.19± 2.19	39.17± 2.07
RDW (%)	14.14± 0.53 <sup>b</sup>	14.68± 1.95 <sup>a</sup>	14.45± 1.51
<b>Platelets Indices</b>			
PLT( $\times 10^9$ /L)	170.50 ±43.78 <sup>b</sup>	193.11± 80.21 <sup>a</sup>	180.48 ± 68.93
PCT (%)	0.11±0.67 <sup>b</sup>	0.14± 0.09 <sup>a</sup>	0.13± 0.085
MPV(fL)	9.51± 0.71	8.96± 1.06	9.19± 0.96
PDW (%)	15.49± 0.91	15.69± 0.91	15.61± 0.90

Significance at ( $P \leq 0.05$ )

a,b means within the same row followed by different superscripts are significantly( $P \leq 0.05$ ) different



**Table (2): White blood cells parameters in Male and Female of German shepherd Dogs in the Sudan (Mean±Sd)**

<b>Parameter</b>	<b>Males</b>	<b>Females</b>	<b>Overall</b>
WBC( $\times 10^9/L$ )	10.49±2.85	9.41 ± 2.78	9.92 ± 2.84
Lymphocyte%	90.10 ± 6.73	88.97 ± 9.19	89.50 ± 8.07
Neutrophil%	79.50 ± 13.73	74.23 ± 13.79	76.73 ± 13.89
Mix cell%	52.77± 12.01	50.61± 9.42	51.63 ± 10.68

Significance level ( $P \leq 0.05$ )

**Table (3): Correlation between Platelets count and Platelets indices of German Shepherd dogs in the Sudan**

Parameter	PLT( $\times 10^9/L$ )	PCT (%)	MPV(fL)	PDW (%)
<b>Over all</b>				
PLT( $\times 10^9/L$ )	1	0.880**	0.046	-0.336
PCT (%)	0.880**	1	0.098	-0.087
MPV(fL)	0.046	0.098	1	0.190
PDW (%)	-0.336	-0.087	0.19	1
<b>Males</b>				
PLT( $\times 10^9/L$ )	1	0.634*	0.454	-0.465
PCT (%)	0.634*	1	0.217	0.283
MPV(fL)	0.454	0.217	1	-0.024
PDW (%)	-0.465	0.283	-0.024	1
<b>Females</b>				
PLT( $\times 10^9/L$ )	1	0.960**	0.001	-0.332
PCT (%)	0.960**	1	0.152	-0.320
MPV(fL)	0.001	0.152	1	0.364
PDW (%)	-0.332	-0.320	0.364	1

\*Significance at ( $P \leq 0.05$ )

\*\*Significance at ( $P \leq 0.01$ )

(-) Negative correlate

**Table (4): Correlation between platelet indices and parallel RBC parameters in Male and Female German shepherd dogs in the Sudan**

Parameter	PCT (%)	MPV(fL)	PDW%	PLT( $\times 10^9/L$ )
<b>Over all</b>				
RBC( $\times 10^{12}/L$ )	0.071	0.053	-0.473**	0.170
HCT (%)	-0.035	0.163	-0.217	0.042
MCV(fL)	-0.245	0.254	0.627**	-0.307
MCH(pg)	-0.101	0.104	0.760**	-0.322
MCHC (%)	0.086	-0.165	0.340	-0.141
RDW (%)	-0.61	-0.434*	-0.544**	0.012
Hb(gm/dl)	-0.051	0.005	-0.041	-0.56
<b>Males</b>				
RBC( $\times 10^{12}/L$ )	0.023	0.048	-0.403	0.263
HCT (%)	-0.062	0.070	-0.271	0.114
MCV(fL)	-0.262	0.038	0.564*	-0.553*
MCH(pg)	0.319	-0.154	0.875**	-0.425
MCHC (%)	0.574*	-0.221	0.631*	-0.106
RDW (%)	-0.425	0.060	-0.476	-0.124
Hb(gm/dl)	0.190	-0.032	0.051	0.042
<b>Females</b>				
RBC( $\times 10^{12}/L$ )	0.160	-0.013	-0.512*	0.191
HCT (%)	0.035	0.141	-0.153	0.070
MCV(fL)	-0.241	0.315	0.694**	-0.241
MCH(pg)	-0.263	0.193	0.726**	-0.285
MCHC (%)	-0.137	-0.150	0.155	-0.166
RDW (%)	-0.070	-0.490*	-0.694**	-0.023
Hb(gm/dl)	-0.077	-0.056	-0.053	-0.036

\*Significance at ( $P \leq 0.05$ )

\*\*Significance at ( $P \leq 0.01$ )

(-) Negative correlate

### **3-4: Effect of the season**

#### **3-4-1: Metrological data**

The highest temperature was recorded in June, while the lowest temperature was recorded in January, while the highest relative humidity was recorded in August and the lowest was recorded in March, The highest rain fall was recorded in the July and August.

#### **3-4-2: Effect of the season on red blood cells and indices**

Table (5) shows the mean  $\pm$  sd of seasonal variation red cell parameters and erythrocytes indices. All the Erythrocytes parameters except Hb and HCT were significantly affected by the season. Red cells count (RBCs) was significantly decreased in winter while the red cell distribution width (RDW) was significantly increased in summer. Both MCH and MCHC were significantly increased during winter.

#### **3-4-3: Effect of the season on white blood cells and platelets**

Table (6) presents the total count and differential count of white blood cells (WBC), platelets count and indices of German shepherd dogs in the Sudan. Lymphocytes increased during autumn and Neutrophil increased in Winter. Whereas WBCs and mix cells were not affected by the season. PLT count increased during summer, while PCT and PDW increased and MPV decreased during winter.

#### **3-4-4: Effect of the season on biochemical parameters**

Total protein, globulin, albumen, triglyceride, urea and calcium were significantly ( $P \leq 0.05$ ) affected by the season, whereas cholesterols and phosphorous were not affected Table (7). Total protein, globulins and calcium were decreased during winter, while albumen decreased during autumn. Urea and triglyceride were increased during summer.

### **3-5: Effect of exercise**

Tables(8), (9) show the effect of exercise on blood constituents of German shepherd dogs .There was a mild numerical decrease in RBC, HCT, MCV immediately and at one hour post exercise. Whereas MCV, MCHC and Hb were numerically increased immediately post exercise.

The WBCs and neutrophil% were showed a mild increase immediately post exercise. On the other hand, lymphocytes decreased immediately and at one hour post exercise.

PLT was significantly  $P \leq 0.043$  increased immediately post exercise, while PCT showed numerical increase immediately post exercise.

**Table (5): Seasonal variation in the erythrocytes indices of German Shepherd dogs in the Sudan (mean  $\pm$  sd)**

Parameter	winter	summer	Autumn
RBC( $\times 10^{12}/L$ )	6.37 $\pm$ 0.79 <sup>b</sup>	7.20 $\pm$ 0.82 <sup>a</sup>	7.11 $\pm$ 0.75 <sup>a</sup>
Hb(gm/dl)	17.36 $\pm$ 3.91	16.00 $\pm$ 1.80	16.02 $\pm$ 1.70
HCT(%)	45.51 $\pm$ 4.99	48.07 $\pm$ 5.35	47.94 $\pm$ 4.98
MCV(fL)	69.92 $\pm$ 7.30	66.86 $\pm$ 2.42	67.44 $\pm$ 2.09
MCH(pg)	28.26 $\pm$ 0.93 <sup>a</sup>	22.25 $\pm$ 0.78 <sup>b</sup>	22.54 $\pm$ 0.82 <sup>b</sup>
MCHC (%)	39.53 $\pm$ 1.79 <sup>a</sup>	33.28 $\pm$ 0.84 <sup>b</sup>	33.41 $\pm$ 0.95 <sup>b</sup>
RDW (%)	13.95 $\pm$ 0.84 <sup>b</sup>	15.07 $\pm$ 1.45 <sup>a</sup>	14.37 $\pm$ 1.07 <sup>ab</sup>

Significance level (  $P \leq 0.05$  )

a,b ,ab means within the same row followed by different superscripts are significantly( $P \leq 0.05$ ) different.

