Sudan University of Science and Technology

College of Graduate Studies

Prevalence of Lieshmania donovani in Dogs in Jubek State - Republic of South Sudan

انتشار الإصابة بداء اللشمانيا الدونوفاتية في الكلاب في ولاية جوبيك - جمهورية جنوب السودان

A Thesis submitted to the College of Graduate Studies in Partial Fulfillment of the Requirement for the Degree of Master in Preventive Veterinary Medicine (M.P.V.M)

By:

Majok Joseph Ajuong Mayuol

B.V.Sc (2010) College of Veterinary Science,

University of Bahr El-Ghazal

Supervisor:

Dr. Sara Basher Taha Mohammed

May 2019

My father told me that

"Education is the best investment"

Dedication

To my father and my mother

To my brothers and sisters

Acknowledgement

First of all, I thank God for blessing me and gaving me full health and strength to complete this work, I praise you for unchaining me.

My gratitude goes to my Father and my Mother, you are the reason I made it this far, thanks for everything you did to me, for teaching me to love everyone equally, to help everyone in need, to give not to take, to love not to hate and thanks for your overwhelming support.

I would like to express my gratitude and deep appreciations to my supervisor Dr. Sara Basher Taha for her advices, direction and continuous guidance. Actually, all this work is handpicked by Dr. Sara Basher Taha, what you did to me I can't pay you back for it and I would be grateful to you for the rest of my life, without you this work could not have been done. You are such a kind and hard working person; words can't express them selves to describe you. I will be your student forever and no one can take that away from me, thanks for believing in me .

My thanks extend to Prof. Mohamed Tajeldeen for his help in data analysis.

I thank Sudan University of Science and Technology, College of Veterinary Medicine for giving me an opportunity to join their master program of Preventive Veterinary Medicine (M.P.V.M).

My thanks are also extended to Mr.Fayad Osman, Institute of Endemic disease, University of Khartoum for his great help during my work.

Special thanks to each and every single one who collaborated in this work .

List of contents

Title	page
My Slogan	I
Dedication	II
Acknowledgement	III
List of contents	IV
List of tables	VI
List of figures	VII
Abbreviations	VIII
Abstract	IX
ملخص الدراسة	X
Introduction	1
1.Chapter one Literature review	4
1.1 Etiology	4
1.2 Taxonomy	4
1.3 Epidemiology of canine leishmaniasis	6
1.4 Transmission of canine leishmaniasis	7
1.5 Life cycle	8
1.6 Pathogenesis, lesions and clinical signs	9
1.7 Leishmaniasis in other animals	11
1.8 Leishmaniasis in Human	11
1.9 Diagnosis of canine leishmaniasis	12
1.9.1 Clinical finding	12
1.9.2 Direct microscopic examination	12
1.9.3 Polymerase Chain Reaction (PCR)	13
1.9.4 Enzyme-Linked Immunosorbent Assay (ELISA)	13
1.9.5 Direct Agglutination Test (DAT)	14
1.10 Treatment	14
1.11 Control	16
1.12 Economic impacts of leishmaniasis	18
2. **Chapter two Material and methods**	19
2.1 Study area	19
2.2 Study design	21
2.3 Sample size	21
2.4 sampling method	21
2.5 Blood samples	21
2.6 Microscopic examination	22
2.6.1 Giemsa stain	22
2.6.2 Leishman stain	22
2.6.3 Microscopic examination test	22

2.7 Polymerase Chain Reaction (PCR) test	22
2.7.1 DNA Extraction	23
2.7.2 PCR amplification	23
2.7.3 Detection of the PCR product	24
2.8 Questionnaire	24
2.9 Statistical analysis	24
3**. Chapter three Results**	25
3.1 Results of clinical examination	25
3.2 The overall prevalence of Canine Leishmaniasis	26
3.3 Prevalence of Canine leishmaniasis in relationship to the general potential risk factors	27
3.3.1 Categories of dogs	27
3.3.2 Sex	28
3.3.3 Age	28
3.3.4 Breed	28
3.3.5 Body condition	28
3.3.6 Hygiene	28
3.3.7 Contacts with other animals	28
3.4 Prevalence of Canine leishmaniasis in relationship to the potential risk factors that related to the domestic dog	30
3.4.1 Type of roaming	30
3.4.2 Occupation of dog	30
3.4.3 Knowledge of canine leishmaniasis	30
3.4.4 Knowledge of zoonotic effect of leishmaniasis	30
3.4.5 Vaccination	30
3.4.6 Visiting of the veterinary clinic	31
4**. Chapter four Discussion**	33
Conclusion	35
Recommendations	35
References	36
Appendix	45

List of tables

Title	Page
Table 1: Taxonomy of Leishmania parasites	5
Table 2: Leishmania species reported in dogs in new and old worlds	7
Table 3: Drugs and combination used	15
Table 4: Commercialized vaccines	16
Table 5: Commercialized topical insecticide	17
Table 6: Summary of clinical signs observed in the examined dogs, Jubek State, Republic of South Sudan(N=103 dogs)	25
Table 7: The number and distribution of animals examined for Canine leishmaniasis	26
Table 8: Summary of univariate analysis for risk factors associated with infection with leishmaniasis in Jubek State, Republic of South Sudan (N=103 dogs) using the Chi-square test	29
Table 9: Summary of univariate analysis for risk factors associated with infection with leishmaniasis in Jubek State, Republic of South Sudan (N=88 dogs) using the Chi-square test	32

List of figures

Title	Page
Figure 1: Based on scheme published by WHO	5
Figure 2: Life cycle of Leshmania parasite	9
Figure 3: Shows Jubek State "the study area" – Map	20
Figure 4: Shows PCR test results	27

List of abbreviations

WHO: World Health Organization

DNA: DeoxyriboNuclic Acid

CanL: Canine Leishmaniasis

CL: Cutaneous Leishmaniasis

MCL: Mucocutaneous Leishmaniasis

VL: Visceral leishmaniasis

PBS: Phosphate Buffered Saline

PH: Potential Hydrogen

WBC: White Blood Cells

Hrs: Hours

Leishmania spp.: Leishmania species

IFT: Immunoflourescense test

Abstract

A cross-sectional study was conducted from January to February 2018 to assess the prevalence of canine leishmaniasis in Jubek State, Republic of South Sudan. In addition to that, risk factors could be associated with the disease were also investigated.

A total of 103 blood samples were collected from cephalic vein of dogs. The overall prevalence of canine leishmaniasis was 0% using microscopic examination of Geimsa and Leishman stained blood smear.

For DNA detection, the overall prevalence of *Leishmania donovani* was also 0% using PCR test.

The analysis of risk factors that could associate with the disease using Chi-square test shown that, there was no significant between these risk factors and the disease.

ملخص الدراسة

اجريت هذه الدراسة المقطعية في فترة ما بين يناير/كانون الثاني و فبراير/شباط 2018م لتقييم انتشار الاصابة بداء اللشمانيا في الكلاب في ولاية جوبيك, جمهورية جنوب السودان . بالاضافة الي ذلك تم التحقق من العوامل الخطر المرتبطة بالمرض.

تم جمع مجموعة 103 عينة من الدم عن طريق الوريد الرأسي للكلاب . كان الانتشار الكلي لداء اللشمانيا في الكلاب 0% باستخدام الفحص المجهري للشرائح المصبوغة بصبغة جمسا او ليشمان. للكشف عن الحمض النووى, كان الانتشار الكلي للشمانيا الدونوفانية في الكلاب 0% باستخدام اختبار تفاعل البوليميراز المتسلسل.

بعد تحليل عوامل الخطر المرتبطة بالمرض باستخدام اختبار مربع كاي, وجدت انه لا توجد علاقة معنوية ما بين عوامل الخطر و المرض.

INTRODUCTION

Leishmaniasis is vector - borne disease caused by flagellated protozoans of the genus Leishmania. The disease is widespread in tropical and subtropical areas and found in 98 countries in Europe, Africa, Asia and America. However, over 90% of new cases occur in just 13 countries (Afghanistan, Algeria, Bangladesh, Bolivia, Brazil, Columbia, Ethiopia, India, Iran, Peru, South Sudan, Sudan and Syria) (WHO, 2016; Alvar *et al*., 2012). The parasite is categorized into two main groups; the old world species occurring in Europe, Africa and Asia, and the new world species occurring in America (Cox, 1993).

About 53 species of parasite have been described from the different region of the world; of these, 31 species are known to be parasites of mammals and 20 species are pathogenic to humans. Many of the Leishmania species infecting humans are zoonotic, having a complex variation in domestic and wild mammal reservoir hosts; while, other species of parasite are anthroponotic, having human-to-human transmission in the presence of the vector (Alvar *et al*., 2012).

Dogs have been found naturally infected by several species of Leishmania. Although their role as reservoir hosts of these parasites is probably negligible; that is, they are more likely to be victims rather than reservoirs. In a published paper emphasizes the utility of PCR in discriminating species of Leishmania infecting dogs, particularly in areas where both visceral and cutaneous leishmaniasis are endemic (Gomes *et al*., 2007; Elbihari *et al*., 1987).

Not only the domestic dogs but canids, in general fulfill the required attributes to be efficient reservoirs of *L.infantum*. Due to its close relationship with humans, the domestic dog has long been implicated as the

main reservoir of *L.infantum* in China, Mediterranean basin and the Americas. (Dantas-Torres and Brandaˇo-Filho, 2006; Alvar *et al*., 2004).

Domestic dogs are also an important reservoir of *L.donovani* in eastern Sudan and other parts of East Africa (WHO, 2002; Dereure *et al*., 2000).

As far it is known, the dog is not the main reservoir host of the aetiological agents of zoonotic cutaneous leishmaniasis; except for *L.Viannia* and *L.peruviana*, the aetiological agent of Uta, and a typical localized ulcerative cutaneous leishmaniasis whose geographical distribution is restricted to the Peruvian Andes (Gramiccia and Gradoni, 2005).

The origin of domesticated dog is not clear; it is known that the dog was the first domesticated species. The domesticated dog is a member of the genus Canis (canines), which forms a part of the Wolf-like canids (Larson and Bradley, 2014; Lindblad-Toh *et al*., 2005).

It is estimated that three-quarter of the world's dogs population living in developing world as feral, village, or community dogs, with pet dogs uncommon (Coppinger, 2001).

In the Republic of South Sudan, dogs are used to protect against thieves as security guard or machine in compounds. Also, they are used by local people to herd cattle, sheep, and goats, and during hunting trips in the bush.

The value of dogs in the Republic of South Sudan differs from area to area depending on the service they deliver to the owner, pastoralist communities such as Dinka, Nuer, Shilluk, Mondari, and Bari people and all other cattle keepers are very serious about their dogs because they help them on the range. For those who live in big cities such as Juba, Wau, Aweil, Malakal and the rest of the big cities of the country they used dogs also as a pet.

Objectives

The objectives of this study are:

1. To determine the prevalence of canine leishmaniasis in Jubek State, Republic of South Sudan.
2. To assess the association between dog infection and the risk factor of the disease.
3. To identify the geographical distribution of the disease in Jubek State

Chapter one

Literature review

1.1 Etiology

Leishmaniasis is a vector-borne disease caused by flagellated protozoans of the genus Leishmania. The disease is spread by sandflies of the genus Phlebotomus in the Old World and the genus Lutzomyia in the New World. At least 93 sandfly species are proven or probable vectors worldwide (WHO, 2010; Myler and Fasel, 2007; WHO, 2003).

