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Research Article

Prevalence of Leishmania donovani Infection in Humans and Dogs in Gadarif State, Sudan: A Diagnostic Comparison

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Abstract

Background: *Leishmania donovani* is a protozoan parasite that causes visceral leishmaniasis, a potentially fatal disease in humans and dogs. The epidemiology and transmission dynamics of *L. donovani* in Sudan are poorly understood. We aim to determine the prevalence and characterization of *L. donovani* in human hospital cases and domestic dogs using different diagnostic methods in two localities in Gadarif State, Sudan.

Methods: A cross-sectional study was conducted from October 2019 to April 2020. Whole blood samples and lymph node aspirates were collected from 69 human and 32 dog participants for parasitological, immunological, and molecular tests.

Results: No parasites were detected in blood samples from either humans or dogs. Lymph node microscopy revealed 71.01% positivity in humans and 9.4% in dogs. The IT Leish test showed 82.6% positivity in humans and 43.75% in dogs. PCR confirmed *L. donovani* infection in all six selected samples (three from humans and three from dogs).

Conclusion: The study confirmed the presence of *L. donovani* in both human and dog populations in the study area, suggesting that dogs may act as reservoirs or hosts for the parasite. The IT Leish kit test was the most sensitive and specific method, while microscopy of blood and lymph node smears was the least sensitive method. Further studies are needed to elucidate the role of dogs in the transmission cycle of *L. donovani* and the risk factors associated with human infection.

Keywords: *Leishmania donovani,* Sudan, dogs, prevalence, molecular diagnosis

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1. Introduction

Leishmaniasis is a group of diseases caused by protozoan parasites of the genus Leishmania, which are transmitted by the bite of infected Phlebotomine sandflies [1–3]. The disease can manifest in different forms, such as cutaneous (CL), visceral (VL), mucocutaneous (MCL), or post-kala-azar dermal leishmaniasis (PKDL), depending on the parasite species and the host immune response. Leishmaniasis is endemic in many regions of Sudan, with CL being the most common form and VL being mainly confined to the eastern, western, and southern parts of the country [4–6]. Leishmaniasis affects 98 countries in Europe, Africa, Asia, and America, with a high burden in 13 countries that account for over 90% of new cases [7, 8].

Various mammals, such as domestic dogs, rodents, sloths, and opossums, can serve as reservoir hosts for Leishmania parasites. Transmission through non-vector routes is possible but uncommon [9]. Leishmaniasis is considered the most lethal and disabling neglected tropical disease (NTD), with an estimated mortality of 50,000 in 2010 [10] and a disability-adjusted life year of 3.3 million [11]. The diagnosis of leishmaniasis can be done by parasitological methods or serological tests. Zijlstra *et al.* [12] compared the diagnostic accuracy of parasitological methods and the direct agglutination test for kala-azar and found that splenic aspiration was the most sensitive method (96.4%), followed by bone marrow aspiration (70.2%) and lymph node aspiration (58.3%).

The recombinant K39 immunochromatographic test (rK39 ICT) is a rapid diagnostic test that is highly sensitive and specific for VL in patients with fever and splenomegaly who have no prior exposure to the disease, but its sensitivity varies geographically and is lower in east Africa than in the Indian subcontinent [13]. Polymerase chain reaction (PCR) is another diagnostic method that can identify the Leishmania species that infect dogs, especially in areas where both VL and CL coexist, as demonstrated by Elbihari *et al.* (1987) [14] and Gomes *et al.* (2007) [15]. PCR has a higher sensitivity than microscopy for detecting Leishmania parasites in lymph nodes and bone marrow aspirates, but its sensitivity for blood samples from parasitologically confirmed VL cases was only 70% [16]. PCR can also serve as a confirmatory test [17].