**Table (6): Seasonal variation of the, WBC count and white blood cells differential count, platelets count and indices of German Shepherd dogs in the Sudan (mean  $\pm$  sd)**

Parameter	winter	summer	Autumn
WBC( $\times 10^9/L$ )	8.93 $\pm$ 2.91	10.15 $\pm$ 3.00	10.68 $\pm$ 2.42
Lymphocyte%	89.35 $\pm$ 8.32 <sup>ab</sup>	84.71 $\pm$ 8.55 <sup>b</sup>	94.45 $\pm$ 3.19 <sup>a</sup>
Neutrophil%	83.16 $\pm$ 15.30 <sup>a</sup>	76.66 $\pm$ 12.33 <sup>ab</sup>	70.36 $\pm$ 11.31 <sup>b</sup>
Mix cell%	51.75 $\pm$ 7.40	55.12 $\pm$ 13.99	48.02 $\pm$ 8.81
PLT( $\times 10^9/L$ )	173.86 $\pm$ 47.92 <sup>b</sup>	205.58 $\pm$ 69.31 <sup>a</sup>	197.21 $\pm$ 57.15 <sup>ab</sup>
PCT (%)	0.120 $\pm$ 0.07 <sup>a</sup>	0.051 $\pm$ 0.091 <sup>b</sup>	0.034 $\pm$ 0.01 <sup>b</sup>
MPV(fL)	9.42 $\pm$ 0.81 <sup>b</sup>	10.35 $\pm$ 0.98 <sup>ab</sup>	11.15 $\pm$ 0.76 <sup>a</sup>
PDW (%)	16.03 $\pm$ 0.55 <sup>a</sup>	13.94 $\pm$ 2.74 <sup>b</sup>	14.63 $\pm$ 2.71 <sup>ab</sup>

Significance level(  $P \leq 0.05$ )

a,b ,ab means within the same raw followed by different superscripts are significantly( $P \leq 0.05$ ) different

**Table (7): Seasonal variation in the concentration of some blood metabolites and serum minerals of German shepherd dogs in the Sudan (mean  $\pm$  sd) .**

Parameter	winter	summer	autumn
T. P(g/dl)	6.94 $\pm$ 1.02 <sup>b</sup>	8.49 $\pm$ 0.75 <sup>a</sup>	8.12 $\pm$ 0.60 <sup>a</sup>
Albumin(g/dl)	3.46 $\pm$ 0.64 <sup>a</sup>	3.45 $\pm$ 0.39 <sup>ab</sup>	3.03 $\pm$ 0.51 <sup>b</sup>
Globulin(g/dl)	3.47 $\pm$ 1.23 <sup>b</sup>	5.04 $\pm$ 0.80 <sup>a</sup>	5.12 $\pm$ 0.80 <sup>a</sup>
Urea(mg/dl)	36.84 $\pm$ 8.05 <sup>b</sup>	44.15 $\pm$ 10.60 <sup>a</sup>	37.15 $\pm$ 6.50 <sup>ab</sup>
Triglyceride(mg/dl)	151.63 $\pm$ 26.54 <sup>ab</sup>	159.37 $\pm$ 20.65 <sup>a</sup>	147.63 $\pm$ 32.26 <sup>b</sup>
Cholesterol(mg/dl)	148.00 $\pm$ 24.44	156.26 $\pm$ 26.20	146.00 $\pm$ 21.56
Calcium(mg/dl)	10.59 $\pm$ 3.27 <sup>a</sup>	9.50 $\pm$ 2.21 <sup>ab</sup>	6.42 $\pm$ 2.09 <sup>b</sup>
phosphorus(mg/dl)	3.26 $\pm$ 0.92	3.64 $\pm$ 0.79	3.76 $\pm$ 0.97

Significance at (P $\leq$ 0.05)

a,b ,ab means within the same raw followed by different superscripts are significantly(P $\leq$ 0.05) different.



**Table (8): Effect of exercise on red blood cell parameters of German shepherd dogs in the Sudan**

<b>Parameter</b>	<b>Before exercise</b>	<b>Immediately after exercise</b>	<b>One hour after exercise</b>
RBC( $\times 10^{12}$ )	6.61 $\pm$ 0.53	6.57 $\pm$ 0.59	6.46 $\pm$ 0.48
Hb(gm/dl)	17.37 $\pm$ 4.27	18.96 $\pm$ 2.21	18.58 $\pm$ 1.93
HCT (%)	47.51 $\pm$ 3.12	46.65 $\pm$ 4.50	45.52 $\pm$ 4.74
MCV(fL)	72.07 $\pm$ 2.64	71.09 $\pm$ 2.61	70.39 $\pm$ 3.52
MCH(pg)	28.65 $\pm$ 1.55	28.77 $\pm$ 1.43	28.68 $\pm$ 1.70
MCHC (%)	39.81 $\pm$ 1.83	40.53 $\pm$ 0.96	40.69 $\pm$ 1.79

Significance level (  $P \leq 0.05$  )

**Table (9): Effect of exercise on white blood cell count, differential count and platelets count and indices of German shepherd dogs in the Sudan**

<b>Parameter</b>	<b>Before exercise</b>	<b>Immediately after exercise</b>	<b>One hour after exercise</b>
WBC( $\times 10^9/L$ )	8.98 $\pm$ 3.13	9.20 $\pm$ 3.34	8.87 $\pm$ 2.26
Lymphocyte(%)	40.16 $\pm$ 20.21	34.79 $\pm$ 12.21	32.97 $\pm$ 11.43
Neutrophill(%)	42.44 $\pm$ 19.57	47.32 $\pm$ 13.74	47.31 $\pm$ 12.46
Mix cell(%)	17.37 $\pm$ 4.27	16.33 $\pm$ 5.70	16.86 $\pm$ 5.05
PLT( $\times 10^9/L$ )	84.20 $\pm$ 33.45 <sup>b</sup>	118.60 $\pm$ 39.67 <sup>a</sup>	114.30 $\pm$ 37.37 <sup>ab</sup>
MPV(fL)	9.11 $\pm$ 0.93	9.18 $\pm$ 0.55	8.96 $\pm$ 0.92
PWD (%)	15.94 $\pm$ 0.75	15.94 $\pm$ 0.44	15.59 $\pm$ 0.71
PCT (%)	0.07 $\pm$ 0.029	0.102 $\pm$ 0.029	0.102 $\pm$ 0.038

Significance level ( $P \leq 0.05$ )

a,b ,ab means within the same raw followed by different superscripts are significantly ( $P \leq 0.05$ ) different

### **3-6: Erythrocytes Osmotic Fragility:**

Hemolysis of erythrocytes started at Nacl concentration of 0.55% in all the dogs (fig 1) .In females it started at Nacl concentration of 0.75% (fig2).

Males erythrocytes were more tolerant than females as the (EOF) occurred at Nacl concentration of 0.50% (fig2).

Males and females showed similar high EOF at Nacl concentration of 0.1%.

### **3-7: Clinical parameters:**

Table (10) exhibits the clinical parameter at different times of the day. No significant differences in rectal temperature, pulse rate or heart rate were seen at the three different time of the day. A significant increase in respiratory rate was found at 12:00 pm.

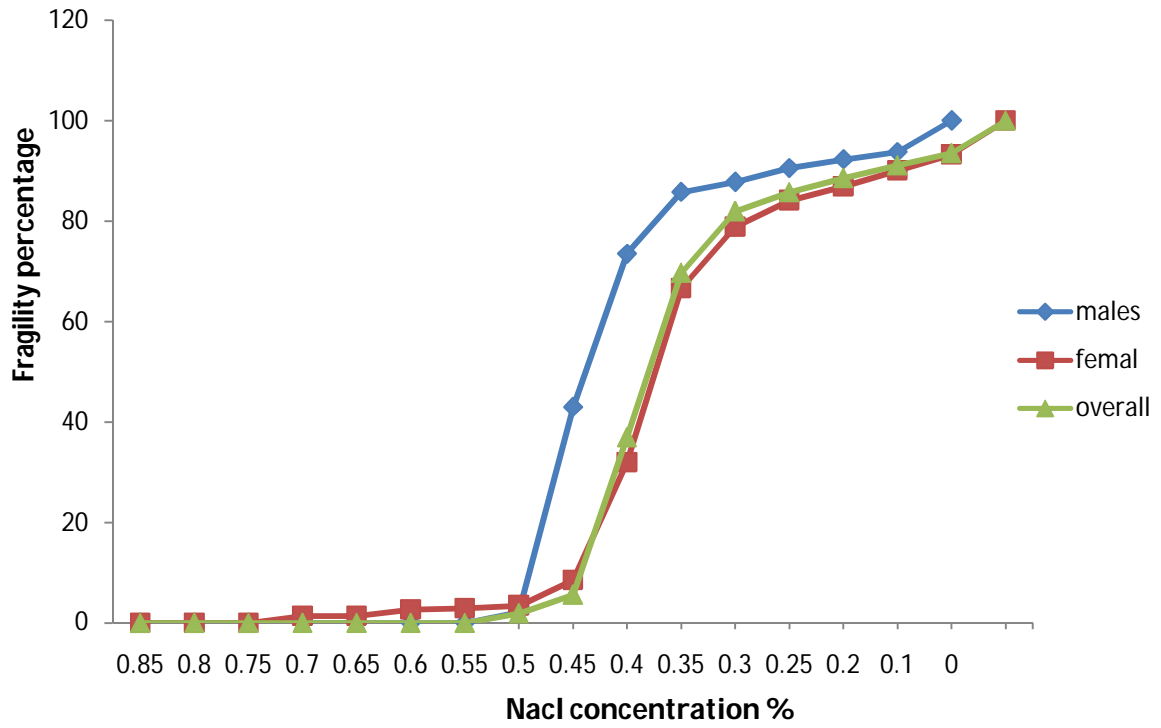


Fig. (1): Over all Erythrocyte Osmotic Fragility of German shepherd dogs in the Sudan