About 70 species of mammals, including humans are considered vertebrate hosts of different species of Leishmania around the world, and some of them are reservoirs of the parasite in nature, although the natural infection in rodents and Canids is more common, the parasite is able to infect felids, odd-toed ungulates and primates (Ribeiro *et al*., 2018).

1.2 Taxonomy

The classification of Leishmania was initially based on ecobiological criteria such as vectors, geographical distribution, tropism, antigenic properties and clinical manifestation. However, biochemical and molecular analysis showed that pathological and geographical criteria were often inadequate and thus other criteria such as the pattern of polymorphism exhibited by Kinteplastic DNA (KDNA) marker, proteins or antigens came to be used to classify Leishmania (Lainson and Shaw, 1987; Barker *et al*., 1986; Le Blaneq *et al*., 1986).

A modern scheme of classification of Leishmania is shown in Figure 1 (based on the scheme published by the WHO, 1990).

All members of the genus Leishmania are parasites of mammals; the two subgenera; *Leishmania* and *Viamnia* are separated on the basis of their location in the vector's intestine, used isoenzyme analysis to define species

complexes within the subgenera (Rioux *et al*., 1990; Lainson and Shaw, 1987).

Table 1: Taxonomy of Leishmania parasites (Bari and Rahman, 2008).

Kingdom	Protozoa
Subkingdom	Protista
Phylum	Sarcomastigophora
Sub-phylum	Mastigophora
Class	Zoomastigophora
Order	Kinetoplastida
Suborder	Trypanosomatina
Genus	Leishmania

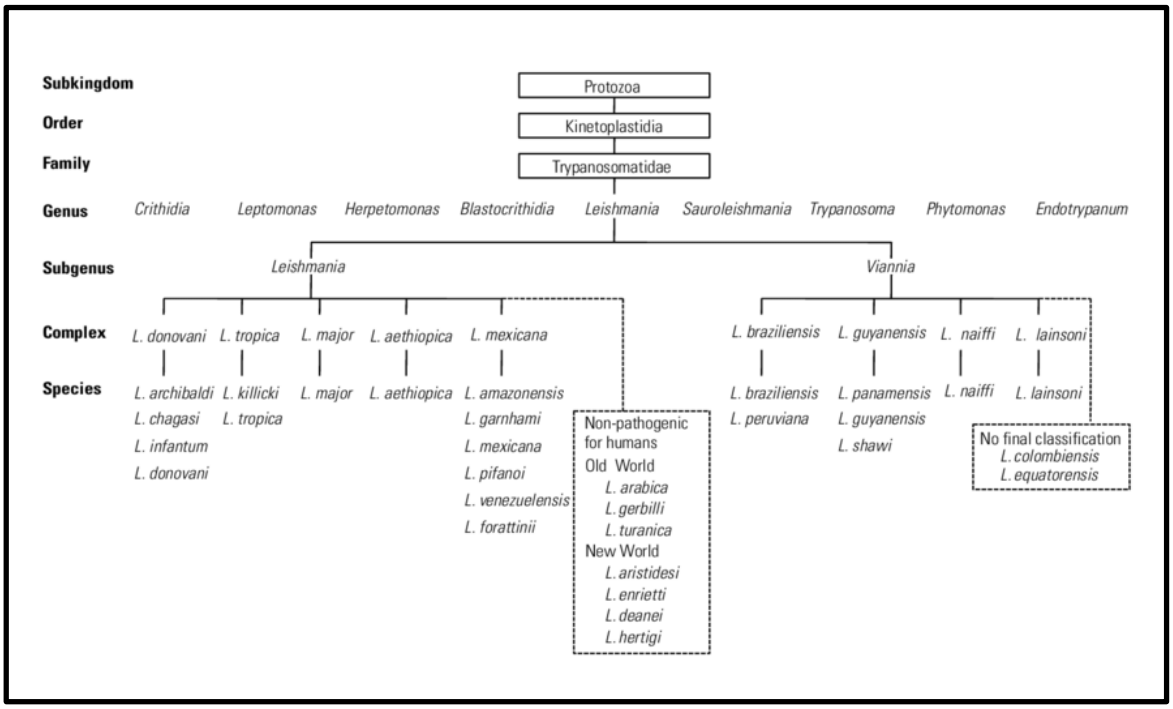

Figure 1: Based on scheme published by WHO (WHO, 1990).

1.3 Epidemiology of Canine Leishmaniasis

Canine leishmaniosis (CanL) is a vector-borne disease caused by protozoa of the genus Leishmania (Kinetoplastida: Trypanosomatidae) that affects dogs from all continents except Oceania. Although dogs have been found infected by at least 13 species of Leishmania (see Table 2), the most important etilogical agent of CanL is *L.infantum* (syn. *L.Chagasi*), which cause visceral and cutaneous disease in humans in some countries in Europe, the Middle East, the Far East, Africa, and Central and South America. CanL is prevalent in approximately 50 countries, occurring mainly in South America and Mediterranean region (Colwell *et al*., 2011; Dantas-Torres, 2009; Solano-Gallego *et al*., 2009; Chappuis *et al*., 2007).

Recent scientific evidence on new data and historical records on CanL indicated the spread of the infection to regions previously regarded as non-endemic, as is the case of some areas of northern Italy, southern Brazil and northern Argentina (Colwell *et al*., 2011; Otranto *et al*., 2009; Tomaz-Soccol *et al*., 2009; Salomon *et al*., 2008).

The epidemiology and distribution of vector-borne diseases may be affected by changes in vector ecology and movements of human and dog populations, which in turn might be linked to factors such as climate changes, deforestation and changes in land use practices. The establishment of CanL in new geographical areas is also associated with increased movement of both infected (e.g., dogs that had traveled to or have been adopted from endemic areas) and susceptible populations.

For instance, the number of reports of *L.infantum*-infected dogs in non-endemic areas in Europe such as Germany and the United Kingdom are on the rise. This currently represents a great concern mainly because veterinarians and physicians in these countries usually do not know how to deal with such an exotic disease (Mencke, 2011; Shaw *et al*., 2009).

Table 2: Leishmania species reported in dogs in new and old worlds.

Leishmania spp. [a]	Geographical distribution [c]	Refs
L.amazonensis	Brazil	(Maroli *et al*., 2012)
L.arabica	Saudi Arabia	(Maroli *et al*., 2012)
L.braziliensis	South America	(Maroli *et al*., 2012)
L.colombiensis	Venezuela	(Maroli *et al*., 2012)
L.guyanensis	Colombia	(Maroli *et al*., 2012)
L.infantum	Africa, America, Asia, Europe	(Maroli *et al*., 2012)
L.major	Egypt, Saudi Arabia	(Maroli *et al*., 2012)
L.mexicana	Ecuador, USA	(Maroli *et al*., 2012)
L.panamensis	Colombia, Ecuador, Panama	(Maroli *et al*., 2012; Valez *et al*., 2012)
L.peruviana	Peru	(Maroli *et al*., 2012)
L.pifanoi	Ecuador	(Maroli *et al*., 2012)
L.tropica	India, Iran, Morocoo, Syria	(Maroli *et al*., 2012)
L.donovani	Southmost western Ghats in India	(Jambulingam *et al*., 2017)

1.4 Transmission of Canine Leishmaniasis

The transmission chain of Leishmania spp. involves complex interaction between parasites, vectors, vertebrate hosts and different ecotopes. They transmitted primarily by the hematophagous activities of the female phlebotomine sand flies belonging to the genera Lutzomyia (New World) and Phlebotomus (Old World) (Kaye and Scott, 2011).

The main route of transmission of parasite is via the bite of female phlebotomine sandfly, the vector ingests the parasite while blood-feeding and then transmits the infective stages during a following blood meal (Naucke *et al*., 2016).

Other than insect route, CanL can be transmitted vertically and venereally and through transfused blood products from infected donors. A suspected mode of transmission is the direct dog-to-dog transmission of the parasite by wounds or dog bites (Karkamo *et al*., 2014; Naucke and Lorentz, 2012; Tabar *et al*., 2008).

1.5 Life cycle

The parasite has digenetic lifecycle, alternating between a mammalian host and insect vectors. In short according to the literature, when a sand fly bites an infected host, it also ingests macrophages infected by rounded and non-motile amastigote forms. Then, the parasites transform from the amastigote to the flagellate promastigote stage, multiply by binary fission in the midgut, and migrate to the foregut and in mouthparts (pharynx, cibarium and proboscis) of the infected sand fly vector. Subsequently, it can be transmitted to other new hosts, where these flies feed on blood meals, and the invertebrate cycle is concluded.

When the infectious promastigote forms are inoculated from vector's proboscis into the host's skin, they are phagocytized by macrophages. They then evolve into the amastigote form, where reproducing asexually and continuously in macrophages until rupture occurs. The parasites spread by invading mononuclear phagocytes in many organs, mostly spleen, liver, bone marrow, lymph node, and other tissues (Bates, 2007).

Other blood-feeding arthropods, such as ticks or fleas have sometimes been suspected of transmitting Leishmania based on the association of CanL with the presence of these alternative vectors (De Oliveira *et al*., 2015).

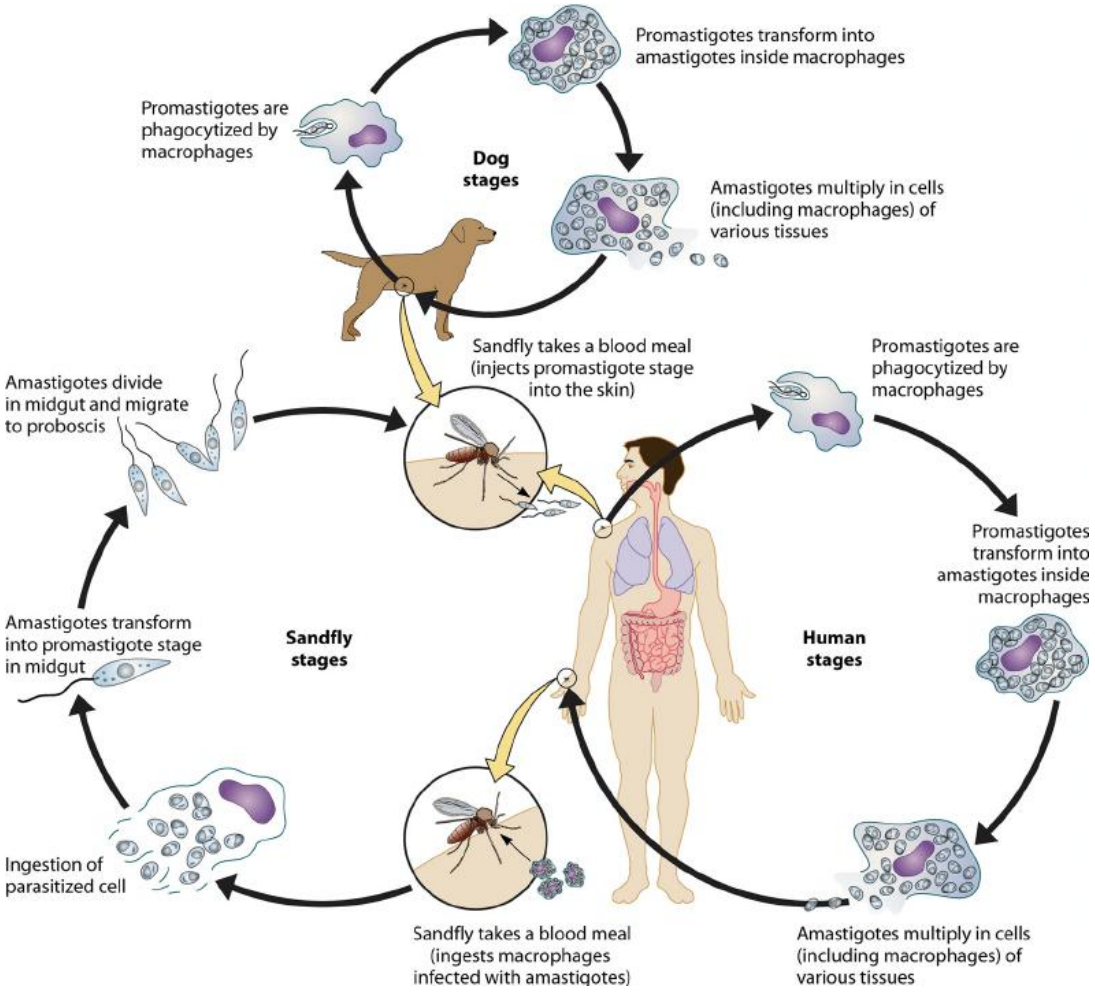

Figure 2: Life cycle of Leishmania parasite (Esch and Petersen, 2013).

