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Molecular survey of *Leishmania donovani* in dogs in Jubek State, Republic of South Sudan

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Abstract
Background: *Leishmaniasis is a zoonotic, vector-borne disease caused by the genus Leishmania and widespread in tropical and subtropical areas. The disease is infecting many species including humans and domestic and wild mammals. Although dogs are naturally infected by several species of Leishmania including species that infect humans, their role as reservoir hosts of these parasites is probably negligible and had not been investigated in several countries. To my knowledge, no study has been conducted previously to investigate the role of dogs as reservoirs for Human Leishmaniasis (Leishmania donovani) in Jubek State, Republic of South Sudan.*
Materials and Methods: *A cross-sectional study was conducted between January and February 2018 to assess the prevalence of Leishmania donovani in the dogs in this city. A total of 103 blood samples were collected from cephalic vein of dogs. Tow diagnostic techniques were used such as blood smear and polymerase chain reaction (PCR).*
Results: *The overall prevalence was 0% (0/103) using the microscopic examination of stained slides and PCR.*
Conclusion: *To conclude, this survey is the primary investigation in Jubek State. The results showed that at least dogs in this area did not play any role in the epidemiology of human leishmaniasis. However, more studies are required in order to have a precise picture of this disease.*
Keywords*: Dog, Jubek State, Leishmania donovani, PCR*

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I. Introduction

Leishmaniasis is vector-borne disease caused by an intracellular parasite of the genus *Leishmania* and is transmitted by the bite of infected female phlebotomine sand flies [1]. The disease is common in tropical and subtropical areas and is found in 98 countries in Asia Africa, Europe, and America. Nevertheless, above 90% of new cases happen in just 13 countries including Bolivia, Brazil, Columbia, Peru, Afghanistan, Algeria, Iran, India, Bangladesh, Ethiopia, South Sudan, Sudan and Syria [2,3]. Around 1 million new cases of leishmaniasis happen yearly in about 100 endemic countries and over 20,000 deaths are attributed yearly to them [4].

Among other species of *leishmania*, *Leishmania donovani* (*L. donovani*) causes the most severe and potentially fatal form of leishmaniasis (kala-azar) and is considered an important disease in Europe, Africa, Asia, and Latin America [5]. This protozoan is primarily maintained in nature by transmission from human to human. However, the transmission of *L. donovani* between humans and animals was documented in several countries [6]. For example, Elnaiem [7] suggest that the Egyptian mongoose could be a possible reservoir host of *L. donovani* in eastern Sudan. In another study conducted in the same area, they also isolated *L. donovani* from samples collected from dogs [8]. A similar observation was reported in India, where *L. donovani* was identified in dogs [9].

It is estimated that three-quarters of the world's dog population lives in the developing world as feral, village, or community dogs, with pet dogs uncommon [10]. In the Republic of South Sudan, dogs have a significant value in the community as they are used as security guards for houses, compounds, and livestock or used during hunting.

Although dogs have been found naturally infected by several species of Leishmania in many countries [11]. Their role as a reservoir host of this parasite is ignored, which reflects negatively on the prevention and

control measures of leishmaniasis particularly in developing countries. Therefore, this study aimed to investigate the possible role of dogs as reservoirs for *L. donovani* in the Republic of South Sudan.

II. Material and Methods

Study area: Jubek state is one of the states of South Sudan, located within the Equatorial region; the state borders include Terkeka State to the north, Amadi State to the west, Yei River State to the southwest, and Imatong State to the east (Fig 1). It contained the national capital, Juba, which is also the largest city in South Sudan and falls between longitude 31° 34' 16.5" E and latitude 4° 51' 33.7" N. The state consists of 14 counties including; Rejaf county, Lobonok county, Mangala county, Liria county, Lokiliri county and Kondokoro county, Lado county, Luri county, Rokon county, Dolo county, Wanduruba county, Bunga county, Ganji county and Juba county. Generally, the climate is similar to a tropical climate and characterised by a rainy season of high humidity and large amounts of rainfall followed by a drier season. The temperature average is always high with March being the warmest month with an average temperature ranging from 23 to 37 º C and July being the coolest month with an average temperature falling between 20 and 30 º C.

Figure 1: Shows Jubek State "the study area" (https://upload.wikimedia.org/wikipedia/commons/thumb/1/12/Jubek_in_South_Sudan_2015.svg/800px-Jubek_in_South_Sudan_2015.svg.png).

Ethical approval: The collection of the sample was performed in line with the principles of the Ministry of Animal Resources and Fisheries, Juba, South Sudan. Other procedures conducted in the study were in accordance with the ethical standards of the Institutional Ethics Committee of Sudan University of Science and Technology, Sudan.

Sample collection: The study was a cross-sectional study performed to estimate the prevalence of *Leishmania donovani* in dogs in Jubek State. One hundred and three dogs were randomly selected by using the multi-stage technique. Approximately 5 ml of blood was taken from the cephalic vein of dog after restraint [12] by using disposable syringe after disinfecting the site of injection. The blood was transferred into tubes containing EDTA (Ethylene Diamine Tetra-acetic Acid).

Blood Smears: The blood films were prepared and stained with Giemsa's and leishman's stain following the procedure described by the OIE [13]. The identification of *Leishmania* was performed according to their morphological characteristics [14]. Fifty microscopic fields were examined under immersion lens oil (100×magnification). The presence of one amastigote was considered a positive case.