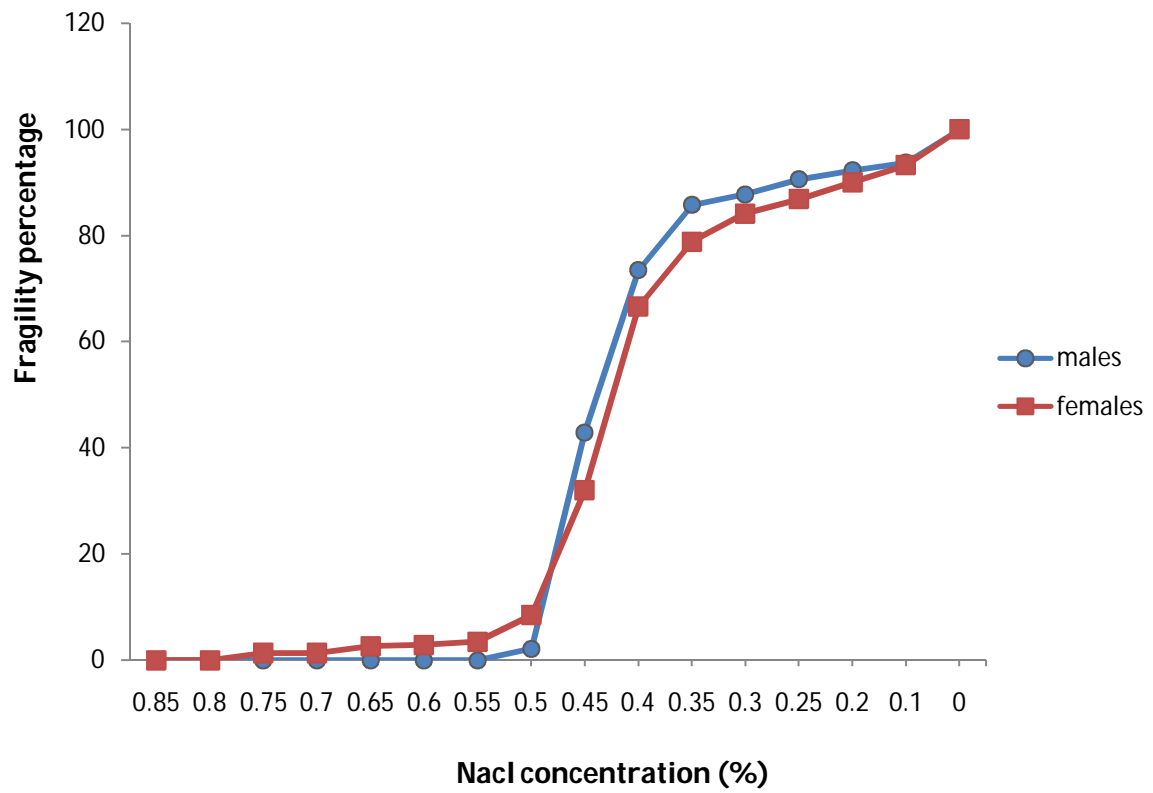


Fig. (2): Effect of sex on erythrocyte Osmotic fragility of German Shepherd dogs in Sudan

**Table (10): Effect of time of the day on some clinical parameters of German Shepherd dogs in the Sudan (mean  $\pm$  sd).**

Parameter	At 8:30 am	At 12:00 pm	At 3:00pm
Rectal temperature C <sup>0</sup>	38.94 $\pm$ 0.49	38.91 $\pm$ 0.49	38.94 $\pm$ 0.40
Pulse rate/minute	69.6 $\pm$ 8.68	65.60 $\pm$ 3.37	63.60 $\pm$ 12.57
Heart rat/minute	70.9 $\pm$ 9.21	74.40 $\pm$ 11.50	66.80 $\pm$ 8.65
Respiratory rate/minute	46.4 $\pm$ 8.38 <sup>b</sup>	75.5 $\pm$ 10.94 <sup>a</sup>	58.7 $\pm$ 18.43 <sup>ab</sup>

Significance level(  $P \leq 0.05$ )

a,b ,ab means within the same raw followed by different superscripts are significantly( $P \leq 0.05$ ) different.

## CHAPTER FOUR

### Discussion

No significant differences were found between male and female for red blood cells count, hemoglobin concentration, HCT,MCV,MCH,MCHC or white blood cells count ,these findings agree with the findings of( Lund *et al*,(2000) ;Gracnier *et al* ,(2007) ; Rovira, (2007) and Shiela *et al*,(2007) ) in dogs. On the contrary Aroch *et al*,(2005) obtained higher RBCs count,Hb,HCT,MCHC values in free ranging males golden jackals than the females. That can be attributed to climatic variation, species, animal habitat, method of sampling and the device used in the examination of the samples.

There is a scarcity of publication regarding the platelets count and indices and correlations between platelet indices and their parallel red cell indices in Shepherd dogs in the Sudan. In the current study a higher platelet counts and slight rise in PCT were found in females German Shepherd dogs in Sudan than the males, this significant difference between male and female is on line with what was reported in humans by ( Giacomoini *et al*,(2001), and Lawrie *et al*,(2009)). This gender variation may be due to sex hormonal profile variations (Bain, 1985). The present study revealed a positive significant correlation between PLT count and PCT in the overall dogs and females and low positive significant correlation between the previous parameters in males. Hussein *et al* ,(2010) found similar result in camels except of that males showed highly significant correlation between the two parameters. On the other hand there was no significant correlation between PLT and

MPV this is on line with Hussein *et al*,(2010) in camels. No significant difference between males and females in PDW and MPV, this is on line with (Botma *et al*,(2012);Punnet *et al*,(2014) and Awad Elkareem *et al*,(2015)), in humans, and in contrast with the findings of Hussein *et al*, (2010) in camels. In the current study there was no significant correlation between PCT and MCV or between MPV and MCV ,this agree with the findings of Wiwanitkit,(2004) in humans ,Hussein *et al* ,(2010) in camels confirming the view that the sizes of RBC and blood platelets are independent of each other (Wiwanitkit, 2004). In concur with these authors, a highly significant negative correlation was found between PDW and RDW in all dogs. More over a highly negative correlation was found between PDW and RBCs in the all dogs, while a highly positive correlation was found between PDW and MCV, this contradicting the findings of Hussein *et al*, (2010) in camels. It must be noted that different instruments give different results, some instruments measure PDW as percentage not as femtoliter as other instruments.

A number of factors such as nutrition, age, sex,race,body weight,season,climate and analytical methods affect hematological and biochemical parameters of clinically healthy dogs( Awah and Noltidge,1998, Shadia,2009). Season is considered a critical factor that can affect the values of hematological parameters, through changes in ambient temperature, rain fall and air humidity. In this study the season did not affect the Hb, HCT, MPV, WBC, Mix cells, as well as cholesterol and phosphorus level,this is on line with Elenica *et al* ,(2013) who found that season had no effect on the values of WBCs ,or differential white blood cell count in stray dogs, and similar to the



findings of Stefania *et al* ,(2013) who found that the similar result in dairy cow. In this study German Shepherd dogs represent mild increased leucocytes and marginal decreased lymphocyte as Elenica *et al* ,(2003) observed in stray dogs, they suggested that indicates an increased level of circulating corticoid due to stress caused by heat release resulting from increased climate temperature and exercise. In summer there was a significant increase in RBCs, RDW when compared with the other season, this is on line with Alia *et al* ,(2007) who reported an increase in RBCs count during this season in camels. In contrast, Hellman *et al* ,(1985);Elenica *et al* (2013) observed a reduction of RBCs and Hb during this season in dogs. In the current study there was a significant reduction of PLT value ( $P \leq 0.05$ ) during winter. The reduction of PLT during winter is in contrast with the findings of Stefania ,(2013) and Mirzadeh *et al*, (2010) in dairy cattle. In the current study there was an increase in the serum total protein concentration during summer which contradicts the findings of Elenica *et al*, (2013) in stray dogs. This variation most probably is due to theirs indigenous dogs at the same climate as they are reared. Nazifi *et al* ,(1999); Abokouider *et al* ,(2001) and Alia *et al* ,(2007) reported similar result in camels, this could be attributed to the stress to which the dogs were subjected under hot condition, while Al-Eisa *et al* ,(2012) in goats;Averos *et al* ,(2007) in pigs; Yokus *et al* (2006) in sheep stated that no seasonal variation in total protein levels.