1.6 Pathogenesis, lesions and clinical signs

Leishmania parasites exist as two developmental forms: intracellular amastigotes in mammalian macrophages and flagellated promastigotes within the sand fly vector. Promastigotes are inoculated into the bite wound of the mammalian host during sand fly blood feeding. Once in the mammal, promastigotes are opsonized with complement component C3. Mac-1, the integrin receptor for iC3b, is present on the surface of macrophages. Surface bound C3 binds to Mac-1 and is followed by phagocytosis of the promastigote. Once internalized, the phagosome, which contains the promastigote, fuses with lysosomes to form a phagolysosome. The mature phagolysosome is the major site of microbicidal activity in macrophages due

to its low pH and production of toxic radicals such as nitric oxide. Promastigotes slow the phagosomal maturation process during which time they are in the process of transforming to amastigotes and are sensitive to acidic pH. 24hours following phagocytosis, amastigotes are formed within the macrophage. Amastigotes survive and proliferate in low pH until eventually the host macrophage lyses and releases amastigotes. Newly released amastigotes are opsonized with host IgG that binds to Fcγ receptors on macrophages, the parasites are quickly distributed to the lymph node and spleen via blood or lymph, and from there they go to the kidney and liver. Later, the parasites spread to the reproductive organs, skin, bladder, digestive and respiratory systems, etc. (Mosser and Brittingham, 2002; Matlashewski, 2002; Molyneux and Ashford, 1983).

The typical histopathologic finding in canine leishmaniasis is granulomatous inflammation associated with a variable number of Leishmania amastigotes with macrophages. Deposition of immune complexes in kidney, blood vessels and joints occur as the infection progresses. Glomerulonephritis associated with the renal immune complexes is a hallmark of this disease. Renal pathology, including glomerulonephritis and interstitial nephritis is evident by histopathology (Merck, 2019).

CanL is a systemic disease that may potentially involve any organ, tissue or body fluid and is often manifested by nonspecific clinical signs. The clinical course varies from a symptomatic infection to a life-threating generalized disease. Skin lesions are the most frequent manifestations. However, dogs can be presented with other clinical signs unrelated to cutaneous lesions. Other common clinical presentations are renal, ocular and articular lesion. In the majority of cases lymphadenomegaly, lethargy, emaciation and muscular atrophy are observed. Chronic proteinuric nephritis that may progress to end-stage kidney disease is the main cause of mortality due CanL (Koutinas, 2014; Solano-Gallego *et al*., 2011).

1.7 Leishmaniasis in other animals

Several species of wild and domestic and synanthropic mammals have been recorded as hosts and/or reservoirs of Leishmania spp. in different parts of the world. Rock hyraxes, Rodents, cats, foxes, jackals, wolves, bats, primates and other domestic animals are among the multihost reservoirs to maintain transmission of leishmaniasis in different localities, however, Leishmania reservoirs are so complex that they show regional and temporal variation, and the only a local studies involving ecological and parasitical analysis can determine whether these animals are playing a role as reservoirs in a given environment (Rohousova *et al*., 2015; Roque and Jansen, 2014; Raymond *et al*., 2003).

1.8 Leishmaniasis in Human

Sixteen well recognised Leishmania species are agents of human leishmanioses, In the New World, tegumentary forms are caused by *L.braziliensis*, *L.guyanensis*, *L.panamenisis*, *L.shawi*, *L.naïffi, L.lainsoni, L.lindenbergi*, *L.peruviana*, *L.mexicana*, *L.venezuelensis* and *L.amazonensis* ; visceral and more rarely cutaneous forms are caused by *L.infantum.*

In the Old World, cutaneous forms are caused by *L.tropica*, *L.major* and *L.aethiopica* (Kuhls *et al*., 2011).

Leishmnaiasis in humans have 3 main forms:

a) Cutaneous leishmaniasis (CL); is the most common form of leishmaniasis , causes skin lesions, mainly ulcers on the exposed parts of the body leaving life-long scars and serious disability. About 95% of CL cases occur in the Americas, the Mediterranean basin, the Middle East and Central Asia. It is estimated that between 600 000 to 1 million new cases occur worldwide annually (WHO, 2014).

b) Mucocutaneous leishmaniasis (MCL); leads to partial or total destruction of mucous membranes of the nose, mouth and throat. Over

90% of mucocutaneous leishmaniasis cases occur in Bolivia, Brazil, Ethiopia and Peru (WHO, 2014).

c) Visceral leishmaniasis (VL); also known as Kala-azar is fatal if left untreated in over 95% of cases. It is characterized by irregular bouts of fever, weight loss, enlargement of the spleen and liver, and anemia. Most cases occur in Brazil, East Africa and South East Asia. An estimated 50 000 to 90 000 new cases of VL occur worldwide each year (WHO, 2014).

1.9 Diagnosis of Canine leishmaniasis

1.9.1 Clinical finding:

The incubation period prior to the appearance of clinical signs may last 3 months up to several years. The intensity of the disease is determined by a set of factors involving parasite strain, parasite genetics and the host immune status (Rebeiro *et al*., 2018; Baneth *et al*., 2008).

The main clinical signs associated with CanL are dermal lesions, lymphadenomegaly, splenomegaly, onychogryphosis and poor condition. An additional finding includes epistaxis, renal failure, decreased appetite, polyuria, polydipsia, vomiting, melena and lameness (Solano-Gallego *et al*., 2009).

1.9.2 Direct microscopic examination

In CanL, isolation and identification of the parasite from biopsies (lymph node, bone marrow, and spleen aspirate) coupled with molecular and immunodiagnostic tests are recommended. Buffy-coat preparation of peripheral blood or aspirate from bone marrow, spleen, lymph nodes or skin lesions should be spread on a slide to make a thin smear and stained with Leishman stain or Giemsa stain (PH 7.2) for 20 minutes. Amastigotes are seen within blood and spleen monocytes, or less commonly in circulating neutrophils and aspirated tissue macrophages. They are small, round bodies 2-4 um in diameters with indistinct cytoplasm, a nucleus and small rod-

shaped Kinetoplast. Occasionally, amastigotes may be seen lying freely between cells (OIE, 2014; Dacie *et al*., 2006).

1.9.3 Polymerase chain reaction (PCR)

PCR methods are available for diagnosis and/or identification of Leishmania from different types of animal samples. Essentially, this technigue developed either to established isolates of Leishmania or to detect organisms from fresh or frozen, formalin-fixed and paraffin-embedded tissue biopsies, include: (a) digestion of material with proteinase K and DNA extraction. These steps can be either performed using in-house protocols and reagents, or by commercial kits that are widely available, (b) standard PCR amplification using oligonucleotide sequences (primers) selected from the small-subunit rRNA gene, kinetoplast DNA minicircles or other highly repetitive genomic DNA sequences, (c) analysis of amplification products by 1–2% agarose gel (Bulle *et al*., 2002; Mathis *et al*., 1995; Maarten *et al*., 1992).

1.9.4 Enzyme-linked immunosorbent assay (ELISA)

The ELISA can be carried out on serum or on a measured volume of blood. The blood is collected by needle-prick on to suitable absorbent paper strips and allowed to dry. The sample is eluted and tested at a single dilution previously determined to give an acceptable sensitivity and specificity. This test can be used for seroepidemiological surveys under field conditions. In the classical method, the antigen is prepared as follows: promastigotes harvested from cultures are washed four times with PBS, PH 7.2, at 1000 *g* for 15 minutes. The packed promastigotes are resuspended in twice their volume of distilled water and then sonicated at medium amplitude in an ice bath. The suspension is left at 4°C overnight to allow the proteins to come into solution. After final centrifugation at 4000 *g* for 10 minutes to eliminate the cellular debris, the overlay, representing the concentrated soluble antigen, is dispensed into vials and stored at –20°C until required. For use in the test, it is reconstituted with PBS to the predetermined optimal protein

concentration (around 20 μg/ml) as measured by Lowry's method. The enzyme (usually horseradish peroxidase)-conjugated reagents consist of anti-dog goat immunoglobulins or Protein A (Hamarsheh *et al*., 2012).

1.9.5 Direct agglutination test (DAT)

The direct agglutination test (DAT) has been described for the diagnosis of CanL. After test improvement, DAT has been validated as a specific and sensitive assay for field investigations. The antigen consists of promastigotes harvested from cultures, washed in PBS, pH 7.2, treated with 0.4% trypsin (for 45 minutes at 37°C and then washed again), and stained with 0.02% Coomassie brilliant blue. Two fold serial dilutions of serum in PBS are made in V-bottomed microtitre-plate wells; 50 μl of antigen preparation is added to each well, and the plate is then carefully shaken by hand and left for 18 hours at room temperature. The test is read visually against a white background. Positive reactions are indicated by typical light-blue aggregates, while negative samples give a clear sharp-edged blue spot (OIE, 2014; Cardoso *et al*., 2004).

A modified DAT for detection of specific anti-leishmanial antibodies in canine reservoir hosts is considered to be highly suitable for wide-scale epidemiological and ecological field work and diagnosis of CanL, having 100% sensitivity and 98.9% specificity. The reliability of the test was improved by treating the test sera with 0.2 M 2-mercaptoethanol and incubating them at 37°C (Harith *et al*., 1988; 1989).

1.10 Treatment

Even though parasitological cures are rarely achieved and clinical recurrences in CanL often occur after therapy, it is necessary to consider that the available protocols can promote clinical cure, increase the life expectancy and improve the quality of life, in addition to reducing the parasite load and infectiousness to sand fly vectors. Thus, the decision to treat a diseased dog is the result of a discussion between the dog owner and

the veterinarian. An important factor analyzed is the owner's ability and/or willingness to comply with the treatment protocol, in addition to the assessment of the dog's potential responsiveness to therapy by a complete serologic, hematologic, and chemical profile and urine analysis in order to evaluate, principally, the bone marrow and renal and hepatic status. According to the literature, the clinical response to treatment can vary from poor to good depending on their overall initial clinicopathological status and their specific response to therapy. For instance, dogs with renal insufficiency are expected to have a lower recovery rate in comparison to those without compromised kidneys or only mild proteinuria. For reasons of public health and to prevent reinfection, the constant use of permethrin spot on and/or flumethrin or deltamethrin impregnated collars in treated dogs and continuous veterinary monitoring is necessary (Gharbi *et al*., 2015; Solano-Gallego *et al*., 2011).

Table 3: Drugs and combination used (Reguera *et al*., 2016).