Domestic dogs are a significant reservoir of *L. donovani* in Sudan and other East African countries [18–20]. This means that dogs can carry the parasite without showing any signs of disease, and they can transmit the parasite to sandflies, which then transmit it to humans. In a rural setting in eastern Sudan, a dog infection was reported from the Atbara River area [18, 21, 22]. This provided the first solid evidence that domestic dogs may play an important role as reservoir hosts of *L. donovani* in eastern Sudan. This hypothesis was also supported by Hassan *et al.*, who used serological and PCR methods to detect Leishmania infection in dogs [20]. However, some studies have found no evidence of Leishmania amastigotes in bone marrow and lymph node aspirates by microscopy or PCR in domestic dogs in Al Gadarif State, eastern Sudan [23, 24]. These studies may have been limited by small sample sizes and the fact that the dogs were not tested for all species of Leishmania parasites.

Therefore, this study aimed to determine the prevalence of *L. donovani* in human hospital cases and domestic dogs using different diagnostic methods in two localities in Gadarif State, Sudan. This information will be used to design and

implement suitable prevention and eradication programs for the disease at the state level.

2. Materials and Methods

2.1. Study design and period

The study was a cross-sectional survey that aimed to determine the prevalence and risk factors of Leishmania infection in dogs and humans in Gadarif State, eastern Sudan. The study was conducted from October 2019 to April 2020.

2.2. Study population and area

The study population consisted of human and domestic dog subjects from Gadarif State, eastern Sudan, which is an endemic area for leishmaniasis. The human subjects were patients who visited the hospital with clinical signs suggestive of leishmaniasis, such as fever, weight loss, anemia, and splenomegaly. The canine subjects were pet dogs that lived in the same areas as the human cases. The study was conducted in El Hawata and Bazoura, two agricultural areas in Gadarif State, eastern Sudan. These areas are located about 200 km southeast of Khartoum, the capital city of Sudan. The main crops grown in these areas are sorghum, sesame, finger millet, and groundnuts. The vegetation consists mainly of native trees such as *Acacia senegal* (hashab), *Balanitls aegyptiaca* (higleeg), *Acacia mellifera* (kiter), *Acacia seyal* (taleh), and *Azadirachta indica* (neem). The area has a semi-arid climate with an annual rainfall of about 400 mm, which occurs mainly from June to October. The dry season lasts from November to May and is characterized by high temperatures and low humidity. The average temperature ranges from 21.9°C to 42.9°C in the dry season and from 22.5°C to 38°C in the rainy season. The relative humidity varies from 10% to 80% throughout the year. The soil is sandy loam with low organic matter and fertility [25]. The area is endemic for leishmaniasis, which is transmitted by the sand fly vector Phlebotomus spp. [26].

2.3. Eligibility criteria

The study included human and domestic dog samples collected from the areas of Al-Hawata and Bazoura in Gadarif State, eastern Sudan. The human samples were obtained from patients aged 18 years or older who visited the hospital with clinical signs suggestive of leishmaniasis, such as fever, weight loss, anemia, and splenomegaly. Patients were excluded if they were taking antiparasitic medications or were pregnant or breastfeeding. Patients were enrolled in the study after obtaining informed consent or assent from their guardians. The canine samples were obtained from pet dogs aged one year or older that lived in the same areas as the human cases. The dogs were selected randomly from the households that agreed to participate in the study. The owners of the dogs gave their verbal consent before the samples were taken.

2.4. Sample size determination

The sample size for this study was determined by convenience sampling, based on the availability and accessibility of the subjects. The sample size was limited by the number of human patients who visited the hospital with suspected leishmaniasis and the number of dog owners who agreed to participate in the study. The sample size was also influenced by the time and resources available for the study. The final sample size consisted of 101 subjects, 69 humans and 32 dogs, from the areas of Al-Hawata and Bazoura in

Gadarif State, eastern Sudan. This sample size may be considered statistically sufficient for a cross-sectional survey, depending on the desired level of precision and confidence. However, it is important to note that a convenience sampling method was used, which may have resulted in a sample that is not representative of the entire population of dogs and humans in Gadarif State.