Albumen concentration was lowest during autumn and no significant difference between summer and winter. This is contradicting the findings of Suntorn *et al*, (2009); Al-Eissa *et al* ,(2012) who reported a reduction in this parameter during summer in goats.

Abdalla *et al* ,(2009) observed a high serum globulin level in autumn in goats which accords with the findings of this study.

Although there are many reports on seasonal changes of lipid and lipoproteins in different animals, but there are limited references in pet animals including dogs. Triglycerides are known to provide the metabolic fuel for most tissues when the animal has energy deficit (Beitz 1993).

In this study triglycerides recorded the highest value in summer, but cholesterol was not affected by season. These findings are on line with the findings of Bahman *et al*,(2014) in German shepherd dogs in Iran ,and Mirghani,(1982); Abokouider *et al* ,(2001) and Alia *et al* ,(2007) in camels . The high value of triglyceride during summer could be related to the reduction of food intake or loss of appetite to regulate internal body temperature. Nazifi *et al*, (2007);Eshratkhah *et al*,(2008) found contradictory findings of the serum concentrations of triglyceride and cholesterol in sheep and goat.

According to this study the highest level of urea was found during summer may be result in greater efficiency in the digestion of dietary protein, this supports Elenica *et al*(2013) result in dogs. Also, Alia *et al*, (2007) recorded similar result in camels during the same season. In contrast to, Youkus *et al* ,(2006) who did not find any seasonal change in urea level in sheep.

In this study Calcium concentration decreased during autumn and there was no significant variation between winter and summer, while Aleissa (2011) stated a high level of Calcium in rabbit during summer. On the other hand season did not affect phosphorus serum concentration.

Alia *et al* ,(2007) observed a marked increase in the concentration of serum phosphorus and calcium in camel during autumn.Sandabe *et al*,(2000);Al-Eissa *et al* ,(2012) reported that phosphorus and magnesium did not show any significant variation with the seasons in goat.

Increased RBCs,HCT and Hb is a normal response to racing (Lassen *et al*,1986;Snow *et al*,1988;Ilkiw *et al*,1989)and agility exercise Rovira *et al*,(2007a,b).By contrast ,these parameters did not change or even decreased during prolonged exercise (Hinchcliff *et al*,1993);Burr *et al*,1997;Mckinzie *et al*2007).The absence of significant changes in RBCs,HCTand Hb in our study indicated that hemo concentration is not a concern for searching and rescue dogs during exercise sessions of one hour or the dogs may be get adapted the exercise.

Platelets numbers showed a significant increase from pre to post-exercise; this is on line with the finding of Gleenson *et al* ,(1995); El-Sayed *et al*,(2000),and Gina *et al* ,(2008) in humans.

In the current study WBCs showed numerical increase immediately post exercise. Gina *et al* ,(2008) found that no difference in WBC numbers between pre to post exercise in humans. The numerical decreased of Mix cells (eosenophills, basophills) in the current study supports the fact that corticoid prohibit eosinophills production, so the increase of their circulation explain the eosenophills reduction( McAdam and Eberhart1972;Wegner and Stott1972).According to Feldman(2000) stress causes corticosteroid release from adrenal glands, which is followed by leukocytosis,neutrophilia eosenopenia and lymphopenia,that corticosteroid acts as immune suppressor which suppressing lymphocytes mainly T lymphocyte(Robson *et al*,1999) , that explain the results that we found immediately after exercise.

Increased EOF usually results when reactive oxygen or nitrogen radicals or other form of oxidants react with integral and other proteins on the erythrocyte membrane leading to destruction of the membrane structure. They may also attack the membrane lipids, resulting in lipid peroxidation, membrane fluiding and ultimate destruction of the bilayer integrity of erythrocyte to membrane (Girotti1985).

To the best of my knowledge studies of erythrocyte osmotic fragility in dogs in the Sudan was not documented. In this study the hemolysis of erythrocytes started at NaCl concentration of 0.55% . While Alia *et al* ,(2007) found the EOF of camels started at 0.4% when the blood samples collected during Summer similar to our samples which were collected in April. There was a significant difference in EOF between males and females, the females erythrocyte membrane was more susceptible to hemolysis at concentrations of NaCl of 0.75 % and 0.70% (have minimum resistance) than males EOF started at 0.5% of NaCl concentration. This contradicting the findings of Oyewale *et al*,(2011) who did not find any significant differences between sexes in camels and donkeys. However donkeys erythrocytes showed higher fragility than camels erythrocytes at NaCl concentrations of 0.5% ( $p \leq 0.01$ ) and 0.3% ( $p \leq 0.001$ ).Also Ayeres *et al*, (2014) found that sex had no influence in Zebu Nellore cattle.

The difference in EOF between the two sexes in the current study may be associated with difference in the hormones activity which affects the metabolic rate (Brunet, 2004). Further more in this study there were no significant EOF differences between the two sexes at NaCl concentration

of( 0.3 0.25,0.20 and 0.10 )% .In contrast females recorded lower EOF than males at Nacl concentration of ( 0.45,0.40 and 0.35)%.

In the current study some clinical parameters were selected to study the effect of the time of the day on these parameters that are heart rate, pulse rate, respiratory rate and rectal temperature .All the parameters were within the normal range. No significant differences in rectal temperature, pulse rate or heart rate at the three different times. A significant increase in respiratory rate was found at 12:00pm.That could be due to the rises of the ambient temperature.

## CONCLUSION

- German Shepherd dog Females showed significant differences in mean of platelets count and RDW than males, whereas, male showed high numerical values of red cells.
- No significant difference in WBC or differential count between the two sexes.
- In two sexes PLT significantly correlates positively with PCT.
- PDW correlates significantly and negatively with RBC and RDW but correlate positively with MCV and MCH in all animals.
- In males PDW correlates significantly and positively with MCV, MCH and MCHC.
- In Winter RBCs, MCV decreased while PCT, PDW, MCH, MCHC and Neutrophil increased.
- PLT and RDW increased in summer. lymphocyte increased in autumn.
- The season did not affect Hb, HCT, WBC and Mix cells.
- The total protein and globulins decreased during winter, but calcium increased.
- Urea and triglyceride increased in summer.
- Albumen decreased during Autumn.
- Exercise increased PLT, PCT, MPV, Hb, MCHC and WBC, Neutrophil, whereas decreased RBC, HCT, MCV, and lymphocyte.
- Hemolysis of erythrocyte started at NaCl concentration of 0.55% in all the dogs.

- Males' erythrocytes were more tolerant than females. Males and females showed similar high EOF at NaCl concentration of 0.1%.
- Males' and females' clinical parameters have no variation at different time of the day, but respiratory rate increased at 12 pm.

## **RECOMMENDATION**

### **Further studies are recommended, to investigate:**

- The effect of prolonged exercise on blood glucose level, and stress hormones.
- The hemostatic parameters such as coagulation time, bleeding time, blood fibrinogen level, Thrombin time (TT), Prothrombin time (PT), and activated Thromboplastin time (aPTT).
- Reproductive performance of males and females German shepherd dogs reared in the Sudan and follow up the off spring through the CBC parameters examination and growth.
- Routine blood examination of imported and locally bred German shepherd dogs.
- The hematological profile of the indigenous Sudanese dog breeds.