Molecular detection

DNA extraction: Genomic DNA was extracted from frozen blood samples using Guanidine Chloride method. Firstly, 700µl of blood was added to 700µl of Red Cell Lysis Buffer (RCLB) in 1.5 ml Eppendorf tube, 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 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 three layers.
400µl of the upper layer was collected into a new 1.5 ml Eppendorf tube 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 was allowed to dry for 2 hours on sterile tissue at room temperature.
The pellet was re-suspended in 30µl dH_2O, then briefly vortexed and put at 4˚C overnight. The DNA was stored at -20˚C.
Polymerase chain reaction (PCR): Two primer pairs [Forward 5'GGTTCCTTTCCTGATTTAGG3'] and [Reverse 5'GGCCGGTAAAGGCCGAATAG 3'] were used to amplify gene sequences of *L. donovani* . PCR was performed in a final reaction volume of 25µl volume containing 18 µl of PCR- water containing 10x PCR buffer, 2 µl of extracted DNA template, 1µl of each primer (10 µM), 2 µl of 50x dNTP mix and 2 µl of the 50x polymerase mix. The amplification was performed with an initial denaturation 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. The PCR products were visualized on 2% agarose gel stained with Ethidium Bromide.

III. Results

A total of 103 blood samples of dogs were selected randomly from four different counties of Jubek State, Republic of South Sudan and examined. The overall prevalence was found to be 0% (0 out of 103 samples) using blood smear technique and PCR test (Table 1 and Figure 1).

Table 1: The number and distribution of dogs examined for *L. donovani*.

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 county	16	0	0
Total	103	0	0

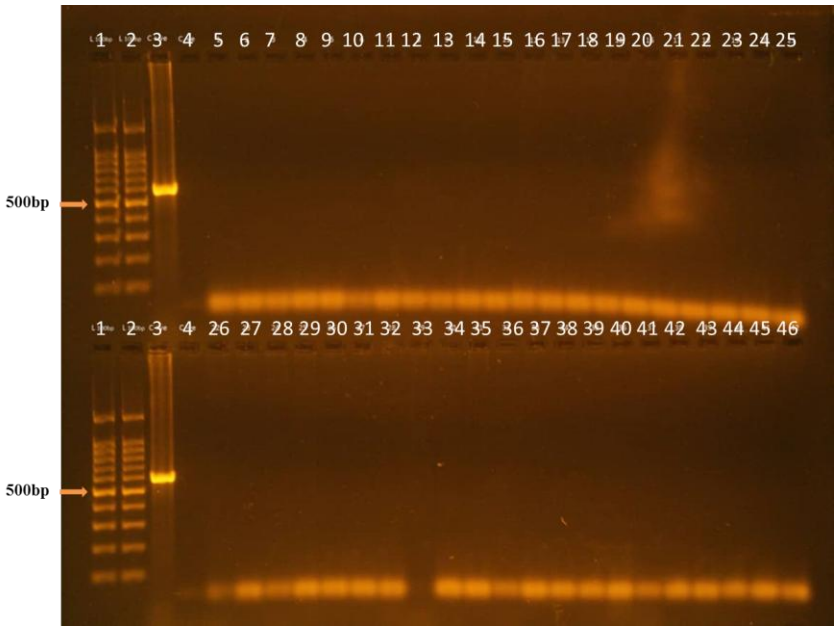

Figure 1: PCR amplification of L. donovani using specific primers. 1& 2: Molecular weight ladder; 3: Positive control (560bp); 4: Negative control; 5-46: Different samples.

IV. Discussion

Understanding the epidemiology of any zoonotic disease including leishmaniasis and then designing control programmes requires a good knowledge of the reservoir hosts and their population density. Nevertheless, the zoonotic transmission cycle of *L. donovani* is not sufficiently understood in several parts of Africa [15].

It has been estimated that approximately 375 million dogs or 75 % of the global dog population are free-roaming, living mostly in Latin America, Africa and Asia [16]. The majority of these dogs are classified as either owned free-roaming dogs or true stray ownerless dogs [17]. Which make them as a major reservoir for several zoonotic pathogens[18].

Several studies have been conducted especially where leishmaniasis is endemic to investigate the potential reservoir host. Previous studies in eastern Sudan have discovered *L. donovani* infection in dogs [19, 20]. Another investigation indicates the role of dogs as reservoir hosts for *L. donovani* in the Western Ghats, India [9]. Clearly, these researchers are indicating that the dog may be an important reservoir host of *L. donovani* in endemic areas.

Our results showed that 0% of the dogs in the study area were positive for *L. donovani* infection. The finding in the present study does not match the results obtained from early studies done in Sudan and Ethiopia [19, 20, 21]. These differences may be due to the timing of sampling following the transmission season. Alternatively, our results may indicate the existence of other nature reservoirs for *L. donovani* rather than dogs in the area. This hypothesis is supported by previous study, which was done by Elnaiem [7] who found that the Egyptian mongoose was acting as a reservoir for *L. donovani* in eastern Sudan. Similar results were reported by [22], who detected *L. donovani* and *L. tropica* in Ethiopian wild rodents.

Since this study is considered the first molecular study investigating *L. donovani* in dogs in Jubek State and the PCR analysis showed negative results in all collected samples. Further experimental and field research involving other potential animal reservoirs must be conducted to gain a comprehensive understanding of the epidemiology of Leishmaniasis in the area.

V. Conclusion

In closing, although our investigations showed negative PCR results for *Leishmania donovani* in dogs, the data on human leishmaniasis in Jubek State suggests additional surveys are required for more information regarding the possible reservoirs for this disease in the State.

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Conflict of interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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