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Measurement of Prothrombin Time, Activated Partial Thromboplastin Time, Fibrinogen Concentration, Platelets count and indices in healthy police dogs in Sudan

قياس زمن البروثرومبين ، زمن الثرومبوبلاستين المنشط الجزئ، تركيز الفيبرينوجين، عدد الصفائح الدموية ومؤشراتها في الكلاب الشرطية السليمة في السودان

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By :Abdullah Abdulslam Abdullah Mohammed

B.V.M. (2014), University of AL- Gezira

Supervisor: Professor / Shadia Abdelatti Omer Mohamed

Department of Biomedical sciences, College of Veterinary Medicine,

Sudan University of science and Technology

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DEDICATION

To the soul of my teacher "Abuagula Yousif"

To my mother (Mona) who surrounded me with love and care.

To my brother and sister for their encouragement and support.

To all my friends….

To all whom I love….

I dedicate this work with great love.

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List of Abbreviations

Abbreviation	Meaning of Abbreviation
ADP	Adenosine Di Phosphate
APTT	Activated partial thromboplastin time
Ca	Calcium
$Cacl_2$	Calcium chloride
DD	D-dimer
DIC	Disseminated intravascular coagulation
EDTA-K_3	Tri-potassium - Ethylene diamine tetra acetate
FDPs	Fibrin Degradation Products
FIB	Fibrinogen concentration
FIB_{Clauss}	Fibrinogen concentration Clauss method
FIB_{PT}	Fibrinogen concentration Prothrombin method
GDV	Gastric dilatation and volvulus syndrome
GM – CSF	Granulocyte -monocyte colony stimulating factor
GP	Glycoprotein
HAC	Hyperadrenocorticism
IL	Interleukin
MPC	Mean platelet component
MPM	Mean platelet mass
MPV	Mean platelet volume
PCDW	Platelet component distribution width
PCT	Plateletcrit
PDW	Platelet distribution width
PL	Phospholipids
PLT	Platelet count
PMDW	Platelet mass distribution width
PT	Prothrombin time
RPM	Round per minutes
S.D	Standard deviation
Sec	Second
SPSS	Statistical Package of Social Science

TF	Tissue Factor
t-PA	Tissue Plasminogen Activator
TT	Thrombin time test
TXA2	Thromboxane A2
U-PA	Urokinase Plasminogen Activator
USA	United states of America
vWF	Von Willebrand Factor

ABSTRACT

The study aimed to determine the normal values of Prothrombin Time (PT) Activated Partial Thromboplastin Time (APTT), Fibrinogen Concentration (FIB), platelets count (PLT), mean platelets volume (MPV), plateletcrit (PCT) and platelet distribution width (PDW) for healthy police dogs in Sudan and to assess the effect of breed and sex on these parameters. Forty six dogs were used; 20 *Labrador Retriever* and 26 *German Shepherd* dogs comprising 21 males and 25 females, 20-83 months old. Blood samples were collected from the cephalic vein. The PT, APTT and FIB were measured in paired citrated plasma by a semi automatic BCA-2000-LED-light coagulometer using Spinreac reagents kits for human medicine. Platelets parameters were determined in whole blood, containing (EDTA- K_3), using BK6100 Auto Hematology Analyzer. Differences in mean values between groups were detected by student's t-test and Spearman correlation was used to determine correlations between platelet count and platelets indices using statistical package for social science (SPSS) version 16 for Statistical analysis.

The overall mean values for PT, APTT and FIB were 7.35±0.91second, 15.12±1.32 second and 341.54±55.16 mg/dl respectively. *German Shepherd* dogs showed significantly (P≤0.05) higher values than *Labrador Retriever* dogs for PT (7.58±1.01 vs. 7.06±0.67 seconds), and APTT (15.28 ±1.58 vs. 14.9±0.85 seconds), respectively. Males showed significantly (P≤0.05) higher values for FIB than the females (346.30±38.75 vs. 337.73± 65.99 mg/dl).

The overall mean values for PLT, MPV, PCT and PDW were: 172.022±52.72 $\times 10^9$/L, 10.2±0.76 FL, 0.173±0.057 L/L and 11.16±0.31% respectively. *German Shepherd* dogs showed significantly (P≤0.05) higher values for MPV than *Labrador Retriever* dogs (10.43±0.71vs. 9.9±0.73 FL),*German Shepherd* females showed significantly (P≤0.05) higher values for MPV than *German Shepherd* males dogs (10.72±0.69 vs.10.13±0.62 FL)**,** and also *Labrador Retriever* males showed significantly (P≤0.05) higher values than *Labrador Retriever* females dogs for MPV(10.41±0.71 vs. 9.55±0.53 FL) and PDW(11.51±0.43vs. 11.07±0.2%), respectively.

Highly positive significant (P≤0.01) correlations were found between PLT and PCT in males**,** females and all the dogs, and between MPV and PDW in all the dogs.

A highly positive significant ($P \leq 0.01$) correlation was found between MPV and PCT in all the dogs.

It is concluded that breed and gender may influence coagulation parameters, platelets count and indices and this should be considered in clinical interpretations. Future studies are recommended using different breeds of dogs to establish wide data base for coagulation parameters.

ملخص الدراسة

هدفت هذه الدراسة لتحديد المعدل الطبيعي لزمن البروثرومبين ، زمن تنشيط الثرومبوبلاستين الجزئ ، تزكيز الفبرينوجين، العد الكلي للصفائح الدموية ، متوسط حجم الصفيحة الدموية، مكداس الصفائح الدموية وعرض توزيع الصفائح الدموية في الكلاب الشرطية السليمة في السودان ، و لتقييم آثار السلالة والجنس على هذه المقاييس .ستة وأربعين كلب أستخدمت في هذ الدراسة ٢٠من كلاب اللابرادورالكندي و٢٦ من كلاب الراعي الألماني تضم ٢١ من الذكور و ٢٥من الإناث تتراوح أعمارهم بين ٢٠-٨٣شهراً .تم جمع عينة دم من الوريد الساعدي .تم قياس مقاييس تجلط الدم في البلازما المقترنة بالسترات باستخدام جهاز semiautomatic BCA-2000-LED-lightcoagulometer-with-Double-channel ومجموعات الكواشف Spinreac للطب البشري . معاملات الصفائح الدموية، تم تحليلها في عينة الدم الكاملة (تحتوي علي $EDTA-K_3$) باستخدام جهاز BK6100 Auto Hematology Analyzer. تم فحص الفروقات بين متوسطات المجموعات ، بإستخدام إختبارT للطالب ، قيس الإرتباط بإستخدام إختبار سبيرمان لمعرفة الإرتباط بين عد الصفائح الدموية ومقاييس الصفائح الدموية،تم تحليل هذة الإختبارات السابقة بإستخدام الحزم الإحصائية للعلوم الإجتماعية(SPSS) نسخة ١٦ للتحليل الإحصائي.

عموماً كانت قيم المتوسطات لكل من زمن تنشيط الثرومبوبلاستين الجزئ ,زمن البروثرومبين وتركيز الفيبرينوجين 15.12±1.32 ثانية 7.35±0.91 ,ثانية, 341.54±55.16 ملجرام/ديسليتر على التوالي .أظهرت كلاب الراعي الالماني زيادة معنوية ($P\leq0.05$) عن كلاب اللابرادور في قيم زمن البروثرومبين (7.58±1.01 vs 7.06±0.67 ثانية) ، و زمن تنشيط الثرومبوبلاستين الجزئي(15.28 ±1.58 vs. 14.9±0.85 ثانية) على التوالي.أظهرت الذكور زيادة معنوية ($P\leq0.05$) في قيم تركيز الفيبرينوجين عنها في الاناث (346.30±38.75 vs. 337.73± 65.99 ملي جرام / ديسيلتر) على التوالي.
عموماً كانت قيم المتوسطات لكل من العد الكلي للصفائح الدموية(10^9/L×)، متوسط حجم الصفيحة الدموية(FL)، مكداس الصفائح الدموية(L/L) وعرض توزيع الصفائح الدموية % 172.022±52.72, 10.2±0.76، 0.173±0.057 و11.16±0.31.