2.5. Samples collection

Whole blood samples were collected from humans and dogs (*n* = 101) as follows: The human blood samples were collected by venipuncture from the antecubital vein using sterile syringes and needles. The dog blood samples were collected by cephalic venipuncture using a labeled disposable 2 ml syringe after disinfecting the injection site. The collected blood samples were transferred to EDTA tubes (ethylene diamine tetraacetic acid) and stored at –20°C until further analysis. The samples were labeled with codes indicating the source, date, and location of collection. The samples were transported to the laboratory of the College of Veterinary Medicine, University of Bahri for parasitological and molecular testing. Blood spots were made on filter papers (Whatman chromatography) for the PCR test. Lymph node aspirates were taken from suprascapular lymph nodes (*n* = 101). The collected samples were taken for the performance of the following tests.

2.5.1. Parasitological (direct microscopy) blood and lymph node smear

Thin smears from peripheral blood were prepared on glass slides, allowed to air dry, fixed with 100% methanol, and labeled with a human or dog number and date. Stained with Giemsas and then examined under oil immersion (100× magnification) for the presence of amastigotes.

Lymph node aspirate: popliteal or suprascapular lymph nodes were grasped between thumb and fingers, a 21-gauze needle attached to a 5-ml syringe was introduced into the lymph node, the lymph node was pressed gently several times, and then the syringe was removed. Thin smears were made from the lymph node aspirate as described above and then examined under the microscope for the presence of Leishman-Donovan bodies (L. D.) [27]. Direct microscopy of blood and lymph node smears is a simple and inexpensive diagnostic test, but it is not as sensitive as other serological and molecular tests (Table 1).

2.5.2. Serological (immunological) tests, IT LEISH (K39)

Leish is an immunochromatographic test to detect the presence of anti-Leishmania spp. with the help of a recombinant antigen (K39) [28, 29]. The test can be performed with whole blood (taken by the prick of the fingertip) or with serum or plasma; the result is obtained in 20 min with whole blood and 10 min with serum or plasma. The IT LEISH (K39) test is a good compromise between sensitivity and specificity (Table 1), and it is relatively easy to perform in field settings. It is the recommended test for the diagnosis of VL in endemic areas. IT LEISH includes all the necessary material for a test (**IT LEISH by BIO-RAD, 710124**).

2.5.3. Molecular–biological (PCR)

PCR is the most sensitive and specific test for the diagnosis of VL, but it is also the most expensive and complex test to perform (Table 1). The testing of samples was performed at Bahri University, Faculty

of Veterinary Medicine, Laboratory of Molecular Biology.

2.5.4. DNA extraction

DNA extraction was done using the guanidine chloride method described by Ciulla *et al.* (1988) [30].

2.5.5. PCR amplification

Two pairs of forward (18SLEISH: 5'GCTGTGCAGGTTTGTTCCTG'3) and reverse (18SLEISH: 5'GGACGCACTAAACCCCTCAA'3) pairs of primers were used to amplify the *L. donovani* gene sequences. From the extracted DNA template, two l were extracted: 1 l of each primer (10 M), 2 l of 50× dNTP mix, and 2 l of 50× polymerase mix. The PCR program was performed for 2 min at 95°C, followed by 30 cycles at 95°C for 30 sec, 59.3°C for 30 sec, 72°C for 40 sec, and a final extension step at 72°C for 5 min [31].

PCR product changed into detected with the aid of electrophoresis; three l of loading answer changed into introduced to the PCR product, loaded onto 0.4 g Agarose gel and stained with three l of ethidium bromide (10 mg/ml), run for 1.5 hr in 1 X TBE buffer (1M Tris, 1M Boric Acid, and 50M EDTA) at 90 V 20 A, and photographed under Neath UV lighting after electrophoresis of three L of response answer Agarose gel and 100-b A DNA ladder (Vivantis®) was used as a marker.