Active ingredient	**Therapeutic protocols**	**Potential adverse effects**
Allopurino	10 mg/kg BID P.O. for at least 6-12 months or lifelong	Xanthine urolithiasis
Amphotericin B deoxycholate	0.5 mg/kg I.V. twice per week for 2 months	Nephrotoxicity
Meglumine antimoniate	75-100 mg/kg SID S.C. for 4 weeks	Nephrotoxicity
Miltefosine	2 mg/kg SID P.O. for 28 days	Digestive disorder
Allopurinol + meglumine antimoniate	10 mg/kg BID P.O. for 12 months; 100 mg/kg SID S.C. for 4 weeks	Urolithiasis and nephrotoxicity
Allopurinol + bmiltefosine	10 mg/kg BID P.O. for 12 months; 2 mg/kg SID P.O. for 28 days	Urolithiasis and nephrotoxicity

1.11 Control

Considering that sand fly bite is the most important route of transmission of CanL, the infection control measures should be primarily focused on preventing contact with the insect vector, either through physical barriers (fine mesh nets in the windows and kennels), chemical barriers (repellents) or handling (avoiding exposure to twilight, eliminating organic peridomicillary material). Predicting a large possibility of failure of these measures, the dog still needs to be able to respond to the infection challenge caused by the bite of infected sand flies, preferentially by an adaptive immune response previously developed through vaccination, or as a last alternative by chemotherapeutics which boost the immune system to help fight infection (Ribeiro *et al*., 2018).

Table 4: Commercialized vaccines

Trade name/licensed	**Antigens/ adjuvant**	**Efficacy in field studies (references)**
Canileish*/Virbac	Excreted-secreted proteins of L.infantum (LiESP)/QA21	68.4% (Oliva *et al*., 2014)
Leish-Tech*/Hertape Calier	Recombinant protein A2 of L.donvani/Saponin	71.4% (Regina-Silva *et al*., 2016)
Leishmune*/Zoetis(marketing temporarily suspended)	Fucose-Manose Ligand (FML) of L.donovani/QS21	76-80% (Palatnik-de Sousa, 2012)
LetiFend*/Leti + MSD-Animal Health	Recombinant Protein Q from L.infantum/none	72% (CVMP, 2016)

Table 5: Commercialized topical insecticide.

Trade name/licensed	Pharmaceutical compounds/application form/duration	Efficacy in field studies (references)
Scalibor/MSD-Animal Health	4% Deltamethrin/impregnated PVC collar/4-6 months	50-86%; 61.8% (Brianti *et al*., 2016)
Seresto/Bayer Animal Health	10% imidacloprid + 4.5% flumethrin/impregnated PVC collar/8 months	88.3% (Brianti *et al*., 2016)
Advantix/Bayer Animal Health	10% imidacloprid + 50% permethrin/spot-on/3 weeks	88-90.4% (Otranto *et al*., 2007)
Exspot/MSD-Animal Health	65% permethrin/spot-on/2-3 weeks	84% (Ferroglio *et al*., 2008)
Frontect or Frontline Tri=Act/Merial	6.76% fipronil + 50.48% permethrin/spot-on/3 weeks	100% (Papadopoulos *et al*., 2017)
Effitix or Fiprotix or Fipratix/Virbac	6.1% fipronil + 54.5% permethrin/spot-on/4 weeks	-
Perfikan/Clement Thekan	6.1% fipronil + 54.5% permethrin/spot-on/4 weeks	-
Caniguard Line on/Beaphar	40% permethrin/spot-on/5 weeks	-
Vectra 3D/Ceva	4.95% dinotefuran + 36.08% permethrin + 0.44% pyriproxyfen/spot-on/4 weeks	-

1.12 Economic impacts of Leishmaniasis

For its potential severity in dogs and its zoonotic nature, the prevention of this infection is not only desirable, but also a must for both dog and human health, the management of this disease is extremely complex (Maroli *et al*., 2013; Dantas-Torres *et al*., 2012)

Medical expenditures, direct non-medical costs comprise expenditure for transport, food costs and other daily expenditures for the patient and accompanying family members. The indirect cost of an episode represented the loss of productivity within the household due to illness (wijerathna *et al*., 2018).

The economic burden of CanL, includes the costs associated with prevention, treatment and mortality losses (Mattin *et al*., 2013).

Chapter two

Material and methods

2.1 Study area

Republic of South Sudan is a landlocked country in East-Central Africa. South Sudan is bordered by Sudan to the north, Ethiopia to the east, Kenya to the southeast, Uganda to the south and the Central African Republic to the west.

South Sudan lies between latitude 3 and 13 ° N and longitudes 24 and 36 °E. It is covered in tropical forest, swamps and grassland. The White Nile passes through the country, passing by Juba.

South Sudan has a climate similar to an Equatorial or tropical climate, characterization by a rainy season of high humidity and large amounts of rainfall followed by a drier season. The temperature average is always high with July being the coolest month with an average of temperature falling between 20 and 30 ° C (68 and 86 ° F) and March being the warmest month with average temperature ranging from 23 to 37 ° C (73 to 98 ° F).

The most rainfall is seen between May and October but the rainy season can commence in April and extend until November. On the average May is wettest month. The season is "influenced by the annual of the inter-tropical Zone" and the shift to southerly and southwesterly winds leading to slightly lower temperatures, higher humidity and more cloud coverage (Ministry of Housing, Physical Planning and Environment, Juba).

A variety of livestock are reared in South Sudan including cattle, goats, sheep and chicken, livestock are the main source of livelihood in many households in the country. Nilotic peoples are the majority of its population (Ministry of Animal Resources and Fisheries, Juba).

Juba is the capital and largest city of the Republic of South Sudan. The city is situated on the White Nile and also serves as the Capital of the Jubek State (see Figure 3).

Jubek state is one of the states of South Sudan, located within the Equatorial region; the state borders include Yei River State to southwest, Amadi State to the west, Terkeka State to the north and Imatong State to the east.

Jubek state consists of 14 counties:

- Eastern bank of the Nile; Rejaf county, Lobonok county, Mangala county, Liria county, Lokiliri county and Kondokoro (is an island) county.
- Western bank of the Nile; Lado county, Luri county, Rokon county, Dolo county, Wanduruba county, Bunga county and Ganji county.
- Juba city council divided; Juba payam, Kator payam and Munuki payam.

Jubek state is what Juba county in the then Central Equatoria State was before the presidential order that to create 28 states on October, 2, 2015 (Ministry of Housing, Physical Planning and Environment, Juba).

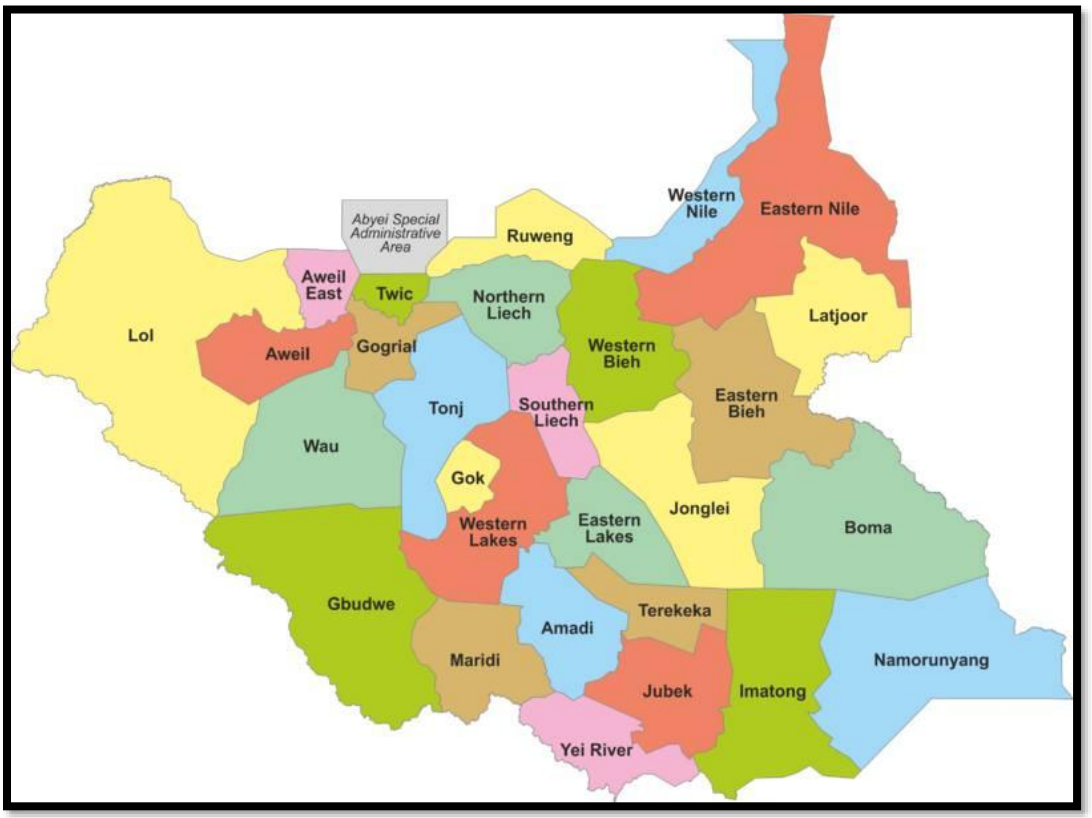

Figure 3: Shows Jubek State "the study area" (Paanluelwel.com).

2.2 Study design

The study was a cross-sectional study performed to estimate the prevalence of canine leishmaniasis and the risk factors associated with the disease.

2.3 Sample size

The sample size of animals was determined using the formula of Thrusfield (2007).

n= 〖 (1.96) 〗^2. Pexp (1-Pexp) /d^2

n= sample size

P_{exp} = expected prevalence

d = desired absolute precision (d=0.05)

One hundred and three samples were taken randomly from dogs in Jubek State.

2.4 Sampling method

The samples were taken from four counties. 103 blood samples were randomly collected by using multi-stage technique. Based on this technique Republic of South Sudan was divided into States, from these States Jubek States was selected, Jubek State was divided into counties, counties were Juba county, Luri county, Kondokoro county and Rejaf county. Samples were taken randomly from these counties.

2.5 Blood samples

The whole blood samples were collected from cephalic vein as is the easiest way to collect blood from dogs after restraint (CTP Veterinary Assistant Website, 2017). Approximately 3-5 ml was taken from cephalic vein by using labeled disposable 2cc syringe after disinfecting the site of injection. The blood was transferred into tubes containing EDTA (Ethylene Diamine Tetra-acetic Acid).

2.6 Microscopic examination

2.6.1 Giemsa stain

Thin smears were prepared by applying one drop of blood onto a microscopic slide near the edge. At the angle of 45 °, another slide was placed and then the blood was spread by gently moving forward of the slide. The smears were first left for air dried, then fixed for 3 seconds using methyl alcohol (absolute methanol) by putting a few drops of methanol on the slide. Then the slide is immersed in a freshly prepared Giemsa stain solution for 20-30 minutes and then flushed with tap water and left to dry.

2.6.2 Leishman stain

Thin smears were prepared as described previously. The smears were first left for air dried, then stained using leishman's stain for 5 minutes.

2.6.3 Microscopic examination test

These slides were transferred to Endemic Disease Institute, University of Khartoum (Khartoum, Republic of Sudan) for microscopic examination.