أظهرت كلاب الراعي الالماني زيادة معنوية ($P\leq0.05$) عن كلاب اللابرادور بالنسبة لمتوسط حجم الصفائح الدموية (9.9±0.73 FL vs. 10.43±0.71)،إيضاً أظهرت إناث كلاب الراعي

الالماني زيادة معنوية ($P\leq0.05$) عن ذكور كلاب الراعي الالماني بالنسبة لمتوسط حجم الصفائح الدموية(10.72±0.69 vs.10.13±0.62 FL) .

أظهرت ذكور كلاب اللابرادور زيادة معنوية ($P\leq0.05$) عن إناث كلاب اللابرادور في قيم لمتوسط حجم الصفائح الدموية (10.41±0.71 vs. 9.55±0.53 FL) ، وعرض توزيع الصفائح الدموية (11.51±0.43vs. 11.07±0.2%) على التوالي. وجد أن هنالك إرتباط عالي معنوي موجب ($P\leq0.01$) بين العد الكلي للصفائح الدموية ومكداس الصفائح الدموية في الذكور ،الإناث و المجموعة الكلية للكلاب وايضاً بين متوسط حجم الصفيحة الدموية وعرض توزيع الصفائح الدموية في المجموعة الكلية للكلاب. يوجد إرتباط عالي معنوي موجب ($P\leq0.01$) بين متوسط حجم الصفيحة الدموية و ومكداس الصفائح الدموية في المجموعة الكلية للكلاب.

يستخلص من هذة الدراسة أنه السلالة والجنس يمكن أن يؤثر علي لإختبارات التجلط و عد ومقاييس الصفائح الدموية وهذا يجب أن يوخذ في الحسبان عند التفسير السريري للنتائج. الدراسات المستقبلية توصي بأن تشمل السلالات المختلفة من الكلاب لوضع قاعدة بيانات واسعة لجميع معلمات التخثر.

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INTRODUCTION

The role of dogs has been progressively emphasized as a companion animal for security purpose, drug trafficking and even warfare.

Some breeds have been adapted and trained to work in police operation to detect and prevent crimes, detect narcotic drugs and explosives by virtue of their intelligence and strong sense of smell (Eatzaz, 2006).

In such condition dogs may be over worked and subjected to a lot of stress and health problems. So there animals should receive proper medical care to ensure that they are free of diseases (Suljević *et al.*, 2016).

Blood is one of the important body fluids for assessment of the health status of animals; it is a mirror of the body which reflects the internal body environmental status.

In dogs like in other animals, blood tests are also used to determine and evaluate disease state, drug effectiveness and organ function. Age, sex, breed and some environmental factors may also affect the normal blood parameters (Larica, 2016).

Dogs are the most studied animals prone to clotting defects , this is attributed to many reasons, including the hereditary deficiency of some coagulation factors such as, fibrinogen, VII, VIII(classic haemophilia), IX(haemopilia B), and X (Kerr, 2008).

In pet clinics, dogs are sometimes brought with bleeding disorder of unknown reasons creating a problem for clinical practitioners. So the examination of clotting factors and platelets indices and knowledge of normal haematological values is of great importance to veterinarians to evaluate and treat such unknown conditions (Sumathi *et al.*, 2012).

Research objectives:

General objective:

-To measure the normal values of some coagulation parameters, platelets count and Platelets indices in healthy police dogs in Sudan.

Specific Objectives:

1- To establish normal values for **PT, APTT** and **FIB** in healthy police dogs.

2- To determine the normal values for **PLT, MPV, PCT** and **PDW** in healthy police dogs and correlate them with **PLT**.

3- To investigate the effect of breed, sex and age on level of the parameters investigated.

CHAPTER ONE

LITERATURE REVIEW

1.1. Domestic dog:

Domestic dog (Canis familiaris)**:** it is believed that the domestic dogs has been domestication from wolves about 14000-15000 years ago (Vila *et al.,* 1997). Since this long history, dogs have played an important role in human civilization, spearhead by many tasks developed over time. On the basis of the role they play in human life dogs are grouped into the following categories (Vila *et al.,* 1997):-

1.1.1. The shepherd dogs: which are commonly used in police services in most of the countries, examples, *German Shepherd* dog.

1.1.2. The hunting dogs: which are used in hunting, examples, *Saluki* dog.

1.1.3. The working dogs: Which are commonly used in work such as grazing cattle, caring for the elderly and the disabled people, examples, *Labrador Retriever* dog.

1.1.4. The toy dogs: which are used to entertain and accompany people, examples, *Maltese lion* puppy. (Vila *et al.,* 1997).

1.2. Police Dogs in Sudan:

The first unit of police dogs in the Sudan was established in 1967 in Burry town Khartoum State (Ministry of Interior Presidency of the Police Forces Criminal Evidence "MIPPFCE" 2017), and the preferred breeds of dogs used are the *German Shepherd, Belgian Shepherd, Dutch Shepherd* and *Labrador Retriever* (MIPPFCE 2017).

In this study we focused on *German Shepherd* and *Labrador Retriever*.

1.2.1. The *German Shepherd* dog: The methodical breeding was started in 1899.

It has been bred from the central German and southern German breeds of the herding dogs existing at that time with the ultimate objective of creating a working dog inclined to high achievement. In order to achieve this objective, the breed standard of the *German Shepherd* dog was determined، this relates to the physical constitution as well as the traits and characteristics (Gaunet, 2010).

The desired height for males at the top of the highest point of the shoulder blade is 24 to 26 inches; and for bitches, 22 to 24 inches. The *German Shepherd* dog is longer than tall, with the most desirable proportion as 10 to 8½. The length is measured from the point of the prosternum or breastbone to the rear edge of the pelvis, the ischial

tuberosity. The desirable long proportion is not derived from a long back, but from overall length with relation to height, which is achieved by length of forequarter and length of withers and hindquarter, viewed from the side (Ensminger, 2011).

1.2.2. The *Labrador Retriever* dog: Originally came from Newfoundland, Canada, and was known as the "St. John's Dog", "St. John's Newfoundland", or "Lesser Newfoundland". Although there are several different accounts of the early history of the breed, it is most widely speculated that the Labrador's origins can be traced to the Greater Newfoundland dog or to the French St. Hubert's dog (Gaunet, 2010).
The use of the *Labrador Retriever* dog in police work began in 1946 in London (Ensminger, 2011).

For dogs above on year the height at the withers for a male dog is 22½ to 24½ inches; for a bitch is 21½ to 23½ inches. Approximate weight of dogs in working condition: males dogs 65 to 80 pounds; bitches 55 to 70 pounds. Length from the point of the shoulder to the point of the rump is equal to or slightly longer than the distance from the withers to the ground. The distance from the elbow to the ground equals to one half of the height at the withers. The brisket extends to the elbows. The body of sufficient length to permit a straight, free and efficient strides; the dog appear low and long or tall and leggy in outline. Labrador Retrievers in working condition are well-muscled and without excess fat (Ensminger, 2011).

1.3. Physiology of haemostasis:

Haemostasis is the mechanism to control blood loss arising from vascular or tissue trauma and fibrinolysis ,it is initiated with the activation of coagulation and is a part of this mechanism. The haemostatic system is a finely balanced mechanism (Elif, 2012).

Traditionally, the haemostatic system has been divided into 3 stages: primary haemostasis, secondary haemostasis and tertiary haemostasis, including fibrinolysis and natural anticoagulants (Hoffbrand and Moss , 2015).

1.3.1. Primary haemostasis:

The activation of coagulation cascade begins with vascular endothelial damage, which leads to the contact between sub endothelial matrix, platelets and coagulation factors. The primary haemostasis process includes vascular response (vasoconstriction) and platelet response (Hall, 2016).

1.3.1.1. Vasoconstriction:

When a blood vessel is damaged an immediate vasoconstriction reaction takes place to avoid extensive bleeding and to slow the blood flow to the area of injury; these reactions include constriction of adjacent small arteries and arterioles. The reduction of blood flow allows contact activation of platelets and coagulation factors. The vasoactive amines and thromboxane A2 liberated from platelets, and the fibrin peptides liberated during fibrin formation, also have vasoconstrictive activity (Hoffbrand and Moss ,2015)

1.3.1.2. Platelets: Platelets (Thrombocytes) are a component of blood whose function (along with the coagulation factors) is to stop bleeding by clumping and clotting blood vessel injuries (Hall, 2016).Platelets are produced in the bone marrow by fragmentation of the cytoplasm of megakaryocytes, one of the largest cells in the body. The precursors of the megakaryocytes are the megakaryoblast which arises by a differentiation from the haemopoietic stem cell (Hoffbrand and Moss ,2015).