2.5.6. Statistical analysis

Data were analyzed using the Statistical Package for Social Science (SPSS) software program version 21. The prevalence of Leishmania infection in dogs and humans was calculated as the percentage of positive samples out of the total number of samples tested.

3. Results

3.1. Parasitological results

The parasitological diagnosis of Leishmania infection was based on the microscopic detection of Leishmania amastigotes in blood and lymph node samples from dogs and humans. The results showed that none of the blood samples from dogs or humans were positive for Leishmania amastigotes by microscopy (Table 2). However, 49 out of 69 (71.01%) human lymph node samples and 3 out of 32 (9.4%) dog lymph node samples were positive for Leishmania amastigotes by microscopy (see Table 2 and Figure **1**). The overall prevalence of Leishmania infection by microscopy was 51.48% (52 out of 101) in dogs and humans combined.

3.2. Immunological results

The immunological diagnosis of Leishmania infection was based on the detection of *L. donovani* antibodies in serum samples from dogs and humans using the IT Leish kit test. The IT Leish kit test results showed that 57 out of 69 (82.6%) human serum samples and 14 out of 32 (43.75%) dog serum samples were positive for *L. donovani* antibodies (see Table 2 & Figure **2**). All 49 human lymph node samples that were positive for Leishmania amastigotes by microscopy were also positive for *L. donovani* antibodies by the IT Leish kit test. The overall prevalence of Leishmania infection by the IT Leish kit test was 70.3% (71 out of 101) in dogs and humans combined.

TABLE 1: Sensitivities and specificities of various methods used for the diagnosis of leishmaniasis.

Diagnostic test	Sensitivity	Specificity	Reference
Direct microscopy (blood smear)	50–60%	80–90%	[32]
Direct microscopy (lymph node smear)	60–70%	80–90%	[32]
IT LEISH (K39)	85–90%	95–99%	[33]
PCR	90–95%	95–99%	[17, 34]

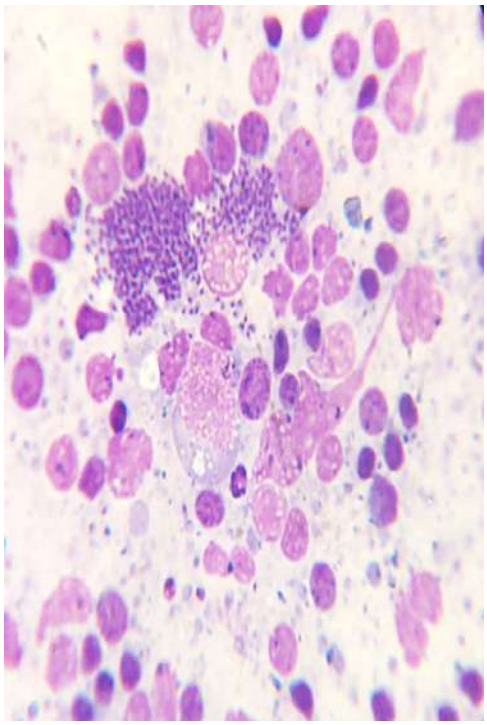


Figure 1: Microphotograph showing intracellular and extracellular *Leishmania donovani* bodies (amastigotes) in lymph node aspirate.