Fifty microscopic filed were examined under immersion lens oil (100×magnification). The presence of one amastigote was considered as positive cases. Amastigote, are small intracellular rounded or oval body, 1.5–3 × 2.5–6.5 μm in size, found in vacuoles within the cytoplasm of the macrophages. There is no free flagellum. The organism has a relatively large nucleus and the kinetoplast consisting of a rod-like body and a dot-like basal body considered positive (OIE, 2014).

2.7 Polymerase chain reaction (PCR) test

After completed the collection, the blood samples were transferred to Endemic Disease Institute, University of Khartoum (Khartoum, Sudan) for PCR test.

2.7.1 DNA extraction

DNA was extracted from the whole blood sample. 700µl from blood was added to 700µl of Red Cell Lysis Buffer (RCLB) in Eppendorf tube (1.5ml) and then vortexed and centrifuged for 10 min at 6000 rpm. 400µl of WBCs Lysis Buffer, 200µl of Guanidine Chloride, 50µl of NH4 and 5µl of proteinase K were added to the mixture and then vortexed and incubated at 37°C overnight .

The mixture was cooled to the room temperature and then 400µl from cold Chloroform (-20˚C) was added then vortexed and centrifuged for 10 min at 6000 rpm. the mixture was separated into 3 layers.

400µl of the upper layer was collected into new Eppendorf tube(1.5ml) and 1 ml of cold absolute ethanol was added then shaken and kept at -20˚C overnight to precipitate the DNA. After that, the mixture was centrifuged at 6000 rpm for 10 min and the supernatant was removed carefully.

The pellet was washed with 400µl of 70% ethanol, then centrifuged at 6000 rpm for 10 min and the supernatant was carefully removed then the pellet allowed to dry for 2 hours on sterile tissue at room tempreature.

The pellet re-suspended in 30µl dH2O, then briefly vortexed and put at 4˚C overnight. The DNA was stored at -20˚C.

2.7.2 PCR amplification

Two primer pairs Forward (5'GGTTCCTTTCCTGATTTAGG3') and Reverse (5'GGCCGGTAAAGGCCGAATAG 3') were used to amplify gene sequences of L. donovani . PCR reactions were performed in 25 µl volume containing 18 µl of PCR- water containing 10x PCR buffer, 2 µl of extracted DNA template, 1 µl of each primer (10 µM), 2 µl of 50x dNTP mix and 2 µl of the 50x polymerase mix. PCR program was comprised at 95°C for 2 min followed by 35 cycles of 95°C for 30 s, 54°C for 30 s, 72°C for 1 min and a final extension step at 72°C for 5 min.

2.7.3 Detection of PCR product

The PCR product was detected by Electrophoresis, 5 µl of loading Buffer were added to PCR product, the loading on 2% of agarose gel and stained with 2 µl Ethidium Bromide (10 mg/ml) and run for 2 hours in 10 X TBE buffer (1M Tris, 1M Boric acid and 50 M EDTA) at 90 V, and photographed under a standard UV transilluminator.

2.8 Questionnaire

Data regarding the characteristic of individual dog, including type, occupation, sex, age, breed, body condition, hygiene, clinical signs of Leishmaniasis and the area, were obtained by asking the owner. These factors were divided into categories described by Thrusfield (Thrusfield, 2007).

2.9 Statistical analysis

The data collected during the study period were stored in Microsoft Excel sheet and then analyzed using statistical software program for Microsoft windows SPSS (SPSS version 16.0). The prevalence of Canine leishmaniasis was calculated by dividing the number of positive cases by the total number of examined dogs. The associations of risk factors like dog type, occupation, sex, age, breed, body condition, hygiene, clinical signs of leishmaniasis were analyzed using Chai-sequare test.

Chapter three

Results

3.1 Results of clinical examination

First, all dogs in this study were investigated clinically. According to our results, the clinical signs were classified into the following categories:

(a) Loss of weight: 17 out of 103 (16.5%) of dogs were suffering from loss of weight, while 86 out of 103 (83.5%) of dogs were not suffering from loss of weight.

(b) Alopecia: 6 out of 103(5.8%) of dogs were suffering from alopecia, while 97 out of 103(94.2%) of dogs were not suffering from alopecia.

(c) Ocular lesion: 103(100%) of dogs were not suffering from ocular lesion.

(d) Skin lesion: 19 out of 103(18.4%) of dogs were suffering from skin lesions, while 84 out of 103(81.6%) of dogs were not suffering from skin lesions.

(e) Lymphadenopathy:1 out of 103 (1%) of dogs was with lymphadenopathy, while 102 out of 103 (99%) of dogs were not (Table 6).

Table 6: Summary of clinical signs observed in the examined dogs, Jubek State, Republic of South Sudan (N=103 dogs).

Clinical signs	Total of animal examined	No. of positive	No. of positive (%)
Weightloss Yes	17	0	(0%)
No	86		
Alopecia Yes	6	0	(0%)
No	97		
Ocular lesion Yes	0	0	(0%)
No	103		
Skin lesion Yes	19	0	(0%)
No	84		
Lymphadenopathy Yes	1	0	(0%)
No	102		

For all risk factors no statistics were computed because infection with leishmaniasis was a constant and Pearson Chi-square was (.[a]).

3.2 The overall prevalence of Canine Leishmaniasis

A total of 103 blood samples of dogs were selected randomly from four different counties of Jubek State and examined. The overall prevalence was found to be 0% (0 out of 103 samples) using blood smear technique and 0% (0 out of 103 samples) using PCR test (Table 7 and Figure 4).

Table 7: The number and distribution of animals examined for Canine leishmaniasis.

Area	**No. of tested**	**No. of positive**	
		Microscopy	PCR
Juba county	76	0	0
Rejaf county	7	0	0
Luri county	4	0	0
Kondokoro Island county	1	0	0
Stray	15	0	0

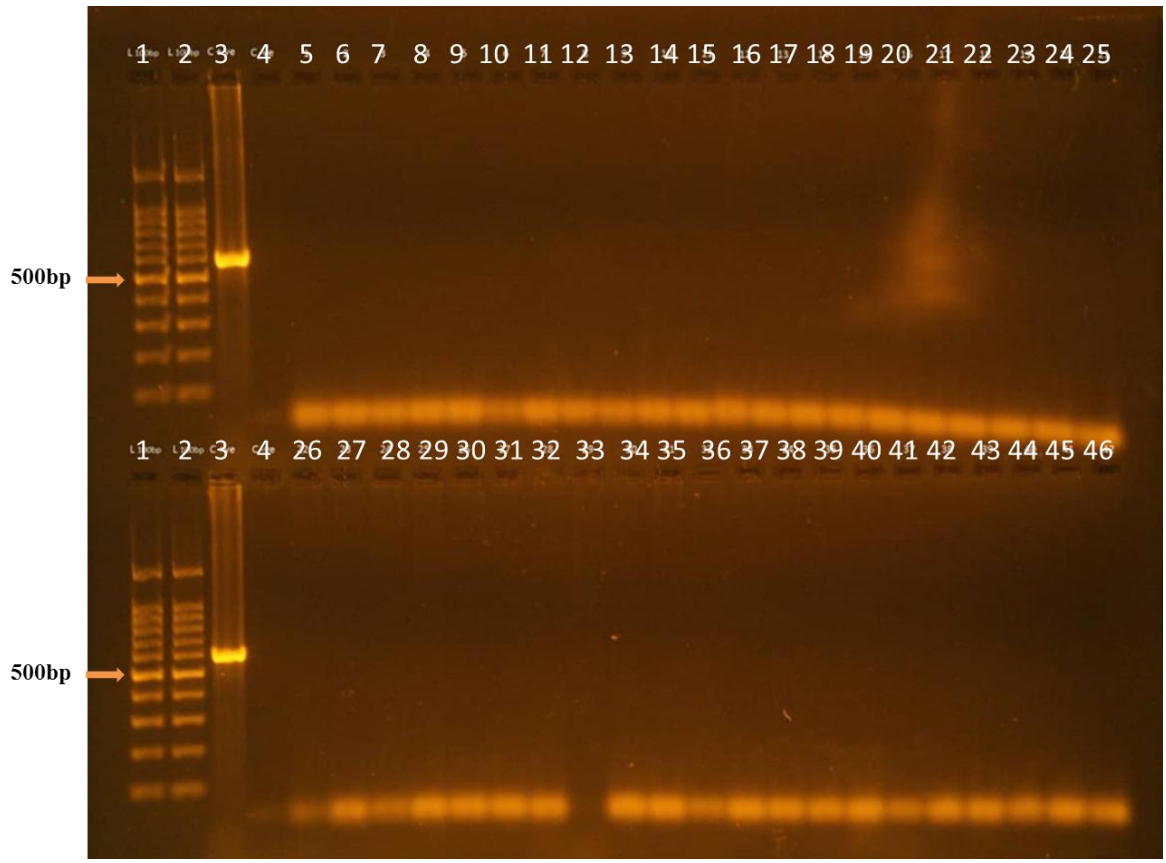

Figure 4: PCR amplification of L. donovani using specific primers. 1& 2: Molecular weight ladder; 3: Positive control (560bp); 4: Negative control; 5-46: Different samples

3.3 Prevalence of Canine leishmaniasis in relationship to the general potential risk factors (Categories of dogs, Sex, Age, breed, body condition, Hygiene and contact with other animals)

3.3.1 Categories of dogs

Dogs examined for infection with leishmaniasis were divided into 2 groups: 88 out of 103 (85.4%) of dogs were domestic, while 15 out of 103 (14.6%) of dogs were stray. In all categories of dogs, no infection with leishmania was detected (Table 8).

3.3.2 Sex

Based on the gender; 86 out of 103(83.5%) of dogs were males, while 17 out of 103(16.5%) of dogs were females. Obtained results revealed that neither male nor female was infected with leishmaniasis (Table 8).

3.3.3 Age

The dogs were divided into 3 groups: young (age less than 3 years (< 3years)), adult (between 3 and 6 years (3-6 years)) and old (greater than 6 years (> 6years)). 60 out of 103(58.3%) of dogs were young, 36 out of 103 (35%) of dogs were adults and 7 out of 103(6.8%) of dogs were old. No infection with leishmania was observed in all age groups (Table 8).

3.3.4 Breed

Based on the breed of dogs, 90 out of 103(87.4%) of dogs were local breed, 6 out of 103(5.8%) of dogs were foreign breed and 7 out of 103(6.8%) of dogs were cross breed. No infection with leishmania was detected in all breeds(Table 8).

3.3.5 Body condition

According to the body condition of examined dogs, 77 out of 103(74.8%) of dogs were in good condition, 22 out of 103 (21.4%) of dogs were in medium condition and 4 out of 103(3.9%) of dogs were in poor condition. In dogs examined no infection with leishmania was reported (Table 8).

3.3.6 Hygiene

The dog's hygiene was classified into 2 groups; poor condition and good condition. 86 out of 103(83.5%) of dogs were in good hygiene while 17 out of 103 (16.5%) of dogs were in poor hygiene. No infection with leishmania was detected neither in the dog with good nor poor condition (Table 8).

3.3.7 Contacts with other animals

75 out of 103 (72.8%) of dogs had a contact with other animals while 28 out of 103(27.7%) of the dogs had not contact with other animals. From these animals no infection with leishmaniasis was observed (Table 8).

Table 8: Summary of univariate analysis for risk factors associated with the infection with leishmaniasis in Jubek State, Republic of South Sudan (N=103 dogs) using the Chi-square test.