When megakaryocytes are stimulated by factors such as thrombopoietin, stem cell factor, interleukin (IL) - 3, IL - 6, IL - 11, IL - 12, granulocyte -monocyte colony stimulating factor (GM - CSF), and erythropoietin, megakaryocytes microtubules and microfilaments aid in breaking apart the megakaryocytes and release the individual platelets into the blood stream (Weiss *et al.,* 2011).

After vascular injury von Willebrand factor (vWF) acts as a bridge between endothelial collagen and platelet surface receptors GPIb and promotes platelet adhesion. The platelet glycoprotein complex I (GP-Ib) is the principal receptor for vWF, and also exposes the GPIIb/IIIa binding sites to fibrinogen and vWF leading to platelet aggregation (Hoffbrand and Moss ,2015).

After adhesion, degranulation from both types of granules (dense and alpha) takes place with the release of various factors. Calcium is released which binds to the phospholipids that appear secondary to the platelet activation and provides a surface for assembly of various coagulation factors (Hoffbrand and Moss ,2015).

Thromboxane A2 (TXA2) produced by activated platelets provide stimulus for further platelet aggregation. TXA2 along with ADP enlarge this platelet aggregate leading to the formation of the platelet plug, which seals off vascular injury temporarily. ADP binding also causes a conformational change in GPIIb/IIIa receptors presented on the platelet surface causing deposition of fibrinogen. Thrombin generation also catalyses the conversion of this fibrinogen to fibrin which adds to the

stability of the platelet plug and is now known as secondary haemostasis (Weiss *et al.*, 2011).Prostacyclin inhibits platelet aggregation (platelet anti aggregating effect) and the balance between TXA2 and prostacyclin leads to localized platelet aggregation thus preventing extension of the clot thereby maintaining the vessel lumen patency (Hoffbrand and Moss , 2015).

1.3.2. Secondary haemostasis or coagulation cascade:

According to the available coagulation tests, the reactions of the secondary haemostasis can be functionally divided into extrinsic, intrinsic and common pathways (Weiss *et al.*, 2011).

1.3.2.1. Extrinsic (Tissue factor) Pathway:

It is considered as the first step in plasma mediated haemostasis. It is activated by tissue factor (TF), which is expressed in the sub endothelial tissue (Weiss *et al.*, 2011). Under normal physiological conditions, normal vascular endothelium minimizes contact between TF and plasma procoagulants, but vascular insult expose TF which binds with factor VIIa and calcium to promote the conversion of factor X to Xa ; the final component from these pathway is enzymatic complex (extrinsic Xase) which consist of VIIa, TF, PL and Ca^{2+} (Hoffbrand and Moss , 2015). This pathway is examined in the laboratory by using a PT test (Stockham *et al.*, 2013).

1.3.2.2. Intrinsic pathway (Contact pathway):

It is a parallel pathway for thrombin activation by factor XII. It begins with factor XII, HMW kininogen, prekallikrein and factor XI (contact family) which results in activation of factor XI. Activated factor XI further activates factor IX, which then acts with its cofactor (factor VIII) to form tenase complex on a phospholipids surface to activate factor X (Hall. 2016).The final component from these pathway is enzymatic complex (intrinsic Xase) which consist of IXa, VIIIa, PL and Ca^{2+} (Hoffbrand and Moss , 2015).This pathway is examined in the laboratory by using an APTT test (Stockham *et al.*, 2013).

1.3.2.3 Common Pathway:

Activated factor X along with its cofactor (factor V), tissue phospholipids, platelet phospholipids and calcium forms the prothrombinase complex which converts Prothrombin to thrombin. This thrombin further cleaves circulating fibrinogen to insoluble fibrin and activates factor XIII, which covalently crosslinks fibrin polymers incorporated in the platelet plug. This creates a fibrin network which stabilizes the clot and forms a definitive secondary haemostatic plug (Hoffbrand and Moss , 2015).

This pathway is examined in the laboratory by using Thrombin time test (TT) and FIB test (Stockham *et al.,* 2013).

1.3.3 Tertiary haemostasis:

Fibrinolytic system is a parallel system which is activated along with activation of coagulation cascade and serves to limit the size of clot**.** To avoid and control the spreading of the initial thrombus formed with the reactions of the primary and secondary haemostasis, an inhibition of coagulation is needed. Fibrinolysis is an enzymatic process that dissolves the fibrin clot into fibrin degradation products (FDPs) by plasmin originating from fibrin bound plasminogen in liver. This reaction is catalyzed by t-PA or urokinase plasminogen activator (u-PA) released from vascular endothelium. The release of t-PA is stimulated by tissue occlusion, thrombin, epinephrine, vasopressin and strenuous exercise .Plasmin activity is tightly regulated by its inhibitor (α-2 antiplasmin) thus preventing widespread fibrinolysis (Antovic and Blomback ,2013). In *vivo* activity of the fibrinolytic system is assessed clinically by measuring the FDP's. D.dimers are produced by digestion of cross linked fibrin and are specific indicators of fibrinolysis used in the assessment and diagnosis of pulmonary embolism, DIC or deep vein thrombosis (Machida *et al.,* 2010).

1.4. Coagulation parameters (PT, APTT and FIB) in dogs:

1.4.1.PT, APTT and FIB in healthy dogs:

There have been many reports on the normal PT,APTT and FIB concentration in dogs in worldwide including Brazil (Lopes *et al.,* 2005),USA(Ameri *et al.,*2011) ,India(Sumathi *et al.,*2012) and Greece(Athanasiou *et al.,*2013[a]). Commercially available kits for human medicine have been used to detect the PT and APTT in healthy dogs due to lack of veterinary kits (Lopes *et al.,* 2005 and Sumathi *et al.,*2012).

Lopes *et al,* (2005) determined the reference range values of PT and aPTT, with manual methods. Forty healthy dogs of no definite breed, or gender ratio and of variable ages were used. The blood samples 2.5ml were drawn from cephalic venipuncture into tubes containing 0.25ml of 3.8% sodium citrate. Blood was centrifuged and plasma separated. PT and aPTT were determinate using commercial kits, “HemoStat Thromboplastin-Sia” and “HemoStat aPTT-Elb”, respectively.PT

range from 4.07 to 9.67 (mean 6.87 ± 1.4) seconds, and aPTT range from 11.9 to 18.3(mean 15.1 ± 1.6) seconds.

Ameri *et al,* (2011) compared the fibrinogen concentration which was determined by the FIB_{Clauss} and FIB_{PT} assays in citrated plasma samples from healthy 40 dogs, 40 monkeys, 26 rabbits and 58 rats, using an automated coagulation analyzer. The results indicated that the mean plasma fibrinogen concentrations measured by the two assays for all four species were significantly different. According to Pearson correlation coefficients, the FIB_{PT} assay displayed a high correlation (0.93 to 0.98) with the FIB_{Clauss} assay for all species. When the FIB_{PT} and FIB_{Clauss} assays were compared by using deeming regression, positive or negative constant and proportional biases emerged for all species. Intra- and interassay coefficients of variation for the FIB_{PT} and FIB_{Clauss} assays were 0.8% to 2.3% and 1.8% to 7.4%, respectively. It was concluded that the FIB_{PT} assay is rapid and economical for estimating fibrinogen concentration in plasma of dogs, monkeys, rabbits, and rats. Commercially available kits for human medicine can be used for FIB analysis in healthy dogs (Ameri *et al.,* 2011).

Sumathi *et al,* (2012) estimated the reference range for PT and aPTT in 15 normal dogs, of both sexes and various breeds using a semiautomatic coagulometer and commercial kits for human medicine. The mean value for aPTT was 43.17±2.39 second and for PT was 12.12±0.57second.

Athanasiou *et al,* (2013) compared two techniques used in measuring fibrinogen concentration in canine plasma , Millar's technique and modified thrombin clotting time Clauss method . Fibrinogen concentrations were measured in the plasma of 85 clinically normal dogs and of 43 dogs previously associated with dysfibrinogenemia. Passing-Bablock's regression and Bland Altman difference plots were used for the comparison of the results of the two techniques. No correlation was found between the results of the two techniques; thus the two techniques cannot be used interchangeably.