## REFERENCE

- Abbas, A. K.; Lichtman, A. H. (2003).** Cellular and Molecular Immunology 5<sup>th</sup> ed. Saunders, Philadelphia.
- Abdalla, M.Abdelatif.; Ibrahim, M,and Yahia, Y.(2009).**Seasonal variation in Erythrocytic and Leukocytic Indices and Serum Proteins of Female Nubian Goats. Middle –East J.of Sci. Res,4(3):168-174.
- Abokouider, S.N.; Dabbag, N. and Schenkel, F. (2001).**Studies on the camels blood parameters in relation to season in Syria.6<sup>th</sup> Annual Conference on animal production under arid condition.Alain,U. A E.
- Adili, N and Melizi, M. (2014).** Preliminary study of the influence of red blood cells morphometry on the species determinism of domestic animals, Vet. World, 7(4): 219-223.
- Aengwanich ,W.;Chantiratikul, A,andPamok ,S.(2009).**Effect of seasonal variation on hematological values and health monitor of crossbred beef Cattle at slaughter house in northeastern part of Thail and.American-Eurasian .J .Agric. Environ. Sci,5:644-648.
- Aengwanich, W.(2002).**Effect of age on hematological values and blood profile of Holestein Friesian cressbred in northern Thailand.Suranaree .J. Sci Technol,9:289-292.
- Aguirre,F.I.;Martin,D.;Grinson,M.;Ruiz,A.;Hager,T.;De paoli,J.;Ihlo,H.A.Farach and poole, C.p.(1998).**Oxiditive damage,plasma antioxidant capacity and glucemic control in early NIDDM patients. Free Radic. Biol.Med,24:580-585.

**AL-Eissa ,M.S.;Saad, Alkahtani.;Al-Farraj ,S.A.;Saud ,A.Alarifi.;Al-Dahmash. B, and Hamad, Al-Yahya.(2012).**Seasonal variation effects on the composition of blood in Nubian ibex(*Capra nubiana*)in Saudi Arabia. Afri. J. of Bio-Technol, 11(5), 1283-1286.

**Aleissa, M.S .(2001).** Effect of Gestation and Season on the Haematological and Biochemical Parameters in Domestic Rabbit (*Oryctologus cuniculus*).Brit.Bio-Tech.J.1 (1):10-17.

**Alia,S.A.Amin.;Khalid,A.Abdonand Abdalla,M.Abdelatif.(2007).** Seasonal variation in Blood Constituents of One-humped Camel (*Camelus dromedaries*),Paki. J. Biol. Sci, 10(8):1250-1256.

**Al-Shami, S. A.(2007).**Comapitive study of haematological and blood biochemical components in milk-fed and conventionally-reared Hassawi breed calves.Sci. J. King Faisal Univ Basic Appl Sci,8:99-106.

**Amin , M.A. ;Amin, A.P ,and Kulkarnite ,H.R.(2004).**Platelet distribution width(PDW) is increased in vaso-occlusive crisis in sickle cell disease. Anna Hematol; 83(6):331-335.

**Anderson, B.H.;Watson, D.L,and Colditz ,J.G.(1999).**The effect of dexamethasone in some immunological parameters in cattle. Vet Res Commun,23:399-413.

**Ansari-Lari, M.A.; Muzny, D.M.; Lu, J. Lu. F.; Lilley, C.E.; Spanos ,S.;Malley, T,and Gibbs, R.A. (1996).** A gene-rich cluster between the CD4 and triosephosphate isomerase genes at human chromosome 12p13. Genome Res. 6 (4): 314–326.

**Arcoh, I.; Shippel, N.Y.; Avidar, Y.; Yakobson, B. King. R and Shamir. M. (2005).** Hematological and biochemical measurements in healthy adult, free- ranging golden jackals (*Canis aureus syriacus*) held in captivity. Vet. Rec.10:317-321.

**Ariel, O.; Orly ,Z.;Saul ,Y, and Gregory, B.(2017).** Biophysical and biochemical Markers of red blood cell fragility.Transfus Med Hemother,44(3):183-187.

**Averos, X.;Herranz, A.;Sanchez, R.;Comella, J,and Gosalvez, L.F.(2007).**Serum stress parameters in pigs transported to slaughter under commercial conditions in different seasons.Vet.Med,52(8):333-342.

**Awad-Elkareem. A.;Israa,I.;Razaz. Y.;Eman ,A. ;Reem, M.;Sara ,M.;Haifa ,A, and Adam, A.(2015).**Reference value of Platelets count and indices in Sudanese using Sysmex KX-21.Inte. j.Of Health Sci. 3(2): 120-125.

**Awah,J.N, and Nottidge,H.O.(1998).**Serum biochemical parameters in clinically healthy dogs in labadan.Tropi. Vet. Medi ,16:123-129.

**Ayeres,M.C.C.;Birgel,E.H.;Birgel,J.R.E.H,andMartins,L.E.P.(2014).** Influence of age, sex and Breed Lines of ZEBU Nellore Cattle on the Erythrocytes Osmotic Fragility, raised in Sao Paulo,Brazil.Inter. J. Of. App.Sci and Tech .4(5).

**Bain ,B.J.(1985).**Platelet count and Platelet size in males and females. Scand J. Hematol,35(1):77-79.

**Bahman,M.;Ali, G. Reza.;Reza,M. Avizch,and Marziae,R.(2014).** Serum concentration of lipids and lipoproteins and their correlations with thyroid hormones in clinically healthy German shepherd dogs:effect of season,sex and age.Iran J of Vet Sci and Tech,2(6):53-61.

**Barbara, J. Bain. (2006).** Blood cells: a practical guide. Wiley-Blackwell. pp. 66 .UK.

**Beitz ,D.C.(1993).** Lipid metabolism in Dukes' physiology of Domestic Animals,11<sup>th</sup> ed, 453-471 Swenson,M.J,and W.O.Reece , Cornell University ,London.

**Bexfield, N.H.; Villiers, E.J,and Herrtage, M.E.(2005)** . Immune-mediated haemolytic anaemia and thrombocytopenia associated with *Anaplasma phagocytophilum* in a dog. J Small Anim Pract; 46:543-548.

**Binder, J.P,and Mayhois, H. (1986).** Osmotic resistance of erythrocytes of Cows. J. Vet. Med , 33: 89-92.

**Botma ,J.; Mogongoa ,L.F.; Jaftha,J. van,and Rensburg ,W.(2012).** Reference ranges for Platelet indices using Sysmex XE-2100 blood analyser.Med Tech S A fr; 26(2):17-12.

**Boyko, A(2009).** Complex population structure in African village dogs and its implication for inferring dog domestication history. Proc. Natl Acad. Sci. USA 106: 13903–13908.

**Bridgett M. vonHoldt; John P. Pollinger; Kirk E. Lohmueller; Eunjung Han;Heidi G. Parker; Pascale Quignon; Jeremiah D. Degenhardt; Adam R. Boyko; Dent A. Earl; Adam Auton; Andy Reynolds; Kasia Bryc; Abra Brisbin;James C. Knowles; Dana S. Mosher; Tyrone C. Spady; Abdel Elkahoun; Eli Geffen; Malgorzata Pilot;Wlodzimierz Jedrzejewski; Claudia Greco; Ettore Randi; Danika Bannasch; Alan Wilton; Jeremy Shearman;Marco Musiani; Michelle Cargill; Paul G. Jones; Zuwei Qian; Wei Huang; Zhao-Li Ding; Ya-ping Zhang; Carlos D. Bustamante; Elaine A. Ostrander; John Novembre, and Robert K. Wayne(2010).**Genome- wideSNP and haplotype analysis reveal a rich history underlying dog domestication. J.Nature 464:10-108.

**Bruce ,A.; Alexander ,J.; Julian, L.; Martin ,R.; Keith ,R, and Peter ,W. (2002).** Leukocyte also known as macrophages functions and percentage breakdown. In molecular biology of the Cell 4<sup>th</sup> ed. Taylor and Francis group. New York: Garland Science.

**Brunet- Rossinni,A.K,(2004).**Reduced free-radical production and extreme longevity in the little brown bat(*Myotis lufugus*)versus two non –flying mammals.Mech.Ageing .Dev.,125:11-20.

**Buchl, S.J,and Howard ,B.(1997).** Hematologic and serum biochemical and electrolyte values in clinically normal domestically bred rhesus monkeys (*Macaca mulatta*) according to age, sex, and gravidity. Lab Anim Sci;47:528–33.

**Bulla ,C.; Takahira ,R.K.; Araújo, J.P.; Trinca, A.L.; Lopes, S.R,and Wiedmeyer, C.E. (2004).** The relationship between the degree of thrombocytopenia and infection with *Ehrlichia canis* in an endemic area. Vet Res; 35(1):141-146.