Risk factor		Total of animal examined	No. of positive	No. of positive (%)
Categories of Dogs				
Domestic		88	0	(0%)
Stray		15		
Sex	Male	86	0	(0%)
Female		17		
Age	Young	60	0	(0%)
Adult		36		
Old		7		
Breed	Local	90	0	(0%)
Foreign		6		
Cross breed		7		
Body condition	Good	77	0	(0%)
Medium		22		
Poor		4		
Hygiene	Good	86	0	(0%)
Poor		17		
Contact with other animal				
Yes		75	0	(0%)
No		28		

For all risk factors no statistics were computed because infection with leishmaniasis was a constant and Pearson Chi-square was (.[a]).

3.4 Prevalence of Canine leishmaniasis in relationship to the potential risk factors that related to the domestic dog (Type of roaming, Occupation of dog, Knowledge of Canine Leishmaniasis, Knowledge of Zoonotic effect of Leishmaniasis, Vaccination and Visiting the veterinary clinic)

3.4.1 Type of roaming

Domestic dogs were classified into two groups; dogs roaming freely and dogs kept inside all the time. From a total of 88 domestic dogs, 19 of domestic dogs were found roaming freely and 69 were found kept inside home all the time. In all dog types, no infection with leishmania was detected. (Table 9).

3.4.2 Occupation of dog

Domestic dogs were classified into the groups based on their occupation; guard, pet dog, police dog and other with specifying. From 88 of domestic dogs, 2 were pet dogs and 86 were used as a guard. No infection was observed in both groups. (Table 9).

3.4.3 Knowledge of canine leishmaniasis

From 88 owners of the domestic dogs, 1 (1.1%) had knowledge about the canine leishmaniasis, while 87 (98.9%) of owners had no knowledge (Table 9).

3.4.4 Knowledge of the zoonotic effect of leishmaniasis

One out of 88 (1.1%) of dog owners knew the information about the zoonotic effect of leishmaniasis (transmission between human and animal), while 87 out of 88 (98.9%) of dog owners were not known (Table 9).

3.4.5 Vaccination

Seventy two out of 88 (81.8%) of domestic dogs were vaccinated, while 16 out of 88 (18.2%) of domestic dogs were not vaccinated. In both groups, no infection was observed (Table 9).

3.4.6 Visiting of the veterinary clinic

Sixty seven out of 88 (76.1%) of domestic dogs were regularly visited the veterinary clinic, 8 out of 88 (9.1%) of domestic dogs were visited the veterinary clinic for sometimes, 2 out of 88 (2.3%) of domestic dogs were visited the veterinary clinic once, and 11 out of 88 (12.5%) of domestic dogs were not visited the clinic yet. No infection with Leishmania was observed in all groups (Table 9).

In this study there was no significant association between all potential risk factors and infection with leishmaniasis as there was no infection was detected in all dogs.

Table 9: Summary of univariate analysis for risk factors that related to the domestic dog and associated with infection with leishmaniasis in Jubek State, Republic of South Sudan (N=88 dogs) using the Chi-square test.

Risk factor	Total of animal examined	No. of positive (%)	No. of positive (%)
Type of roaming			
Roaming freely	19	0	(0%)
Kept inside all the time	69		
Occupation			
Guard	86	0	(0%)
Pet	2		
Knowledge of canine leishmaniasis			
Yes	1	0	(0%)
No	87		
Transmission to human			
Yes	1	0	(0%)
No	87		
Vaccination status			
Yes	72	0	(0%)
No	16		
Are visit the veterinarian with dog(s)?			
Regularly	67	0	(0%)
Sometimes	8		
Once	2		
Not yet	11		

For all risk factors no statistics were computed because infection with leishmaniasis was a constant and Pearson Chi-square was (.[a]).

Chapter four

Discussion

CanL is a zoonotic chronic disease transmitted mostly by infected sand flies and can be potentially fatal to human and dogs. Their epidemiological, clinical and laboratory aspects are very variable, which makes it difficult for veterinary practitioners to complete a diagnosis and then treat and control the disease especially due to lack of more effective drugs and vaccines. However, considerable efforts are being made by professionals from multidisciplinary areas in order to improve the knowledge about this parasitic disease, so that prevention, treatment and control may be improved in the future (Ribeiro *et al*., 2018).

CanL constitutes a considerable veterinary challenge, as well as an important public health problem because of infected dogs, ill or asymptomatic act as reservoir hosts for the human disease (Boussa *et al*., 2014).

To my knowledge, this is the first prevalence study of Canine leishmaniasis conducted on dogs in the Republic of South Sudan.

Of the 103 cases enrolled in this study, 86 dogs (83.5%) were males and 17 dogs (16.5%) were females. 88 dogs (85.4%) were domestic and 15 dogs (14.6%) were stray. 90 dogs (87.4%) were local breed while 6 dogs (5.8%) were foreign breed and 7 dogs (6.8%) were cross breed .

Regarding our results, the overall prevalence of canine leishmaniasis in this study using Geismsa and Leishman stain for microscopic examination was 0%. Moreover, the overall prevalence of Leishmania donovani using PCR test is also 0%.

This study disagrees with previous study conducted in a low endemic area in Tunisia; where the prevalence was 20.9% (Chargui et al., 2009). This might be due to infection with other Leishmania spp. as the PCR test in our study detected only *L.donovani's* DNA.

The current study also disagrees with the previous study conducted in Sichuan Province, southwestern China; as the prevalence was to be 24.8% (Shang et al., 2011). This difference might be due to the samples size in this study were unrepresentative. Moreover, our results are in contrary to the study conducted in the Northeast region of Brazil; where the prevalence of the positive cases was 8.4% using IFT and 4.3% using ELISA (Brito et al., 2016). This might be due to variations in the test used to diagnose the infected cases.

Furthermore, a recent study conducted in an endemic area of Brazil showed that the prevalence of the Leishmania was 19% (Pimentel et al., 2014), which is in the opposite of our finding in this study. This variance could be due to variance in the environmental condition.

The number of positive cases might increase more than 0% if we could obtain the samples from the skin, lymph node, conjunctiva and bone marrow of dogs. The reasons we didn't obtain them because we deal with stray dogs and their control is not very easy and they are very aggressive, and if we also insist to collect the samples in that way the procedure required surgical removal which would lead to death and that's against veterinary rules.

Additional, the PCR test was carried out using biopsy material, as it is well known that the parasite level in the blood is lower compared with other tissues (Alvar *et al*., 2004).

In general, the prevalence of canine leishmaniasis in this study is lower than that in other studies conducted in different countries.

Conclusion

In conclusion, this study was thought to provide answers to the question about the prevalence and risk factors of canine leishmaniasis. This study indicates that the overall prevalence of canine leishmaniasis was 0% using microscopic examination of Giemsa and Leishman stained blood smear. For DNA detection, the overall prevalence of *Leishmania donovani* was also 0% using PCR test.

Our study showed there was no significant association between risk factors and canine leishmaniasis.

Recommendations

Structured surveys and studies must be conducted within national and regional programs, to evaluate, monitor and control of the distribution and dynamics of the vector. The interplay of different parasites and their virulence must be determined, and investigation of the carrier states.

Prevention of phlebotomine sand-fly bites by applying repellents/insecticides to dogs in the form of impregnated collars or spot-on and spray formulations can be useful. Dogs should be housed especially at dawn and dusk, between April and November when the sand-flies are most likely to bite.

To avoid an extension of endemic areas, leishmania-infected dogs should be translocating to the non-endemic areas where sand-flies or other vectors may be present, testing and treatment of dogs prior to their movement from an endemic to non-endemic area.

Recommendation on monitoring of disease must be developed in areas where leishmaniasis is prevalent, provides a starting point for discussion and serological testing. Using strategic insecticides for treatment.

Recommendation on further studies of canine lieshmaniasis, must be conducted within national and regional programs.

Recommendation on using vaccines, Vaccination can be provided to dogs over 6 months of age and is based on an initial course of three doses at 3-weekly intervals following by annual revaccination.

References

Alvar, J.; Canavate, C.; Molina, R.; Moreno, J. and Nieto, J. (2004). *Canine leishmaniasis*. Adv Parasitol. 2004; 57: 1-88. Doi: 10.1016/s0065 308x(4)57001-x.

Alvar, J.; Ve´lez, I. D.; Bern, C.; Herrero, M.; Desjeux, P.; Cano, J.; Jannin, J.; Den Boer, M. and the World Health Organization Leishmaniasis Control Team (2012). *Leishmaniasis worldwide and global estimaes of its incidence*. PLoS ONE 7(5): e35671. Doi: 10.1371/journal.pone.0035671

Baneth, G.; Koutinas, A. F.; Solano-Gallego, L.; Bourdeau, P. and Ferrer, L. (2008). *Canine leishmaniosis – new concepts and insights on an expanding zoonosis: part one*. Trends Parasitol. 24,324–330

Bari, A. U. and Rahman, S. B. (2008). *Cutaneous leishmaniasis: an overview of parasitology and host-parasite-vector inter relationship*. Journal of Pakistan Association of Dermatologists 18: 42-48

Barker, D. C.; Gibson, L. J.; Kennedy, W. P.; Nasser, A. A. and Williams, R. H. (1986). *The potential of using recombinant DNA species-specific probes for the identification of tropical Leishmania*. Parasitology 92(supplement 1), S139–S174

Bates, P. A. (2007). *Transmission of Leishman metacyclic promastigotes by phlebotommine sand flies*. International journal for parasitology, Vol. 37, No.10, pp. 1097-1106

Boussa, S.; Kasbari, M.; El Mzabi, A. and Boumezzough, A. (2014). *Epidemiological Investigation of Canine Leishmaniasis in Southern Morocco*. Article ID 104697, 8pages. http://dx.doi.org/10.1155/2014/104697

Brianti, E.; Napoli, E.; Gaglio, G.; Faslone, L.; Giannetto, S.; Solari Basano, F.; Nazzari, R.; Latrofa, M. S.; Annoscia, G.; Tarallo, V. D.; Stanneck, D.; Dantas-Torres, F. and Otranto, D. (2016). *Field evaluation of twodifferent treatment approaches and their ability to control fleas and prevent canine leishmaniosis in a highly endemic area*.PLOS Neglected Tropical Diseases, vol. 10, no. 9, Article ID e0004987

Brito,F. G.; Langoni, H.; Da Silva, R. C.; Rotondano, T. E. D.; De Melo, M. A. and Da Paz, G. S. (2016). *Canine visceral leishmaniasis in the Northeast Region of Brazil*. Journal of Venomous Animals and Toxins including Tropical Diseases (2016) 22:15. DOI: 10.1186/s40409- 016-0069-4

Bulle, B.; Millon, L.; Bart, J. M.; Gallego, M.; Gambarelli, F.; Portus, M.; Schnur. L.; Jaffe, C. L.; Fernandez-BArredo ,S.; AlundA, J. M. and Piarroux, R. (2002). *Practical approach for typing strains of Leishmania infantum by microsatellite analysis*. J. Clin. Microbiol., 40, 3391–3397

Cardoso, L.; Schallig, H. D.; Neto, F.; Kroon, N. and Rodrigues, M. (2004). *Serological survey of Leishmania infection in dogs from the municipality of Peso da Regua (Alto Douro, Portugal) using the direct agglutination test (DAT) and fast agglutination screening test (FAST).* Acta Trop., 91, 95–100

Chappuis, F.; Sundar, S.; Hailu, A.; Ghalib, H.; Rijal, S.; Peeling, R. W.; Alvar, J. and Boelaert, M. (2007). *visceral leishmaniasis: what are the needs for diagnosis, treatment and control?* Nat. Rev. Microbiol. 5, 873–882

Chargui, N.; Haouas, N.; Gorcii, M.; Lahmar, S.; Guesmi, M.; Ben Abdelhafidh, A.; Mezhoud, H. and Babba, H. (2009). *Use of PCR, IFAT and in vitro culture in the detection of Leishmania infantum infection in dogs and evaluation of the prevalence of canine leishmaniasis in low endemic area in Tunisia*. Parasite, 2009, 16, 65-69. http://dx.doi.org/10.1051/parasite/2009161065

Colwell, D.D.; Dantas-Torres, F. and Otranto, D. (2011). *Vector borne parasitic zoonoses: emerging scenarios and new perspectives*. Vet. Parasitol. 182, 14–21

Coppinger, R. and Coppinger, L. (2001). *Dogs: a startling new understanding of canine origin, behavior and evolution*. New York: Scrbner. ISBN 978-0-684-85530-1

Cox, F. E. G. (1993). *A text book of parasitology*(2nd edn). Blackwell scince Ltd, London.