1.4.2. PT, APTT and FIB in diseased dogs:

Warfarin poisoning ,vitamin K deficiency, liver disease, Hyperadrenocorticism and disseminated intravascular coagulopathy (DIC) all these acquired pathological conditions lead to complications in the coagulation and also genetic disorders of some

coagulation factors(I,VII, VIII, IX ,X and vWF) can lead to this complications (Kerr, 2008).

1.4.2.1. Liver disease: liver disease affects most of the body's functions, including haemostasis**;** because it produces pro-coagulant factors (fibrinogen, II, V, VII, VIII, IX, X, XI, and XIII,), anticoagulants and fibrinolytic proteins (Mischke, 2000).

Liver disease associated with haemostatic abnormalities leads to an abnormal coagulation test result value, abnormal PT, APTT and FIB (Prins *et al.*, 2010).

Infection of some other parts of the body may affect some liver function especially the production of proteins example fibrinogen which is one of the acute phase proteins (APP) (Cerón *et al.,* 2005), present in plasma during an acute phase response (APR). (Murata *et al.,* 2004). The response can be due to inflammation, stress, traumas , tissue damage or infection (Zapryanova *et al.,* 2013).

1.4.2.2. Disseminated intravascular coagulation (DIC):

DIC in the dog is a secondary disease (acute or chronic) .that is always initiated by an underlying primary pathologic process leading to continuous change in the results of the coagulation tests, which are mostly not specialized. (Vlasin *et al.,* 2004).The combination of fibrinogen/fibrin degradation products (FDPs) and D-dimer (DD) has been reported to offer the best molecular markers for understanding the process of systemic activation of the procoagulant and secondary fibrinolytic systems in DIC (Nelson and Andreasen ,2003).

D-dimer (DD) concentration in the dog is pure cross-linked fibrin degradation products. Therefore, an increase in DD concentration is specific for the combined presence of coagulation and fibrinolysis (physiologic or pathologic) (Machida *et al.,* 2010).

Gastric dilatation and volvulus syndrome (GDV) in dogs is associated with changes in haemostatic profiles leading to decreased activity of plasma anticoagulants (protein C, protein S, antithrombin) or up regulation of tissue factor and plasminogen activator inhibitor, This leads to the occurrence DIC (Uhrikova *et al.,* 2013).

1.4.2.3. Hyperadrenocorticism (HAC) or Cushing's syndrome:

HAC in dogs can be associated with vascular thrombosis because the increased cortisol level in the blood stimulates the formation of clots by increasing coagulation factors and reducing fibrinogen. Anti-inflammatory drugs such as prednisone corticosteroid are used to treat this condition (Romão *et al.,* 2013).

1.4.3. Factors Affecting Coagulation Tests (PT, aPTT and FIB):

There are some factors that influence the outcome of coagulation tests (PT, APTT and FIB) (Adcock *et al.,* 1997).

The values of PT, APTT and FIB increases with storage time until it reach the stability stage. (Walton *et al.,* 2014).

It is known that in coagulation tests the sodium citrate anticoagulant is used in concentrations of 3.2% or 3.8%, these concentrations affect the values of the coagulation tests, where the normal ranges of PT and APTT higher when samples are drawn into 3.8% citrate compared with 3.2% citrate (Adcock *et al.,* 1997).

There are significant differences among the laboratories results for coagulation tests . This may be attributed to many reasons ,such as the quality of the lab, type of blood collection container, specimen transport, storage conditions, incubation time ,temperature and type of kits used, whether for humans or specific for dogs (Nagler *et al.,* 2013).

1.5. Platelets count and indices:

Platelet indices are biomarkers of platelet activation. These include mean platelet volume (MPV), plateletcrit (PCT) and platelet distribution width (PDW).These parameters are available on many automated cell counters (Willard and Tvedten , 2011).

1.5.1. Mean platelet volume (MPV):

It is machine-calculated measurement of the average size of platelets (Weiss *et al.,* 2011). The MPV is sometimes ordered in conjunction with a platelet count. The MPV indicates the uniformity of size of the platelet population. It is used for the differential diagnosis of thrombocytopenia (Stockham *et al.,* 2013). A high MPV is usually a sign that there are more young platelets circulating in the bloodstream (Chandrashekar, 2013). In addition, a low MPV can be seen with high, low or normal platelet counts in sepsis, spleenomegaly, chronic kidney failure, or treatment with drugs that suppress blood production (Chandrashekar, 2013).

1.5.2. Plateletcrit (PCT):

It is measurement of total platelets mass (Weiss *et al.*,2011).Values varies depending on MPV and PLT and calculated according to the formula PCT = PLT × MPV / 10,000 (Chandrashekar, 2013).

1.5.3. Platelet distribution width (PDW):

It directly measures variability in platelet size, change with platelet activation, and reflects the heterogeneity in platelet morphology (Weiss *et al.,* 2011). It increases in the presence of platelet anisocytosis (Chandrashekar, 2013).

1.5.4. Platelets count and indices in healthy animals:

Hussein *et al*.(2010) determined the reference intervals values for platelet count and indices in Arabian dromedary camels and reported PCT mean value significantly (P≤0.05) higher in males compared with females, PDW mean value significantly lower (P≤0.05) in males compared with females. Hussein *et al.* (2012) Also reported of platelet count and indices in camels and found that the mean MPV value was significantly (P≤0.05) higher in females compared with males.

Awad-Elkareem *et al*. (2015) studied the platelet count and indices in 300 apparently healthy Sudanese; 150 males and 150 females, with ages range between 10 and 60 years. The platelet count and PCT mean values were significantly (P≤0.05) higher in males compared with females .

Schneider and Mischke, (2016) studied the influences of age, breed and sex on platelet parameters (PLT, MPV, PDW, PCT, mean platelet component (MPC), platelet component distribution width (PCDW), mean platelet mass (MPM), platelet mass distribution width (PMDW), large platelets and platelet clump count),in 166 healthy dogs of different breeds (Havanese dogs, Rhodesian Ridgebacks, *Labrador Retrievers*, *Golden retrievers* and *German shepherds*).Significant breed differences were observed for PLT, PDW, PCT, MPC, MPM and PMDW. Significant sex differences were only seen for mean PLT (females, 320 × 103/μL; males, 272 × 103/μL; p = 0.003) as well as median MPV (females, 10.5 fl; males, 11.0 fl; p = 0.043).

Suljević *et al*, (2016) in his study of haematological parameters of working police dogs, reported that the PLT range value were higher in *Labrador Retriever* compared with *German Shepherd.*

Nidaa *et al*, (2017) studied the platelets count and indices of 33 healthy *German Shepherd* dog in sudan .They showed that the platelets indices range values were

higher in females compared with males, and significant sex differences were observed for platelet count ($P \leq 0.05$) .

1.5.5. Platelets count and indices in diseased dogs:

Platelets indices are commonly used to diagnose thrombocytopenia and other disease condition in dogs (Prins *et al.*,2009, Fabisiak *et al.,* 2010,Temizel *et al.*,2011,Vojta *et al.*,2012 ,Schwartz *et al.,* 2014, Żmigrodzka *et al.,* 2014 and Reddy *et al.,* 2015).

Baranidharan *et al*, (2017) studied the platelets indices (PLT, MPV, PCT and PDW) in dogs, and if it can be useful to evaluate in Ehrlichiosis, Babesiosis and Leptospirosis, as thrombocytopenic index. They found that mean values of PLT,MPV,PCT and PDW in normal dogs were 448, 8.35, 0.29 and 16.07 respectively, when it compared with those of diseased dogs with Ehrlichiosis and Leptospirosis did not have any diagnostic role. Where as an increased MPV was detected in Babesiosis infected dogs.

Platelet indices can also be used as inflammatory indicators (Santimone *et al.,* 2011), and to diagnose liver disease in dogs, because the liver produces platelet activating factors and this affects platelet indices (Prins *et al.,* 2009).