**Bunn, H.F.(2011).** Approach to the anemias. In: Goldman ,L; Schafer, AI, eds. Cecil Medicine . 24<sup>th</sup> ed. chap 161 Saunders Elsevier . Philadelphia,

**Burr, J.R.;Reinhart, G.A.;Swenson ,R.A.;Swaim ,S.E.;Vaughn, D.M,and Bradley, D.M.(1997).**Serum biochemical values in sled dogs before and after competition in long-distance races.J. of Am Vet. Med Assoc,211(2):175-179.

**Cannon, J.G.( 2000).** Inflammatory Cytokines in Non pathological States. News. Physiol. Sci. 15: 298–303.

**Clark, P.(2004).** Haematology of Australian mammals. Collingwood: Csiro Publishing.

**Costanzo, S. Linda. (2007).** Physiology board review series,saunders text and review. Williams & Wilkins, Lippincott.

**David ,C.Lewis , and Kenneth, M.Meyers. (2008).** Canine idiopathic thrombocytopenic purpuraJ. Vet.Inter.Medi 101(4) version of research on line.

**David ,C. Dugdale.(2012).**CBC Medline plus medical Encyclopedia Medline plus U. S .Natio. Libr of med.

**David, A.Padgett, and Ronald ,Glaser .(2003).**How stress influences the immune response. Trends in immunology 24:8.

**Dukes.(2005).**Physiology of domestic animals 12<sup>th</sup> ed ,William O,Reece. Panima publishing corporation .New Delhi-Bangalore.

**Dukes.(2015).**Physiology of Domestic Animals, 13<sup>th</sup> ed, William O. Reece , Howard H. Erickson ,Jesse P. Goff), Etsuro E. Uemura . Wiley-Blackwell.Danvers.U.K.

**Elenica, D.;;Jetmira, A.;;Vasilika, D, and Xhilda, R.(2013).**The influence of environmental temperature on the blood parameters of stray dogs(*Canis lupus familiaris*).Natur. Mont.Podgorica,12(2):521-530.

**El-Sayed ,M.S.;Sale, C.;Jones, P.G, and Chester, M.(2000).**Blood hemostasis in exercise and training.Medic. Sci. Sports .Exer,32:918-925.

**Erica ,C.Mckenzie.;Eduard, J. Cunilleras.;Kenneth ,W.Hinchcliff.;Todd, C.Holbrook.;Christopher, R.;Mark, E.Payton.;Kathy, W.;Stuart ,N.;Michael ,D.Willard ,and Michael, S .Davis.(2007).**Serum chemistry alteration in Alaskan sled dogs during five successive days of prolonged endurance exercise. Scientific report .J. Am. Vet .Med .Assoc, 230:1486-1492.

**Eshratkhah, B.; Sadaghian, M.; Khajeye, M.; Ahmadi, H, and Mostafavi, H. (2008).** Evaluation of non-electrolytes normal values in blood of Makuei sheep breed. *J. of Anim. Vet and Adv*, 7:327–329.

**Falcone, F.; Haas, H, and Gibbs, B. ( 2000).** The human basophil: a new appreciation of its role in immune responses. *J. of .Am. Soci. of .Hem.* 96 (13): 4028–4038.

**Feldman,B.R.;Zinkl, J.G, and Jain ,N.C(2000).** In *Shalm's veterinary hematology* 5<sup>th</sup> ed, Lippincott, Williams and Wilkins, Baltimore, Maryland 1344.

**Figueiredo, A.P.M.; Medeiros, R.M.T.; Dantas, F.P.M.; Leite, A.L.D.; Figuera, R.A, and Riet-Correa, F. (2012).** Experimental poisoning by *Indigofera suffruticosa* in goat and sheep. *Pesq. Vet. Bras* 32: 126-130.

**Fischbach, F. Talaska, and Dunning, M. Barnett.(2008).** A manual of laboratory and diagnostic tests <sup>8th</sup> ed. Lippincott Williams and Wilkins.p.116.Canada.

**Funda .Ö, and Kerem .U.(2014).** Thrombocyte indices in dogs infected with *Ehrlichia canis* and *Anaplasma phagocytophilum*. *Revista MVZ Cordoba* .19 :3 Córdoba .

**Furie ,B, andFurie,B.C.(2008).** Mechanisms of thrombus formation. *New England J . Medic.* 359 (9): 938–49.

**Ganji,V, and Kafai, M.R.(2009).** Hemoglobin and hematocrit values are higher and prevalence of anemia is lower in the post-folic acid fortification period than in the pre-folic acid fortification period in US adults. *Am. J. Clin. Nutr.* 89 (3):63–71.

**Giacomoini, A.; Legovivi ,P, and Gessoni ,G.(2001)** Platelet count and parameters determined by the Bayer ADVIA TM 120 in reference subjects and patients. *Clin .Lab. Hematol*;23:181-186.

**Giber, U.; Harvey, J. W.; Yamaguchi, R. A. et al (1985).** Inherited phosphor fructo kinase deficiency in dogs with hyperventilation induced hemolysis: increased invivo alkaline fragility of erythrocytes. *Blood*, 65:354-351.

**Gina, G. Wardyn.; Stephen, I. Rennard.; Susan, K. Brusnhan.; Timothy, R. McGuire.; Mary, L. Carlson.; Lynette, M. Smith.; Sandra, McGranaghan, and John, G. Sharp. (2008).** Effect of exercise on hematological parameters, circulating side population cells, and Cytokines. *Exp. Hematol*, 36:216-223.

**Girotti, A. W. (1985).** Mechanisms of lipid peroxidation. *J. Free Radic. Biol Med*, 1(2):87-95.

**Gleeson, M.; Blannin, A. K.; Sewell, D. A, and Cave, R. (1995).** Short term changes in the blood leukocyte and platelet count following different durations of high-intensity treadmill running. *J. Sport. Sci*, 13:115-123.

**Gomez, K. A and Gomez, A. A. (1984).** Statistical procedure for Agricultural research, 2<sup>nd</sup> ed. Wily and Sons, Inc. Toronto, Canada.

**Goodman, S. R.; Kurdia, A.; Ammann, L.; Kakhniashvili, D, and Daescu, O. (2007).** The human red blood cell proteome and interactome. *Exp Biol and Med*. 232 (11): 1391–408.

**Gracner, D.; Bedrica, L.; Labura, C.; Maticic, D.; Gracner, G. G. and Samardzija, M. (2007).** Blood Groups and Hematology. In *Istrian Pointers .Vet .Arhiv*, 77:95-102.

**Greer, P. John. (2008).** Wintrobe's clinical hematology. Lippincott Williams and Wilkins. P.805. Philadelphia, USA.

**Gregory, T. R. (2001).** The bigger the C-value, the larger the cell: genome size and red blood cell size in vertebrates. *Blood Cells, Mol and Dis*. 27 (5): 830–43.



**Guyton, A.C, and Hall, J.E.** (2006). Textbook Of Medical physiology. 11<sup>th</sup> ed: Elsevier Saunders. 32:401-428. Philadelphia, Pennsylvania.

**Harewood, W.J.; Gillin, A.; Hennessy, A.; Armistead, J.; Horvath, J.S, and Tiller, D.J.** (1999). Biochemistry and haematology values for the baboon (*Papio hamadryas*): the effects of sex, growth, development and age. J. Med. Primatol, 28:19–31.

**Heit, J.A.** (2007). Thrombophilia: common questions on laboratory assessment and management. Am. Soc. Hematol. Educ. Program, 1: 127–135.

**Hellman, P.E.; Scott, N.R, and Tienhoven, A.V.** (1985). Physiological responses and adaptations to hot and cold environmental In: Yousef, M.K, (Ed), stress physiology in live stock. Vol.3-CRS Press Boca Raton, Florida: 1-71.

**Hinchcliff, K.W.; Olson, J.; Crusberg, C.; Kenyon, J.; Long, R.; Royale,; Weber, W, and Burr, J.** (1993). Serum biochemical changes in dogs competing in a long-distance sled race. J. Am. Vet Med. Assoc, 202:401-405.

**Howell, S.; Hoffman, K.; Bartel, L.; Schwandt, M.; Morris, J, and Fritz, J.** (2003). Normal hematologic and serum clinical chemistry values for captive chimpanzees (*Pan troglodytes*). Comp Med, 53:413–23.

**Hussein, M. ; Aljumaah, R.; Alhaidary, A.; Alshaikh, M.; Mohammed, O.; Omer, S.; Gar-Elnabi, A, and Macasero, W.** (2010). Platelets indices of the dromedary camel (*camelus dromedaries*). Vet. Archive 80 (3), 375-382.

**Ilkiw, J.E.; Davis, P.E., and Church, D.B. (1989).** Hematologic, biochemical, blood gas and acid-base values in greyhounds before and after exercise. *Am. J. of. Vet. Resch*, 50:583-586.