CTP Veterinary Assistant Website. Cephalic and Saphenous Veins. (Site accessed August 2017). www.nc3rs.org.uk/dog-cephalic-vein-non- surgical

CVMP: Committee for Medicinal Products for Veterinary Use (2016). European Public Assessment Report (EPAR) for LETIFEND," European Medicines Agency

Dacie, J. V.; Bain, B, J. and Bates, I. (2006). *Dacie and Lewis practical hematology*. Philadelpha: Churchil Livingstone/Elsevier. ISBN 918-0 443-06660-3

Dantas-Torres, F. (2009). *Canine leishmaniosis in South America*. Parasite Vectors 2 (Suppl. 1), S1

Dantas-Torres, F. and Brandaˆo-Filho, S. P. (2006). *Visceral leishmaniasis in Brazil: revisiting paradigms of epidemiology and control*.Rev. Inst. Med. trop. S. Paulo, 48(3): 151-156

Dantas-Torres, F.; Solano-Gallego, L.; Baneth, G.; Ribeiro, V. M.; de Paiva-Cavalcanti, M. and Otranto, D. (2012). *Canine leishmaniosis in the Old and New Worlds: unveiled similarities and differences*. Trends Parasitol. 28, 531–538

De Oliveira, V. V. G.; Alves, L. C. and Da Junior, V. A. (2015). *Transmission routes of visceral leishmaniasis in mammals*. Cienca rural, Santa Maria, V.45, N.9, p.1622-1628

Dereure, J.; Boni, M.; Pratlong, F.; Osman, M.; Boucheton, B.; El Safi, S.; Feugier, E.; Musa, M. K.; Daroust, B.; Dessein, A. and Dededi, J. P. (2000). *Visceral leishmaniuasis in Sudan: first identification of Leishmania from dogs*. Trans R Soc Trop Med Hyg. 2000; 94: 154-155. Doi: 10.1016/s0035-9203(00)90253-0

Elbihari, S.; Cheema, A. M and El-hassan, A.M. (1987). *Leishmania infecting man and wild animal in Saudi Arabia. 4. Canine cutaneous leishmaniasis in the Eastern Province*. Transaction of the royal society of tropical medicine and hygiene, volume 81, issue 6, 1 November 1987, pages 925-927, https://doi.org/10.1016/0035-9203(87)90353-1

Esch, K. J. and Petersen, C. A. (2013). *Transmission and epidemiology of zoonotic protozoal diseases of campanion animals*. Clin microbial rev. 2013 Jan, 26(1):58-85. Doi:10.1128/CMR.00067-12.

Ferroglio, E.; Poggi, M. and Trisciuoglio, A. (2008). *Evalution of 65% permethrin spot-on and deltamethrin-impregnated collars for canine Leishmania infantum infection prevention*. Zoonoses and Public Health. Vol.55, No.3, pp. 145-148

Gharbi, M.; Mhadhbi, M.; Rejeb, A.; Jaouadi, K.; Rouatbi, M. and Darghouth, M. A. (2015). Leishmaniosis (*Leishmania infantum* infection) in dogs. Revue Scientifique et Technique de l'OIE, vol. 34, no. 2, pp. 613–66

Gomes, A. H. S.; Ferreira, I. M. R.; Lima, M. L. S. R.; Cunha, E. A.; Garcia, A. S.; Araujo, M. F. L. and Pereira-Chioccola, V. L. (2007). *PCR identification of leishmania in diagnosis and control of canine leishmaniasi*. Veterinary Parasitology, volume 149, issues 3-4, 10 November 2007, pages 298, https://doi.org/10.1016/j.vetpar.2006.10.008

Gramiccia, M. and Gradoni, L. (2005). *The current status of zoonotic leishmaniases and approach to disease control*. Int J Parasitol. 2005 oct; 35(11-12): 1169-80

Hamarsheh, O.; Nasereddin, A.; Damaj, S.; Sawalha, S.; Al Jawabreh, H.; AzmI, K.; Amro, A.; Arekat, S.; Abdeen, Z. and Al Jawabreh, A. (2012). *Serological and molecular survey of Leishmania parasites in apparently healthy dogs in the West Bank, Palestine*. Parasit. Vectors, 5, 183

Harith, A. E.; Slappendel, R. J.; Reiter, I.; Van knapen, F.; Korte, P. D.; Huigen, E. and Kolk, A. H. J. (1989). *Application of a direct agglutination test for detection of specific anti-Leshmania antibodies in the canine reservoir*. J. Clin. Microbiol., **27**, 2252–2257

Harith, A. E.; Kolk, A. H. J.; Leeuwenburgh, J.; Muigai, R.; Huigen, E.; Jelsma, T. and Kager, P. A. (1988). *Improvement of a direct agglutination test for field studies of visceral leishmaniasis*. J. Clin. Microbiol., **26**, 1321–1325

https://paanluelwel.com

Jambulingam, P.; Pradeep Kumar, N.; Nandakumar, S.; Paily, K. P. and Srinivasan, R. (2017). *Domestic dogs as reservoir hosts for Leishmania donovani in the southernmost Western Ghats in India*. Acta Trop. 2017 Jul; 171: 64-67. Doi: 10.1016/j.actatropica.2017.03.006.

Karkamo, V.; Kaistinen, A.; Nareaho, A.; Dillard, K.; Vainio Siukola, K.; Vidgren, G.; Tuoresmaki, N. and Anttila, M. (2014). *The first report of autochthonous non-vector-borne transmission of canine leishmaniasis in the Nordic countries*. Acta Vet Scand. 2014; 56: 84. Doi: 10.1186/s13028-014-0084-9

Kaye, P. and Scott, P. (2011). *Leishmaniasis: complexity at the host pathogen interface*. Nat. Rev. Microbiol. 9, 604–615

Koutinas, A. F. and Koutinas, C. K. (2014). *Pathologic mechanisms underlying the clinical finding in canine leishmaniasis due to Leishmania infantum/chagasi*. Vet Pathol. 2014; 51: 527-538. Doi: 10.1177/0300985814521248

Kuhls, K.; Alam, M. Z.; Cupolilo, E.; Ferreira, G. E.; Mauricio, I., L.; Oddone, R.; Felliciangeli M. D.; Wirth, T.; Miles, M. A. and Schönian, G. (2011). *Comparative microsatellite typing of new world Leishmania infantum reveals low heterogeneity among populations and its recent old world origin*. PLoS Negl. Trop. Dis., 5 (6), e1155

Lainson, R. and Shaw, J.J. (1987). *Evolution, classification and geographical distribution*. In: The Leishmaniases in Biology and Medicine, Vol. 1(W. Peters and R. Killick-Kendrick, eds), pp. 1–120. London: Academic Press.

Larson, G. and Bradley, D. G. (2014). *How much is that in dog year? The advent of canine population genomics*. PLoS Genet 10(1): e1004093. Doi: 10.1371/journal.pgen. 1004093

Le Blancq, S.M.; Cibulskis, R.E. and Peters, W. (1986). *Leishmania in theOld World: 5. Numerical analysis of isoenzyme data*. Transactions of the Royal Society of Tropical Medicine and Hygiene 80, 517–524

Lindblad-Toh, K.; Wade, C. M.; Mikkelsen, T. S.; Karlsson, E. K.; Jaffe, D. B.; Kamal, M.; Clamp, M.; Chang, J. L.; Kulbokas 111, E. J.; Zody, M. C.; Mauceli, E.; Xie, X.; Breen, M.; Wayne, R. K.; Ostrander, E. A.; Ponting, C. P.; Gilbert, F.; Smith, D. R.; deong,P. J.; Kirkness, E.; Alvarez, P.; Biagi, T.; Brockman, W.; Butler, J.; Chin, C.; Cook, A.; Cuff, J.; Daly, M. J.; DeCaprio, D.; Gnerre, S.; Grabherr, M.; Kellis, M.; Kleber, M.; Bardeleben, C.; Godstadt, L.; Heger, A.; Hitte, C.; Kim, L.; Koepfli, K.; Parker, H. G.; Polinger, J. P.; Searle, S. M.J.; Sutter, N. B.; Thomas, R.; Webber, C.; Broad Institute Genome Sequencing Platform and Lander, E. S. (2005). *Genome sequence, comparative analysis and haplotype structure of the domestic dog*. Nature. 438(7069): 803-819. Doi: 10.1038/nature04338

Lorenz, T. C. (2016). *Polymerase Chain Reaction: basic protocol plus troubleshooting and optimization strateges*. J Vis Exp. 2012; (63): 3998. Published online 2012 May 22. Doi: 10.3791/3998

Maarten, H. L.; De Brujin, M. H. L. and Barker, D. C. (1992). *Diagnosis of New World leishmaniasis: specific detection of species of the Leishmania braziliensis complex by amplification of kinetoplast DNA.* Acta Trop., 52, 45–58

Maroli, M.; Feliciangeli, M. D.; Bichaud, L.; Charrel, R. N. and Gradoni, L. (2012). *Phlebotomine sand flies and the spreading of leishmaniases and other diseases of public health concern*. Med. Vet. Entomol., http://dx.doi.org/10.1111/j.1365-2915.2012.01034.x

Maroli, M.; Feliciangeli, M. D.; Bichaud, L.; Charrel, R. N. and Gradoni, L. (2013). *Phlebotomine sand flies and the spreading of leishmaniases and other diseases of public health concern*. Med. Vet. Entomol. 27, 123–147

Mathis, A. and Deplazes, P. (1995). *PCR and in vitro cultivation for detection of Leishmania spp. in diagnostic samples from humans and dogs*. J. Clin. Microbiol., 33, 1145–1149

Matlashewski, G. (2002). *Leishmania infection and macrophage function*. In: Black SJ, Reed JR, editors. World Class Parasites: Volume 4. Leishmania. Boston, MA: Kluwer Academic Publishers; 2002. p. 105 13

Mattin, M.; Brodbelt, D.; Wylie, C.; Antonanzas, M. C.; Solano-Gallego, L.; Espejo, L.; Costard, S. and Zagmutt, F. (2013). *Data collection to characterize the impact of canine leishmaniosis and modeling of the role of animals inspreading Leishmania infantum within the European Union*. Supporting publications 2013: EN-466. 246pp.

Mencke, N. (2011). *The importance of canine leishmaniosis in nonendemic areas, with special emphasis on the situation in Germany*. Berl. Munch. Tierarztl. Wochenschr. 124, 434–442

Merck Manual (2019). *Merck Manual Veterinary Manual*. Merck sharp & Dohme Crop., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA.

Ministry of Animal Resources and Fisheries (Republic of South Sudan, Juba).

Ministry of Housing, Physical Planning and Environment (Republic of South Sudan, Juba).