1.5.6. Factors Affecting on Platelets count and indices:

Platelet indices are affected by gender (Nagata *et al.,* 2003 ,Adekola *et al.,* 2015, Suljević *et al.,* 2016 and Nidaa *et al* ., 2017), age (Santimone *et al.,* 2011 and Suljević *et al.,* 2016), breed (Lavoué *et al.,* 2014, Adekola *et al.,* 2015, Çayir and Kozat, 2016, Schneider and Mischke, 2016 and Suljević *et al.,* 2016), and environmental factors (Adebiyi *et al.,* 2014 , Adekola *et al.,* 2015 and Nidaa, 2018).

Interlaboratory differences can effect on Platelets count and indices :There are significant differences among the laboratories results for coagulation tests ,this may be attributed to many reason ,such as the quality of the lab, type of blood collection container, specimen transport, storage conditions, temperature and the technique which use to detect PLTautomated or manual can effect on platelets count (Athanasiou *et al.,* 2013[b] and Tan *et al.,* 2014), also the type of auto haematology analyzer may affect the results(Awad-Elkareem *et al* .,2015 and Offutalu *et al.,* 2016).

1.6. Correlation between platelets count and platelets indices:

Nidaa *et al*. (2017) studied the correlation between platelet count and platelet indices in the 33 *German Shepherd* dogs in sudan, and reported significant (P≤0.01) correlation between PLT and PCT in female and all the dogs. No significant correlation was found between PLT and MPV or PDW in the two sexes.

Wiwanitkit (2004) studied the correlation between platelet count and platelet indices in 215 persons .He reported a positive significant (P≤ 0.01) correlation between PLT and PCT , but a negative significant (P≤ 0.01) correlation between PCT and PDW.

Hussein *et al*. (2009) studied the correlation between platelet count and platelet indices in eight captive Arabian mountain gazelle. The correlation analysis revealed highly significant correlation for PCT with PLT (P≤0.0001). Similarly Hussein *et al*, (2010) reported a highly significant correlation between PLT and PCT (P≤0.001) in male, female and all camels and a significant correlation between MPV and PDW (P<0.05) in male and all camels. More over Hussein *et al*, (2012) studied the correlation between platelet count and platelet indices in 221 Saudi Arabian dromedary camels of difference breeds and both gender. A highly significant (P≤0.005) correlation was found between the following parameters PLT and PCT, PLT and PDW, PCT and MPV and MPV and PDW .A significant (P<0.05) correlation was also found between PLT and MPV and between PCT and PDW.

CHAPTER TWO

MATERIALS AND METHODS

2.1. Study type, place and period:

This is an analytical study conducted in Khartoum State in the period January - April in 2017 , to determine coagulation parameters platelets count and indices for two foreign breeds of dogs (*Labrador Retriever* dogs and *German Shepherd* dogs), and to evaluate the effect of gender and breed on them. Coagulation parameters (PT, APTT, and FIB), platelets count and indices all these tests will be carried out at EL-Rayaida for Medical Laboratory-Khartoum.

2.2. Animals:

The dogs belong to the Police Directorate for Dogs at Khartoum State, Burry and there were selected on the base of their medical history and normal health parameters.

Forty-six apparently healthy police dogs, 20 *Labrador Retriever* and 26 *German Shepherd* dogs ,comprising 21 males (8 *Labrador Retriever* and 13 *German Shepherd*) and 25 females (12 *Labrador Retriever* and 13 *German Shepherd*) 20-83 months old .

The dogs were individually housed in kennels and fed on an imported dogs' food (Best Signor - Netherlands), and from time to time they were offered an entertainment meal consisting of meat soup and noodles, the access to water were free.

2.3. Blood collection and analysis:

2.3.1. Blood collection:

Seven ml were drawn from the cephalic vein ; 2 ml were collected into tube containing 3.2% trisodium citrate (9NC; Marina pharma, China) to give a citrate to blood ratio of 1:9 for determination of PT, APTT and FIB , and 5ml were collected into tube containing Ethylenediamine tetra acetate –Tri-potassium (EDTA-K_3) Medical tube (HNTE-DISPO, China) for platelets indices determination.

Following collection, the tubes were gently inverted 3-5 times to guarantee mixing of the sample with the anticoagulant. The samples immediately transported to the laboratory and analyzed. The citrated plasma was separated by centrifuging citrated blood samples immediately for 15 min at 3000 RPM at room temperature.

2.3.2. Coagulation tests:

PT, APTT and FIB were measured in paired citrated plasma, using a semi automated- BCA-2000-LED-light-coagulometer-with-Double-channel **(**Biobase, China) and Spinreac reagents (Spinreac, Spain**).**

2.3.2.1. Coagulometer:

Semi automated coagulometer is an apparatus in the form of a graduated tube, used to determine the rapidity of coagulation of any given specimen of blood, or the coagulating used to measure the ability of the blood to coagulate. It calculates some of coagulation tests like PT, APTT, FIB and TT.

2.3.2.2. Prothrombin time test (PT):

Principle:

The principle of the test consists of the use of calcium thromboplastin to measure the clotting time of the dog's plasma and to compare it with that of a normal standard. The test measures, as a whole, the activity of extrinsic coagulation factors: factor II (Prothrombin), factor V (Labile factor), factor VII (proconvertin), and factor X (Stuart – Prower factor) (Stockham *et al.,* 2013).

Procedure (Spinreac reagent procedure):

Heating the sample (citrated plasma) and the reagent (Rabbit brain thromboplastin, calcium chloride and heparin inhibitor) in the coagulometer incubator to 37$^{\circ C}$ during no more than 10 min, after that start the PT test by adding 100µl of heated sample in clean sterile cuvette in detector hole of coagulometer and then added 200µl of heated reagent to the sample, then the device records the time from the addition of the reagent to the formation of the clot (Fibrin filaments) per (sec).

2.3.2.3. Activated partial thromboplastin time test (APTT):

Principle:

The APTT involves the recalcification of plasma in the presence of a standardized amount of platelet substitute and a specific activator. This procedure minimizes test variables by standardizing the contact activation and optimizes the concentration of platelet-like phospholipids. The APTT explores the intrinsic coagulation pathway factor XII (Hageman factor), factor XI(Plasma thromboplastin antecedent factor), factor IX(Christmas factor),factor VIII(Antihaemophilic factor), factor X (Stuart – Prower factor), factor V(Labile factor), factor II(Prothrombin) and factor I (Fibrinogen) (Stockham *et al.,* 2013).

Procedure (Spinreac reagent procedure):

Heating the sample (citrated plasma) and the reagents (R1 Activator (Ellagic acid Buffers and preservatives), R2Starter (Calcium chloride ($CaCl_2$)) with the coagulometer incubator to 37^{oC} during no more than 10 min, after that start the APTT test by adding 100µl of heated sample in clean sterile cuvette in detector hole of coagulometer and then added 100µl of heated reagent1(Activator) to the sample mixed and incubated in detector hole exactly for 5 min,at 37^{oC} (activation time),Then added 100µl of heated reagent 2(Starter) to the mixture from step 2, after that The device then records the time from the addition of the reagent 2 to the formation of the clot (Fibrin filaments) per (sec).

Control values for PT and APTT will be based on (Lopes *et al.,* 2005).

2.3.2.4. Fibrinogen concentration "Clauss method":

Principle:

In the presence of an excess of thrombin, the clotting time of diluted plasma has a direct bearing on the level of plasma fibrinogen (Stockham *et al.,* 2013).

Procedure (Spinreac reagent procedure):

Heating the sample (citrated plasma) and the reagents (R2 Imidazole buffer) (R3 caolin solution) with the coagulometer incubator to 37^{oC} during no more than 10 min, after that diluted the heating citrated plasma with heating Imidazole buffer (50 µL plasma + 450 µL Imidazole buffer). Diluted sample was treated in 10 minutes , after that start the FIB test by adding 200µl of heated diluted solution (sample and Imidazole buffer) in clean sterile cuvette in detector hole of coagulometer and then added20µl (R3), and incubated the solution at 37^{oC} for 5 minutes, then added 100µl of non heated Bovine thrombin (R1) to the mixture from step 3, after that the device then records the time from the addition of the (R1) to the formation of the clot (Fibrin filaments) per **(sec)**, then comparing the time of clotting with the fibrinogen concentration table to determine the concentration of fibrinogen per **(mg/dl).**

2.3.3. Examination of platelet indices:

Platelet parameters were determined in whole blood (EDTA-K3) samples. The whole blood samples were analyzed using BK6100 Auto Hematology Analyzer-(Biobase,China), The following parameters were determined: platelet count (PLT), plateletcrit (PCT), mean platelet volume (MPV) and platelet distribution width (PDW).