**Jacquelyn, M. Wahl; Stephanie, M. Herbst; Leigh, A. Clark; Kate, L. Tsai; Keith, E. Murphy. (2008)** .A review of hereditary diseases of the German shepherd dog. *J. of Vet. Behav*( 3), 255-265.

**Jain, A. K. (2007).** Textbook of Physiology , 3<sup>rd</sup> edition. Lea and Febiger, Philadelphia.

**Jain, N.C. (1986a).** Normal values in blood of laboratory, fur-bearing and miscellaneous zoo, domestic, and wild animals. In Schalm's *Vet. Hemato* 4<sup>th</sup> ed, Lea and Febiger, Philadelphia , 274-349.

**Jain, N.C. (1986 b).** Hematology techniques .In Shalm's *vet hemato*. 4<sup>th</sup> ed: Lea and Febiger, , Philadelphia, 64-71.

**Jain, N.C. (1993).** Comparative hematologic features of some avian and mammalian species .*Essentials of veterinary hematology*. 1<sup>st</sup> ed. Lea and Febiger, Philadelphia, 19-53.

**Janeway, C.; Travers, P.; Walport, M., and Shlomchik, M. (2001)** *Immunobiology* 5<sup>th</sup> ed. New York and London: Garland Science.

**John, P. Greer, and Maxwell M. Wintrobe. ( 2008).** *Hematology method in Wintrobe's clinical hematology* 12<sup>th</sup> ed. Lippincott Williams & Wilkins. Philadelphia 173.

**Kane, J.D.; Steinbach, T.J.; Sturdivant, R.X., and Burks, R.E. (2012).** Sex-associated effects on hematologic and serum chemistry analytes in sand rats (*Psammodromus obesus*). *J. Am. Assoc Lab. Anim. Sci*; 51:769–74.

**Kenneth ,K. (2010).** Williams hematology 8<sup>th</sup> ed. New York: McGraw-Hill Medical.

**Khan,S.A.;Epstein,J.H.;Olival,K.J.;Hassan,M.M.;Hossain,M.B.; Rahman,K.B.M.A.;Elahi,M.F.;Mamun,M.A.;Haider,N.;Yassin, G,and Desmond,J.(2011).** Hematology and serum chemistry reference values of stray dogs in Bangladesh.Open vet.J.1:13-20.

**Koubkova,M.I.;Knizkova,I.;Kunc,P.;Hartlova,H.;Fluser,J,AND Dolezal,O.(2002).** Influence of high environmental temperatures and evaporative cooling on some physiological, haematological and biochemical parameters in high-yielding dairy cows. Czech J Anim Sci,47:309-318.

**Kumar,B and Pachauri, S.P.(2000).**Hematological profile of crossbred dairy cattle to monitor herd health status at medium elevation in central Himalaya. Res Vet Sci, 69:141-145.

**LaFleur-Brooks, M. (2008).** Exploring Medical Language: A Student-Directed Approach 7<sup>th</sup> ed. St. Louis, Missouri, US: Mosby Elsevier. P. 398.

**Lassen ,E.D.;Craig, A.H,and Blythe, L.L.(1986).**Effect of racing on hematological and serum biochemical values in greyhounds.J.of Am. Vet. Med. Assoc,188:1299-1303.

**Lawrie, D.;Coetzee, L.M.;Becker, P.;Mahlangu, J.; Stevens, W, and Glencross ,D.K. (2009).**Local reference ranges for full blood count and CD4 lymphocyte count testing.S. Afr .Med J;99:243-248.

**Le, Tao.; Bhushan, V,and Vasan, N. (2010).** First Aid for the USMLE Step 20<sup>th</sup> Anni ed.: The McGraw-Hill Companies, USA 124 .

**Lewis, S.M.;Bain, B.J, and B,J. I. (2006).**Dacie and Lewis practical Hematology10<sup>th</sup> ed:Churchill Livingstone, Pheladelphia.

**Lund, C.; Kuhl, S.; Mischke, R, and Gunzel-Apel, A.R.(2000).** Reference values of the red blood profile in Beagles, German shepherd and Golden retriever puppies. Berl Munch Tierarztl Wochenschr, 113; 447-453.

**Lysann ,S,and Reinhard ,M. (2016).** Platelet variables in healthy dogs: reference intervals and influence of age, breed and sex.Compar Clini, 25 (6): 1097-1106.

**MacAdam,W.R,and Eberhart, R.J.(1972).**Diurnal variation in plasma corticosteroid concentration in dairy cattle. J of Dairy Sci, 55:1792-1795.

**Maton, D.; Hopkins, J.; McLaughlin, C.h. W.; Johnson, S.; Warner, M. Q.; LaHart, D, and Wright, J. D, Deep V. Kulkarni (1997).** Human Biology and Health. Englewood Cliffs, New Jersey, US: Prentice Hall.

**Mazepa ,A.W.; Kidd, L.B.;Young ,K.M,and Trepanier, L.A. (2010).** Clinical presentation of 26 *Anaplasma phagocytophilum* seropositive dogs residing in an endemic area. J .Am .Anim .Hosp Assoc; 46(6):405-412.

**McGregor, B.A.;Murphy ,K.M.;Albano, D.L,andCeballos, R.M.(2016).** Stress, Cortisol, and B-Lymphocytes: A Novel Approach to Understanding Academic Stress and Immune Function.PMC, 19(2):185-191.

**McKenzie, E.C.;Jose-Cunilleras, E.;Hinchcliff, K.W.;Holbrook T.C.;Royer. C.;Patyon, M.E.;Williamson. K.;Nelson, S.;Willard M.D,and Davis, M.S(2007).**Serum chemistry alternations in Alaskan sled dogs during five successive days of prolonged endurance exercise. J. of. Am. Vet .Med .Assoc,230:1486-1492.

**Melanie, J. Percy.;Quan ,Z.; Adrian, F.;Claire, H.;Terence, R. J. Lappin.;Patrick ,H. Maxwell.; Mary ,F. McMullin, and Frank, S. Lee.(2006).** A family with erythrocytosis establishes a role for prolyl hydroxylase domain protein 2 in oxygen homeostasis.Proc.Natl.Acad.Sci.U.S.A,103(3):654-659.

**Michaelson, S.M.; Scheer ,K,and Gilt, S.(1966).** The blood of the normal beagle. J. Am .Vet Med. Assoc;148:532–534.

**Miller, A.; Korem, M.; Almog, R.,and Galboiz, Y. (2005).** Vitamin B<sub>12</sub>, demyelination, remyelination and repair in multiple sclerosis. J. of .Neuro .Sci. 233 (1–2): 93–97.

**Mirghani .T.(1982).**Effect of fasting on camel serum lipids. Sudan J.Vet.Sci.Anim.Husb, 23:73-76.

**Mirzadeh,K.;Tabatabaei,S.;Bojarpour,M,and Mamoei,M.(2010).** Comparative study of Haematological parameters according strain,age,sex,physiological status and season in Iranian cattle.J Anim .Vet. Adv,9(16):2123-2127.

**Mohammed,A.K.;Mohammed,G,andAkerejola,O.O.(2007).** Hemato logical and serum biochemical changes in Bunaji work bulls after farm land ridging exercise in Kuduna State,Nigeria.J Anim Vet Adv,6:576-579.

**Nazifi, S.;Saeb, M.; Rowghani, E.; Hasankhani, M.; Hasankhani, F,and Ghafari, N. (2007) .**Studies on the relationship between thyroid hormones, serum lipid profile and erythrocyte antioxidant enzymes in clinically healthy Iranian fat-tailed sheep. Bulgar J of Vet Medi,10: 161-167.

**Nazifi,S.;Gheisari,H.R,and Poorabbas,H.(1999).**The influence of thermal stress on serum biochemical parameters of dromedary camels and their correlation with thyroid activity. *Comp inter hematol*, 9:49-53.

**Oyewale,J.T.;Dzenda,L.;Yaqub,D.;Akanbi,J.;Ayo,O.;Owoyele,N ,and Minka,T.Dare.(2011).**Alternation in the osmotic fragility of camel and donkey erythrocytes caused by temperature,PH and blood storage. *Vet.arhiv* 81,459-470.

**Ozkan ,S.;Miyase C,and Sevket, A.(2015).**Changes in selected hematology and serum biochemistry in Turkish Angora Cats(*Felis catus*) during growth period.*J.Adv.Vet.Anim.Res.*2(1):34-39.