Molyneux, D. H. and Ashford, R. W. (1983). *The biology of Trypanosoma and Leishmania*. 1983, Taylor and Francis, London.

Mosser, D. M. and Brittingham, A. (2002). *The interaction of Leishmania spp. with phagocytic receptors on macrophages: the role of serum opsonins*. In: Black SJ, Reed JR, editors. World Class Parasites: Volume 4. Leishmania. Boston, MA: Kluwer Academic Publishers; 2002. p. 89-103

Myler, P. J. and Fasel, N. (2007). *Leishmania after genome*. Caister Academic Press, Norfolk, UK. ISBN: 978-1-904455-28-8

Naucke, T. J.; Amelung, S. and Lorenz, S. (2016). *First report of transmission of canine leishmaniosis through bite wounds from a naturally infected dog in Germany*. Parasites & Vectors. Vol.9, No.1. Article no.256

Naucke, T.J. and Lorentz, S. (2012). *First report of venereal and vertical transmission of canine leishmaniosis from naturally infected dogs in Germany*. Parasit. Vectors 5, 67

OIE: World Organization for Animal Health (2014). Leishmaniosis. OIE terrestrial Manual 2014

Oliva, G.; Nieto, J.; Foglia Manzillo, V.; Capiello, S.; Fiorentino, E.; Di Muccio, T.; Scalone, A.; Moreno, J.; Chicharro, C.; Carrillo, E.; Butaud, T.; Guegand, L.; Martin, V.; Cuisinier, A. M.; McGahie, D.; Gueguen, S.; Canavate, C. and Gardoni, L. (2014). *A randomised, double-blind, controlled efficacy trial of the liesp/qa-21 vaccine in na¨ıve dogs exposed to two Leishmania infantum transmission seasons*. PLOS Neglected Tropical Diseases, vol. 8, no. 10

Otranto, D.; Dantas-Torres, F. and Breitschwerdt, E. B. (2009). *Managing canine vector-borne diseases of zoonotic concern: part one*. Trends Parasitol. 25, 157–163

Otranto, D.; Paradies, P.; Lia, R. P.; Latrofa, M. S.; Testini, G.; Cantacessi, C.; Mencke, N.; Galli, G.; Capelli, G. and Stanneck, D. (2007). *Efficacy of a combination of 10% imidacloprid/50% permethrin for the prevention of leishmaniasis in kennelled dogs in an endemic area*. Veterinary Parasitology, vol. 144, no. 3-4, pp. 270–278

Palatnik-de-Sousa, C. B. and Day, M. J. (2011). *One health: The global challenge of epidemic and endemic leishmaniasis*. Parasit Vectors 4, 197

Papadopoulos, E.; Angelou, A.; Diakou, A.; Halos, L. and F. Beugnet, F. (2017). Five-month serological monitoring to assess theeffectiveness of permethrin/fipronil (Frontline Tri-Act_) spoton in reducing the transmission of *Leishmania infantum* in dogs. Veterinary Parasitology: Regional Studies andReports, vol.7, pp. 48–53

Pimentel, D. S,; Ramos, R. A. N.; Santana, M. D.; Maia, C. S.; De Carvalho, G. A.; Da Silva, H. P. and Alves, L. C. (2014). *Prevalence of zoonotic visceral leishmaniasis in dogs in an endemic area of Brazil*.Trop. Vol.48, No.4, http://dx.doi.org/10.1590/0037-8682-0224-2014

Raymond, R. W.; McHugh, C. P.; Witt, L. R. and Kerr, S. F. (2003). *Visceral leishmaniasis in Sudan: first identifications of Leishmania from dogs*. Trans R Soc Trop Med Hyg 94: 154-155

Regina-Silva, S.; Feres, A. M. L. T. .; Franc¸a-Silva, J. C.; Dias, E. S.; Michalsky, E. M.; De Andrade, H. M.; Coelho, E. A.; Ribeiro, G. M.; Fernandes, A. P. and Machado-Coelho, G. L. (2016). *Field randomized trial to evaluate the efficacy of the Leish-Tec_vaccine against canine visceral leishmaniasis in an endemic area of Brazil*. Vaccine, vol. 34, no. 19, pp. 2233–2239

Reguera, R. M.; Mor´an, M.; P´erez-Pertejo, Y.; Garc´ıa-Estrada, C. and Bala˜na-Fouce, R. (2016). *Current status on prevention andtreatment of canine leishmaniasis*. Veterinary Parasitology, vol. 227, pp.98–114

Ribeiro, R. R.; Michalick, M. S. M.; Da Silva, M. E.; Dos Santos, C. C. P.; Frézard, F. J. G. and Da Silva, S. M. (2018). *Canine Leishmaniasis: An Overview of the Current Status and Strategies for Control*. Hindawi BioMed Research International.Volume 2018, Article ID 3296893, 12 pages https://doi.org/10.1155/2018/3296893

Rioux, J.A.; Lanotte, G.; Serres, E.; Pratlong, F.; Bastien, P. and Perie`res, J. (1990). *Taxonomy of Leishmania. Use of isoenzymes. Suggestions for a new classification*. Annales de Parasitologie Humaine et Compare´e, 65,111–125

Rohousova, I.; Talmi-Frank, D.; Kostalova, T; Polanska, N.; Lestinova, T.; Kassahun, A,; Yasur-Landau, D.; Maia, C.; King, R.; Votypka, J.; Jaffe, C. L.; Warburg, A.; Hailu, A.; Volf, P. and Baneth, G. (2015). *Exposure to Leishmania spp. and sandflirs in domestic animals in northwestern Ethiopia*. Parasit Vectors 8:360

Roque , A. L. R. and Jansen, A. M. (2014).*Wild and synanthropic reservoirs of Leishmania species in the Americas*. Int J Parasitol Parasites Wildl 3: 251-262

Salomo´n, O.; Sinagra, A.; Nevot, M.; Barberian, G.; Paulin, P.; Estevez, J.; Riarte, A. and Estevez, J. (2008). *First visceral leishmaniasis focus in Argentina*. Mem. Inst. Oswaldo Cruz 103, 109-111

Shang, L.; Peng, W.; Jin, H.; Xu, D.; Zhong, N.; Wang, W.; Wu, Y. and Liu, Q. (2011). *The prevalence of canine Leishmania infantum infection in Sichuan Province, southwestern China detected by real time PCR.* Parasites & Vectors 2011, 4:173 http://www.parasitesandvectors.com/content/4/1/173

Shaw, S. E.; Langton, D. A. and Hiliman, T. J. (2009). *Canine leishmaniosis in the United Kingdom: a zoonotic disease waiting for a vector?* Vet. Parasitol. 163, 281–285

Solano-Gallego, L.; Mir´o, G.; Koutinas, A.; Cardoso, L.; Pennisi, M. G.; Ferrer, L.; Bourdeau, P.; Oliva, G. and Baneth, G. (2011). *LeishVet guidelines for the practical management of canine leishmaniasis*. Parasites & Vectors, vol. 4, article 86

Solano-Gallego, L.; Mir´o, G.; Koutinas, A.; Cardoso, L.; Pennisi,M. G.; Ferrer, L.; Bourdeau, P.; Oliva, G. and Baneth, G. (2011). *LeishVet guidelines for the practical management of canine leishmaniasis*. Parasites & Vectors, vol. 4, article 86

Solano-Gallego, L.; Koutinas, A,; Miro, G.; Cardoso, L.; Pennisi, M. G.; Ferrer, L.; Bourdeau, P.; Oliva, G. and Baneth, G. (2009). *Directions for the diagnosis, clinical staging, treatment and prevention of canine leishmaniosis*. Vet. Parasitol. 165, 1–18
Solano-Gallego, L.; Koutinas, A,; Miro, G.; Cardoso, L.; Pennisi, M. G.; Ferrer, L.; Bourdeau, P.; Oliva, G. and Baneth, G. (2009). *Directions for the diagnosis, clinical staging, treatment and prevention of canine leishmaniosis*. Vet. Parasitol. 165, 1–18

Tabar, M. D.; Roura, X.; Francino, O.; Altet, L. and Ruiz de Gopegui, R. (2008). *Detection of Leishmania infantum by real-time PCR in canine blood bank*. J Small Anim Pract. 2008; 49: 325-328. Doi: 10.1111/j.1748-5827.2008.x.

Thrusfield , M (2007).*Veterinary Epidemiology*.(3rd Edition). UK, Blackwell Science Ltd. pp 274 – 282

Tomaz-Soccol, V.; Castro, E. A.; Navarro, I. T.; de Farias, M. R.; de Souza, L. M.; Carvalho, Y.;Bispo, S.; Membrive, N. A.; Minozzo, J. C.; Truppel, J.; Bueno, W. and Luz, E. (2009). *Allochthonous cases of canine visceral leishmaniasis in Parana´, Brazil: epidemiological implications*. Rev. Bras. Parasitol. Vet. 18, 46–51

Ve´lez, I. D.; Carrillo, L. M.; Lopez, L.; Rodriguez, E. and Robledo, S. M. (2012). *An epidemic outbreak of canine cutaneous leishmaniasis in Colombia caused by Leishmania braziliensis and Leishmania panamensis*. Am. J. Trop. Med. Hyg. 86, 807–811

Wijerathna, T.; Gunathilaka, N, and Gunawardena, K. (2018). *The economic impact of cutaneous leishmaniasis in Sri Lanka*. Volum 2018, article ID 302585, 9 pages. https://doi.org/10.1155/2018/3025185

World Health Organization (1990). Control of the Leishmaniases. Geneva: World Health Organization, Technical Report Series, No. 793.

World Health Organization (2002). Weekly epidemiological record, Nº77, 44, 1 November 2002

World Health Organization (2003). The world health report : 2003: shaping the future. ISBN: 92-4-1562439

World Health Organization (2010). Control of the leishmaniases.World Health Organ Tech Rep Ser949: 1-186

World Health Organization (2014). "leishmaniasis fact sheet Nº375". Archived from the original on 21February 2014. Retrieved 17 February 2014

World Health Organization (2016). Visceral leishmaniasis: who publishes validation document as countries approach elimination

Appendix

Sample form

Date: …….. County:……

Dog ID: ……

Name of owner:……

Address and telephone number:

……

Category of Dog:

Domestic ⬡ **Stray** ⬡

Type of roaming: roaming freely ⬡ **kept inside all the time** ⬡

Occupation of Dog:

Game dog ⬡ **Guard** ⬡ **Pet dog** ⬡

Police dog ⬡ **other** ⬡ **(specify)** ⬡

General Information

Sex: ⬡ **Male** ⬡ **Female**

Age:……

Breed:

Local ⬡ **Foreign** ⬡ **cross breed** ⬡

Body condition: ⬡ **Good** ⬡ **Medium** ⬡

Hygiene: ⬡ **Good** ⬡ **Poor** ⬡

Clinical sign(s) of leishmaniasis:

Weight loss: Yes ⬡ No ⬡

Alopecia: Yes ⬡ No ⬡

Ocular lesion: Yes ⬡ No ⬡

Skin lesion: Yes ⬡ No ⬡

Lymphadenopathy: Yes ⬡ No ⬡

Contacts with other animals: Yes ⬡ No ⬡

Knowledge of canine leishmaniasis: Yes ⬡ No ⬡ No ⬡

Knowledge of zoonotic effect of leishmaniasis:

Yes ⬡ No ⬡

Vaccination: Yes ⬡ No ⬡

Visiting of the veterinary clinic?

Regularly ⬡ Sometimes ⬡ ⬡ ⬡