2.3.4.Quality control:

Quality control of platelets was done using blood smear which was stained by Leishman's stain , microscope (Olympus, Japan) was used to determine platelets clumps in dogs blood smear**,** If we find a large number of clumps the sample will be rejected (Weiss *et al.*,2011). Leishman's stain was done in the following steps , The air dried blood film was covered with a Leishman's stain and left for a minute to 3 minutes to fix it, the stain on the smear was dilute with double the volume of buffered distilled water and stain for 5 to 15 minute, the mixture was blow on gently, the film was washed with distilled water until the film has a pinkish tinge, the back of the stain/the slide was wipe and allow to dry in upright position (kemal, 2014).

2.4. Statistical Analysis:

Differences in mean values between groups were detected by student's t-test as described by Gomez and Gomez (1984), and Spearman correlation was used to determine correlations between platelet count and platelets indices using statistical package for social science (SPSS) version 16 for statistical analysis**.**

CHAPTER THREE

RESULTS

3.1. Coagulation parameters:

The mean values, range and median of PT, APTT and fibrinogen concentration, for all dogs are displayed in Table (1). Effect of the breed is presented in Table (2). **PT** and **APTT** were significantly higher (P≤0.05) in *German Shepherd* than in *Labrador Retriever* dogs , **FIB** did not show any significant variation between the two breeds (P>0.05).

Sex related differences in dogs coagulation parameters are presented in Table (3)**. FIB** was significantly (P ≤ 0.05) higher in males than in females in all the dogs **. PT** and **APTT** did not show any significant variation with the sex (P> 0.05).

Age related differences in dogs coagulation parameters were presented in Table (4)**. PT** , **APTT** and **FIB** did not show any significant variation with the age (P> 0.05).

Table (1): PT, APTT and FIB values in police dogs .

Parameters	**NO.**	**Mean ± SD**	**Min-Max.**	**Median**
PT(second)	46	7.35±0.91	6-10	7.25
APTT(second)	46	15.12±1.32	13-18	14.80
FIB(mg/dl)	46	341.54±55.16	210-417	361

Table (2): Effect of the breed on PT, APTT and FIB values in police dogs .

Parameters	**Labrador Retriever** **mean ± SD** **min-max**	**German Shepherd** **mean ± SD** **min-max**	**P. value**
PT(second)	7.06±0.67 6-8	7.58±1.01 6-10	0.042
APTT(second)	14.9±0.85 13-16	15.28±1.58 13-18	0.01
FIB(mg/dl)	344.41.±50.63 254-417	339.44±59.15 210-416	0.499

Table (3): Effect of sex on PT, APTT and FIB in values police dogs.

Parameters	**Breed**	**Females** **mean ± SD** **min-max**	**Males** **mean ± SD** **min-max**	**P. value**
PT(second)	**Overall**	7.38±0.88 6-10	7.31±0.97 6-9	0.81
	Labrador Retriever Female N=12 Male N=8	7.12±0.65 6-8	6.95±0.73 6-8	0.583
	German Shepherd Female N=13 Male N=13	7.62±1.01 6-10	7.54±1.06 6-9	0.851
APTT(second)	**Overall**	15.02±1.27 13-18	15.23±1.39 13-18	0.598
	Labrador Retriever Female N=12 Male N=8	14.79±0.66 14-16	15.08±1.1 13-16	0.481

	German Shepherd Female N=13 Male N=13	15.23±1.65 13-18	15.32±1.57 13-18	0.885
FIB(mg/dl)	**Overall**	337.73±65.99 210-417	346.30±38.75 284-402	0.021
	Labrador Retriever Female N=12 Male N=8	353.64±53.12 254-417	328.59±45.12 284-398	0.294
	German Shepherd Female N=13 Male N=13	323.04±75.08 210-416	355.85±32.69 312-402	0.167

Table (4): Effect of age on PT, APTT and FIB in values police dogs.

Parameters	**18 – 45 month (age)** **mean ± SD** **min-max**	**46 – 83 month (age)** **mean ± SD** **min-max**	**P. value**
PT(second)	7.25±0.94 5.9 -9.7	7.49±0.87 6.2-10	0.384
APTT(second)	14.92±1.32 12.8-18.1	15.36±1.31 13.6-18.2	0.273
FIB(mg/dl)	345.4±56.02 210-417	336.71±55.12 243-416.06	0.605

3.2. Platelets count and indices:

The mean values, range and median of PLT, MPV, PCT and PDW for all dogs, are displayed in Table (4).Effect of the breed is presented in Table (5). **MPV,** was significantly ($P \leq 0.05$) higher in *German Shepherd* than in *Labrador Retriever* dogs **.**

PLT, PCT, and **PDW,** did not show any significant variation between the two breeds (P>0.05).

Sex and related differences in dog's platelet count and indices are presented in Tables (7) . **MPV,** was significantly (P ≤ 0.05) higher in *German Shepherd* females than in *German Shepherd* males dogs, also **MPV** and **PDW** were significantly (P ≤ 0.05) higher in *Labrador Retriever* males than in *Labrador Retriever* females dogs **PLT** and **PCT,** did not show any significant variation with the sex (P>0.05).

Age related differences in dog's platelet count and indices are presented in Tables (8) respectively. **PLT, MPV**, **PCT** and **PDW,** did not show any significant variation with the age (P>0.05).

Table (5): Platelets count and indices values in police dogs .

Parameters	**NO.**	**Mean ± SD**	**Min-Max.**	**Median**
PLT($x10^9$/L)	46	172.02±52.72	65-290	176
MPV(FL)	46	10.2±0.76	8.7-12.1	10.1
PCT(L/L)	46	0.173±0.057	0.06-0.32	0.17
PDW (%)	46	11.16±0.31	10.8-12.01	11.11

Table (6): Effect of breed on platelets count and indices values in police dogs.

Parameters	**Labrador Retriever** **mean ± SD** **min-max**	**German Shepherd** **mean ± SD** **min-max**	**P. value**
PLT(x10^9/L)	171.3±63.07 65-290	172.58±44.49 71-276	0.936
MPV(FL)	9.9±0.73 8.7-11.4	10.43±0.71 9-12.1	0.017
PCT(L/L)	0.159±0.06 0.06-0.32	0.184±0.054 0.08-0.29	0.131
PDW (%)	11.25±0.38 10.8-12.01	11.10±0.24 10.8-11.84	0.136

Table (7): Effect of sex on platelets count and indices values in police dogs.

Parameters	**Breed**	**Females** **mean ± SD** **min-max**	**Males** **mean ± SD** **min-max**	**P. value**
PLT(x10^9/L)	**Overall**	168±57.03 65-290	176.81±48.03 94-279	0.578
	Labrador Retriever Female N=12 Male N=8	166±72.82 65-290	178±48.63 112-279	0.688
	German Shepherd Female N=13 Male N=13	169±40.49 71-229	175±49.62 94-276	0.722

MPV(FL)	**Overall**	10.164±0.85 8.7-12.1	10.243±0.65 9-11.4	0.729
	Labrador Retriever Female N=12 Male N=8	9.55±0.53 8.7-10.2	10.41±0.71 9.1-11.4	0.006
	German Shepherd Female N=13 Male N=13	10.72±0.69 9.9-12.1	10.13±0.62 9-11.3	0.032
PCT(L/L)	**Overall**	0.166±0.06 0.06-0.27	0.182±0.06 0.09-0.32	0.34
	Labrador Retriever Female N=12 Male N=8	0.142±0.06 0.06-0.22	0.184±0.06 0.12-0.32	0.125
	German Shepherd Female N=13 Male N=13	0.187±0.05 0.08-0.27	0.181±0.06 0.09-0.29	0.749
PDW (%)	**Overall**	11.08±0.22 10.8-11.84	11.26±0.37 10.8-12.01	0.056
	Labrador Retriever Female N=12 Male N=8	11.07±0.2 10.8-11.38	11.51±0.43 11-12.01	0.023
	German Shepherd Female N=13 Male N=13	11.09±0.25 10.8-11.84	11.10±0.23 10.8-11.77	0.847

Table (8): Effect of age on platelets count and indices values in police dogs.