**Parpart, A.K.; Lorenz, P.B,and Parpart, E.R. (1947) .**The osmotic resistance (fragility) of human red cells. *J. Clini .Investigation*, 26: 636-640.

**Peinado ,V.I.;Celdra, J.F,and Palomeque, J.(1999).**Basic hematological values in some wild ruminants in captivity. *Comp Biochem. physiol A*, 124:199-203.

**Perk, K.; Frei, Y.F,and Herz. A. (1964) .**Osmotic fragility of red blood cells of young and mature domestic and laboratory animals. *Am J Vet Resea*, 25: 1241-1248.

**Pernille, J. Vinholt.; Anne-Mette, H,and Mads, N.(2014).** An overview of platelet indices and methods for evaluating platelet function in thrombocytopenic patients. *Europ J. Hemat* 92: 367–376.

**Pillay, J.; Den Braber, I.; Vrisekoop, N.; Kwast, L. M.; De Boer, R. J.; Borghans, J. A. M.; Tesselaar, K,and Koenderman, L. (2010).** In vivo labeling with  $^2\text{H}_2\text{O}$  reveals a human neutrophil life span of 5.4 days. *Blood*. 116 (4): 625–7.

- Punnet, S.;Gagandeep, K.;Sabita, B, and Anita, T. (2014).** Assessment of factors affecting the platelet yield using continuous flow cell separator. *Inter.J of .Biomed. Resea .No 0976-9633*(online).
- Ravi, P. Sama.(1990).** Red cell indices (152) *Clinical methods: the history, physical and laboratory examinations 3<sup>rd</sup> ed.* Butterworths. Boston.
- Rich, P. R. (2003).**The molecular machinery of Keilin's respiratory chain. *Bioch. Soci. Transactions.* 31 (6):1095–1105.
- Rita, P.; Raffaella, P.;Agatina ,A. Liotta,and Rosanna,A.(2015).** Platelet function test:a comparative test. *Vasc .Health. Risk Manag.* 11: 133–148.
- Robson, P.J.;Blannin, A.K.; Walsh, N.P.; Castell, L.M,and Gleeson, M. (1999).** Effects of exercise intensity, duration and recovery on invitro neutrophil function in male athletes. *Inter J of Sports Med.* 20 (2): 128–135.
- Robert, L.Hall ,and Nancy, E.Everds.(2003).**Factors affecting the interpretation of canine non human primate clinical pathology. *Toxic pathol,*31(1):6-10.
- Rodak,B. F .(2007).***Hematology :clinical principles and applications 3<sup>rd</sup> ed.* Elsevier Health Sciences .p.291.USA.
- Rovira, S.; Munoz ,A,and Benito, M.(2008).**Effect of exercise on physiological, blood and endocrine parameters in search and rescue-trained dogs. *Vet Med*53(6):333-346.
- Rovira, S.;Munoz, A,and Benito, M.(2007a).**Fluid and electrolyte shifts during and after agility competitions in dog. *Vet Med Sci,*69:32-35.

**Rovira, S.; Munoz, A, and Benito, M.(2007b).**Hematologic and biochemical changes during canine agility competitions. Vet Clin pathol, 36:30-35.

**Rovira, S.; Munoz ,A, and Benito ,M. (2007).** Hematologic and biochemical changes during canine agility competitions. Vet Clin Pathol. 36: 30-35.

**Saladin, K. (2012).** Anatomy and Physiology: the Unit of Form and Function 6<sup>th</sup> ed. McGraw Hill. New York.

**Sandabe, U.K.; Chaudhary ,S.U.(2000).**Effect of environmental temperature on some biochemical values in female Sahel goats.Pak Vet J,20(1):10-12.

**Saror,D.; Coles,E.H.(1973).**The blood picture of White Fulani (Zebu)and White Fulani/Fresian(cross breed)dairy cow.Bulli of epizo disea of Afri,21:485-487.

**Schalm,O.W.; Jain ,N.C, and Carrol, E.J. (1975).**Schalm's veterinary hematology 3<sup>rd</sup> ed .Lea and Febger,philadelphia.

**Schwartz,D.L.; Sharkey ,P.J. ;Armstrong,C, and Kundson, K.(2014).** Platelet volume and plateletcrit in dogs with presumed primary immune-mediated thrombocytopenia.J. of Vet. Inte. Medi,28:1575-1579.

**Shadia ,A.Omer. (2009).**Normal values of some serological parameters in male and female German shepherd dogs in Sudan.Assiut Vet. Med.J, 55:120.

**Sherwood, L.; Klansman, H, and Yancey, P.(2005).**Animal Physiology from genes to organisms 2<sup>th</sup> ed . Brooks/Cole, Cengage Learning.USA.

**Shiela, R.E.; Brennan, S.F.; O'Rourke, L.G.; McCullough, M, and Mooney, C.T. (2007).** Hematologic values in young pre training healthy Greyhounds. Vet. Clin. Pathol. 36(3): 274-277.



**Snow, D.H.;Harris, R.C,and Stuttard, E.(1988).**Changes in hematology and plasma biochemistry during maximal exercise in greyhounds. *Vet Rec*,123(19):487-489.

**Stefania,C.;Salvator,S.;Alessandro,Z.;Vincenzo ,M.;Francesco ,F, and Giuseppe, P.2013).** Effect of seasonal variation in Mediterranean area on haematological profile in dairy cow. *Comp Clin pathol*,22:691-695.

**Suntorn ,W.;Thongluan, N,and Charoen, S.(2009).**A study on feed management, feed nutritive values and blood metabolites of pony horses in different seasons northern Thailand. International conference on the role of Universities in Hands-On Education Rajamangala University of Technology Lanna, Chiang-Mai, Thailand, pp.23-29.

**Swirski, F.K.; Nahrendorf, M.; Etzrodt, M.; Wildgruber, M.; Cortez-Retamozo, V.; Panizzi, P.; Figueiredo, J-L.; Kohler, R.H.; Chudnovskiy, A.; Waterman, P.; Aikawa, E.; Mempel, T.R.; Libby, P.; Weissleder, R,and Pittet, M.J(2009).** Identification of Splenic Reservoir Monocytes and their Deployment to Inflamm.Sites. *Sci.* 325 (5940): 612–616.

**Testa, U (2004).** Apoptotic mechanisms in the control of erythropoiesis. *Leukemia* 18: 1176-1199.

**Uzoigwe, C. (2006).**The human erythrocyte has developed the biconcave disc shape to optimise the flow properties of the blood in the large vessels. *Med .Hypoth.* 67 (5): 1159–1163.

**Vinay ,K.; Abul, K.; Abbas ,N.;Fausto, R,and Mitchell, N. (2007).** Lysis of dogs erythrocytes in mildly alkaline isotonic media. In *Robbins Basic Pathology* 8<sup>th</sup> ed. Saunders. Elsevier, USA186:339-342.

**Weber, R.E, and Vinogradove, S.N. (2001).**Nonvertebrate hemoglobins: function and molecular adaptations.Physiol Rev.81 (2):569-628.

**Wegner, T.N, and Stott, G.H.(1972).**Serum minerals, leukocyte profile, and plasma corticoids in dairy heifers after an injection of corticotropin.J. Of dairy sci, 55:1464-1469.

**Wheater, P. R,and Stevens, Alan. (2002).** Basic histopathology 4<sup>th</sup>ed Churchill Livingstone. Edinburgh.

**Wintrobres. (2009).** Clinical Hematology. 12<sup>th</sup> ed Vol 1, Lippincott Williams & Wilkins, Philadelphia.

**Wirz, A.; Truppa ,V,and Riviello ,M.C.(2008).** Hematological and plasma biochemical values for captive tufted capuchin monkeys (*Cebus apella*). Am J Primatol, 70:463–72.

**Wiwanitkit ,V.(2004 ).** Plateletcrit, mean platelet volume, platelet distribution width: its expected values and correlation with parallel red blood cell parameters.Clin and Appl Thrombo ,10(2)175-178.

**Wlodzimierz, J.; Claudia, G.; Ettore ,R.; Danika ,B.; Alan, W.;; Jeremy ,S.; Yip ,R.;Johnson, C, and Dallman ,P.R.(1984).** Age-related changes in laboratory values used in the diagnosis of anemia and iron deficiency. Am. J. Clin. Nutr; 39:427–436.

**Yokus ,B.;Cakir, D.U.;Kanay, Z.;Gulten ,T.;Uysal, E.(2006).** Effect of Seasonal and physiological variations on the serum Chemistry,Vitamins and Thyroid Hormone Concentrations in Sheep.J. Of Vet. Med, 53:271-276.