Parameters	**18 – 45 month (age)** **mean ± SD** **min-max**	**46 – 83 month (age)** **mean ± SD** **min-max**	**P. value**
PLT(x10^9/L)	178.31±51.95 65-290	197.65±52.53 114-279	0.054
MPV(FL)	10.2±0.82 8.7-12.1	10.19±0.69 9-11.4	0.969
PCT(L/L)	0.174±0.05 0.06-0.27	0.197±0.05 0.11-0.32	0.09
PDW (%)	11.19±0.35 10.8-12.01	11.13±0.25 10.8-11.77	0.52

3.3.Correlation between Platelets count and Platelets Indices:

In this study, correlation between platelets count (PLT(x10^9/L)), and platelets indices (MPV (FL), PCT (L/L) and PDW (%)) were investigated for males, females and all the dogs . The results are presented in Table (9). A highly significant positive ($P \leq 0.01$) correlation was found between PLT and PCT in males**,** females and all the dogs .Also there was a highly significant positive ($P \leq 0.01$) correlation between MPV and PDW in all the dogs, and a low significant positive ($P \leq 0.05$) correlation in males. A highly significant positive ($P \leq 0.01$) correlation was also found between MPV and PCT in all the dogs, and was also a low significant positive ($P \leq 0.05$) correlation in males and females. A low significant positive ($P \leq 0.05$) correlation between PCT and PDW was found in all the dogs. No significant correlation was found between PLT and MPV or PDW in all the dogs.

Table (9): Correlation between Platelets count and Platelets indices in police dogs.

Parameters	PLT($x10^9$/L)	MPV(FL)	PCT(L/L)	PDW (%)
Overall				
PLT($x10^9$/L)	1	0.290	0.874**	0.289
MPV(FL)	0.290	1	0.492**	0.405**
PCT(L/L)	0.874**	0.492**	1	0.343*
PDW (%)	0.289	0.405**	0.343*	1
Males				
PLT($x10^9$/L)	1	0.343	0.980**	0.164
MPV(FL)	0.343	1	0.439*	0.536*
PCT(L/L)	0.980**	0.439*	1	0.205
PDW (%)	0.164	0.536*	0.205	1
Females				
PLT($x10^9$/L)	1	0.213	0.795**	0.312
MPV(FL)	0.213	1	0.492*	0.265
PCT(L/L)	0.795**	0.492*	1	0.347
PDW (%)	0.312	0.265	0.347	1

*correlation significant at the ($P \leq 0.05$) .

**correlation significant at the ($P \leq 0.01$).

CHAPTER FOUR

DISCUSSION

Effect of breed ,sex and age on coagulation parameters (PT, APTT and FIB) of police dogs has been investigated in this study because; of lack of result on this aspect in previous reports on dogs (Vlasin *et al.,* 2004, Lopes *et al.,* 2005, Machida *et al.,* 2010, Prins *et al.,* 2010, Sumathi *et al* ., 2012, Athanasiou *et al.,* 2013[a], Romão *et al* ., 2013 Uhrikova *et al.,* 2013 and Zapryanova *et al.,* 2013) , a however effect of sex on FIB is reported by (Ameri *et al.,* 2011).

The mean PT value in this study was 7.35±0.91seconds with minimum and maximum values of 6 and 10 seconds respectively. This value is near to the values reported by Machida *et al*.(2010) 7.9 ± 0.67 with range of (6.6-9.3) and Prins *et al*.(2010) 7.4 with range of (6.7-9.5).Lower mean values than reported here were reported by Vlasin *et al.*(2004) 8.12± 0.48 with range of (6.4-7.4) and Lopes *et al.* (2005) 6.87 ± 1.4 with range of (4.07-9.67). Higher mean values of PT than of in this study were reported by Sumathi *et al.* (2012) 12.12 ±0.57 with range of (6.7-16.6), Romão *et al*.(2013) 11.80 with range of (11.03-13.05) and Uhrikova *et al*.(2013)10.7 with range of (9-12.8).
APTT mean value in this study was 15.12±1.32 seconds with a minimum and maximum of 13 and 18 seconds respectively. This value is near to the values reported by Lopes *et al.* (2005) 15.1±1.6 with range of (11.9-18.3) and Machida *et al*. (2010) 15.8±3.0 with range of (9.8-21.8). Lower values APTT than of in this study were reported by Vlasin *et al.* (2004) 11.64±1.54 with range of (9.5-10.5) and Prins *et al*. (2010)14.3 with range of (10-17.2).Higher values of APTT than of in this study were reported by Sumathi *et al.* (2012) 43.17 ±2.39 with range of (22.6-56.6) Romão *et al.* (2013) 18 with range of (15.75-19.21) and Uhrikova *et al.* (2013)17.5 with range of (16.4-19.6).

These differences in PT value and APTT among the different researchers could be due to differences in the number, sex or breed of the studied animals. There are also many interlaboratory differences which may have caused these variations, such as the method of blood collection, specimen transport, storage conditions, incubation time and temperature, and type of kits used (Nagler *et al.,* 2013).Trisodium citrate concentration was described by (Adcock *et al.,* 1997) as an important factor which

can affect the coagulation tests. Also the type of coagulometer, whether semi automatic or automatic, may affect the results.

PT and APTT were significantly higher ($P \leq 0.05$) in *German Shepherd* dog than in *Labrador Retriever* dog, but PT and APTT did not show any significant variation with the sex and age ($P > 0.05$). To the best of our knowledge, there is no published data on the effect of breeds ,sex and age on above parameters in dogs to compare with .

The FIB mean value in this study was 341.54±55.16 mg/dl with a minimum and maximum of 210 and 417mg/dl respectively which is in accord with the mean value (348 mg/dl) found by Walton *et al*. (2014) .Higher mean value (409.595± 3.554 mg/dl) with range of (262.2-644.6 mg/dl) than reported in this work are given by Athanasiou *et al.* (2013).Lower mean values of FIB concentration than in this work were reported by Vlasin *et al*.(2004) 257± 0.526 mg/dl and Uhrikova *et al*.(2013)240 mg/dl. However their obtained results are within the normal range (200-400) described by (Stockham *et al.,* 2013).

This variation in results could be due to variation in physiological state of studied animals as ; pregnancy and lactation are found to affect the FIB concentration (Antovic and Blomback, 2013).

The present study showed a significant increase in FIB in males, which is in line with the results of Ameri *et al*, (2011). The total white blood cells count has a reverse effect on the concentration of fibrinogen Stockham *et al*. (2013),so this may explain the higher concentration of FIB in the males than the females.

FIB did not show any significant variation with the breed and age ($P > 0.05$), to the best of our knowledge, there is no published data on the effect of breeds on FIB in dogs to compare with .

The mean **PLT**($x10^9$/L) value in this study was 172.022±52.72 with minimum and maximum values of 65 and 290 respectively. This value is near to the values reported by (Prins *et al.,* 2010)153 with range of (144-163), (Adekola *et al.,* 2015) 187±0.10 and (Nidaa *et al.,* 2017) 183.52± 59.97. Higher mean values than in this work were found by (Temizel *et al.,* 2011) 399 ± 33, (Weiss *et al.*,2011) with range of 200 – 500 ,(Schwartz *et al.,* 2014) 260±934,(Żmigrodzka *et al.,* 2014) 282±68.70 with range of (210– 518) and (Baranidharan *et al.,* 2017) 448.

The mean **MPV** (FL) value in this study was 10.2±0.76 with minimum and maximum values of 8.7 and 12.1 respectively. This value is near to the values reported by (Fabisiak *et al.,* 2010) 9.75, (Prins *et al.,* 2010)11.3 with range of (7.5– 15.1), (Vojta *et al.,*2012) with range of 6.7-11.1, (Schwartz *et al.,* 2014) 10.5 and (Nidaa *et al.,* 2017) 9.19± 0.96. Lower mean values of MPV than of in this study were reported by (Weiss *et al.,* 2011) with range of 6.7 – 11.1, (Żmigrodzka *et al.,* 2014), 8.77 with range of (7.10– 9.80), and (Baranidharan *et al.,* 2017) 8.35±0.19. A higher mean value also was found by (Temizel *et al.,* 2011) 12.8 ± 0.6.

The mean **PCT** (L/L) value in this study was 0.173±0.057 with minimum and maximum values of 0.06 and 0.32 respectively. This value is near to the value reported by (Nidaa *et al.,* 2017) 0.13± 0.038. A higher mean values than in this work were found by (Temizel *et al.,* 2011) 0.54 ± 0.04, (Schwartz *et al.,* 2014) 0.267, (Żmigrodzka *et al.,* 2014) 0.25 with range of (0.17– 0.37), and (Baranidharan *et al.,* 2017) 0.286±0.06.

The mean **PDW** (%) value in this study was 11.16±0.31 with minimum and maximum values of 10.8 and 12.01respectively. This value is near to the value reported by(Schwartz *et al.,* 2014) 12.12±1.99 .A higher range values than in this work were found by (Temizel *et al.,*2011) 19.4 ± 0.4,(Baranidharan *et al.,* 2017) 16.07±0.16 and (Nidaa *et al.,* 2017) 15.61± 0.90 .

Effect of breed and age on PLT were reports by (Adekola *et al.,* 2015, Çayir and Kozat, 2016, Schneider and Mischke, 2016 and Suljević *et al.,* 2016).The effect of breed on MPV,PCT and PDW was reported by (Schneider and Mischke, 2016), and in *German Shepherd* by (Nidaa *et al.,* 2017). The effect of sex in dogs on Platelets count and indices was reports by (Adekola *et al.,* 2015, Schneider and Mischke, 2016 and Nidaa *et al.,* 2017) and in camels by (Hussein *et al.,*2010, 2012) ,and Awad-Elkareem *et al* .(2015) in Humans.

MPV mean value was significantly ($P \leq 0.05$) higher in *German Shepherd* dogs compared with the *Labrador Retriever* dogs, which contrast with Schneider and Mischke, (2016) who found no significant difference in MPV mean value between *German Shepherd* and *Labrador Retriever* dogs, also MPV was significantly ($P \leq 0.05$) higher in *Labrador Retriever* males than in *Labrador Retriever* females dogs ,this is in line with (Schneider and Mischke, 2016), and contrast with the results of (Awad-Elkareem *et al .,* 2015),and Nidaa *et al.* (2017). MPV was significantly ($P \leq$

0.05) higher in *German Shepherd* females than in *German Shepherd* males dogs this is contrast with the results of Awad-Elkareem *et al* . (2015), (Schneider and Mischke, 2016), and Nidaa *et al.* (2017).

PDW mean value was significantly ($P \leq 0.05$) higher in *Labrador Retriever* males than in *Labrador Retriever* females dogs, this is contrast with the results of Awad-Elkareem *et al* . (2015), (Schneider and Mischke, 2016), and Nidaa *et al.* (2017).

PLT, MPV and PCT values were higher in *German Shepherd* compared with the *Labrador retriever.* This can be due to genetic predisposition and other exogenous and endogen factors, such as the type of work, duration of training, metabolic activity and general physical condition (Suljević *et al.*, 2016).

In the current study there was no significant difference between males and females in PLT this agrees with (Hussein *et al.*,2010, 2012) in camels , Adekola *et al.* (2015), Awad-Elkareem *et al* . (2015) in human and Schneider and Mischke, (2016) in dogs and in contrast with the finding of Nidaa *et al,* (2017) in dogs .

Furthermore Platelets count and indices mean values were higher in males compared with the females. This can be attributed to hormonal profiles amongst the gender (Nagata *et al.,* 2003).The process by which megakaryocytes proceed to proplatelet formation and platelet production is reported to increase under influence of autocrine estrogen ,and less so under the influence of Progesterone hormone,(Nagata *et al.*, 2003) because of that the physiological status(estrus, pregnancy and lactation) can effect on platelets indices. Also the number of members of any breed in any group (Females and Males).

All platelet indices varied with age, in particular platelet count and plateletcrit decreased with age in both males and female(Santimone *et al* .,2011), Age groups for dogs in this study females were larger than males .So that may explain the higher concentration of platelets indices in the males than the females .

The present study revealed a positive significant correlation between PLT and PCT in males, females and all the dogs, this is on line with Wiwanitkit, (2004) in human, Hussein *et al,* (2009) in Arabian mountain gazelle ,(Hussein *et al.*, 2010 and Hussein *et al.*, 2012)in camels .Nidaa *et al,* (2017) found similar result in *German Shepherd* dogs except of that males showed low positive significant correlation between the two parameters. The highly positive significant correlation between MPV and PDW

in all the dogs, and the low positive significant correlation in males are in line with(Hussein *et al.,* 2012) in camels

Hussein *et al,* (2010) found similar result in camels except that all the camels showed low positive significant correlation between the two parameters.

Wiwanitkit, (2004) in human, Hussein *et al,* (2009) in Arabian mountain gazelle and Nidaa *et al,* (2017) in *German shepherd* dogs, found dissimilar results.

A highly positive significant correlation was found between MPV and PCT in all the dogs, low positive significant correlation were found in males and females, this is on line with (Hussein *et al.,* 2012) in camels and in contrast with the finding of (Wiwanitkit, 2004 in human, Hussein *et al.,* 2009 in Arabian mountain gazelle, Hussein *et al,* (2010) in camels and Nidaa *et al,* (2017) in *German Shepherd* dogs.

No significant correlation was found between PLT and MPV, PDW in all the dogs and this is agrees with (Wiwanitkit, (2004) in human, Hussein *et al,* (2009) in Arabian mountain gazelle ,Hussein *et al,* (2010) in camels and Nidaa *et al,* (2017) in *German Shepherd* dogs , and is in contrast with the finding of (Hussein *et al.,* 2012) in camels.

This differences in Platelets count and indices values among the different researchers could be due to differences in the studied animals like the number of animals, sex, breed (Nagata *et al.,* 2003 ,Adekola *et al.,* 2015, Çayir and Kozat, 2016,Schneider and Mischke, 2016, Suljević *et al.,* 2016 and Nidaa *et al.,* 2017), age (Santimone *et al.,* 2011, Schneider and Mischke, 2016 and Suljević *et al.,* 2016),physiological status of dogs,(Nagata *et al.,* 2003 and Suljević *et al.,* 2016) and the environmental factors (Adebiyi *et al.,* 2014 , Adekola *et al.,* 2015 and Nidaa, 2018). There are many interlaboratory differences (Offutalu *et al.,* 2016) which may have caused this variation in the results obtained by the different researchers, such as the type of blood collection container, specimen transport, storage conditions, temperature, also the type of auto haematology analyzer and the technique which were used may affect the results(Athanasiou *et al.,* 2013[b] ,Tan *et al.,* 2014 and Awad-Elkareem *et al* ., 2015)**.**

These differences among studies in (Correlation between Platelets count and Platelets indices) may be attributed to species of animal (Camels, Mountain gazelle and dogs) and Humans , number of males and females in each study, animal ages , climate and other factors that can be affected Platelets count and indices.

CONCLUSION

The results of the study could be concluded in the following**:**

1. All the results were within the international reference value**.**

2. Breed can affect in **PT**, **APTT** and **MPV** mean values**.**

3. Gender can affect in **FIB** mean value**.**

4. A revealed significant correlation between Platelets count and Platelets indices was found**.**

RECOMMENDATIONS

1.Future research should be under taken to examine the coagulation parameters and factors in healthy dogs and take other factor like (breeds ,ages, season, and physiological status, disease and management practice) under Sudanese conditions.

2. Examination of Platelets count and indices and its relationship to diseases in all dogs.

3.Effect of breed and sex should be considered in clinical interpretation of the dogs coagulation profile, platelet indices**.**

4. Establish a research center to study Sudanese dogs and foreign dogs under Sudan conditions.

CHAPTER FIVE

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APPENDIX

One scientific paper extracted from the thesis

(Accepted for publication)

1. Abdullah, A.A. and Shadia.A.Omer (2018). Measurement of the prothrombin time , activated partial thromboplastin time and fibrinogen concentration in healthy dogs in Sudan. In *journal of science and Technology*.