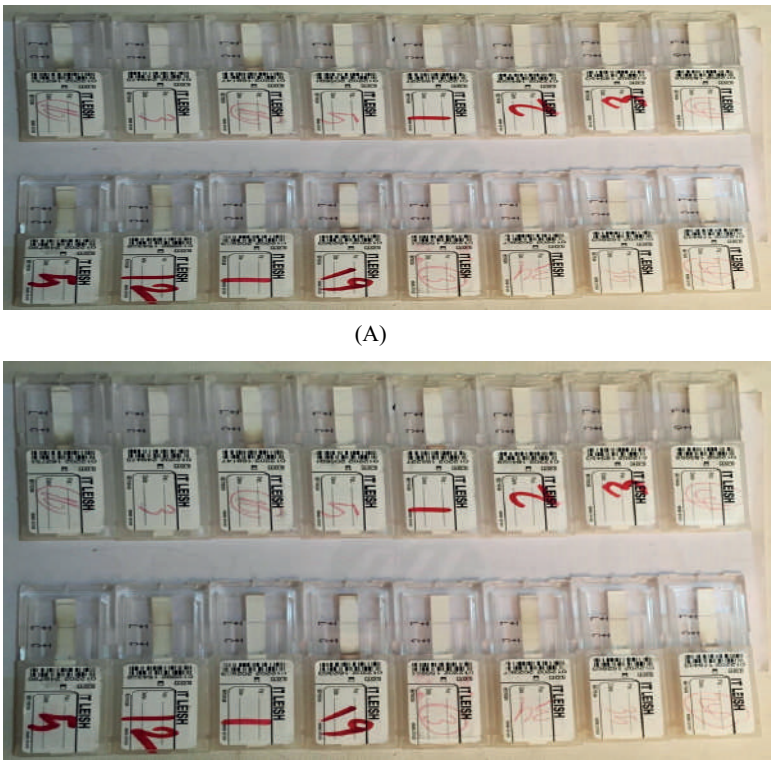
3.3. Molecular study results

The molecular diagnosis of Leishmania infection was based on the amplification of the 18S rRNA gene of *Leishmania donovani* using PCR. The results showed that all the positive lymph node aspirate samples by microscopy and the IT Leish kit test were confirmed as positive by PCR. The PCR results are shown in Figure **3**, which displays a band at 357 bp for *L. donovani*. The overall prevalence of Leishmania infection by PCR was 70.3% (71 out of 101) in dogs and humans combined, which was consistent with the IT Leish kit test results (Figure **3**).

4. Discussion

In Sudan, leishmaniasis is an endemic disease, with reports all over the country. However, there is only a little data about the use of different diagnostic methods available, and few studies in Sudan have studied the zoonotic nature of the disease or the role of dogs in disease transmission. Thus, the present study investigated the prevalence and characterization of *L. donovani* in human patients and domestic dogs in two localities in southern part of Gadarif state namely Hawata and Bazoura, using a variety of diagnostic methods.

The results of this study showed that the prevalence of Leishmania infection in humans



(A)

(B)

Figure 2: The positive IT LEISH kit test results. (A) Human-positive samples. (B) domestic dog's positive samples.

Table 2: Comparison of diagnostic methods for leishmaniasis in human and dog samples.

Tests	Species no (%)		Total
Blood film	**Human**	**Dogs**	
+ve (%)	0 (0.0)	0 (0.0)	0 (0.0)
–ve (%)	69 (70.4)	32 (29.6)	101 (100)
Total	69 (68.3)	32 (31.7)	101 (100)
Lymph node			
+ve (%)	49 (94.2)	3 (5.8)	52 (51.5)
–ve (%)	20 (40.8)	29 (59.2)	49 (48.5)
Total	69 (68.3)	32 (31.7)	101 (100)
IT leish			
+ve (%)	57 (80.3)	14 (19.7)	71 (70.3)
–ve (%)	12 (40.0)	18 (60.0)	30 (29.7)
Total	69 (68.3)	32 (31.7)	101 (100)

Note: The methods include a blood smear, a lymph node smear, and an i mmunochromatographic test (IT Leish) based on the K39 antigen. The results are shown as positive (+) or negative (–) for each method and sample type.

and dogs in the southern part of Gadarif State, eastern Sudan, was higher than the country-wide prevalence [6], likely due to the combination of environmental and socioeconomic factors. Gadarif State is a known endemic area for Leishmania, with favorable ecological conditions for the transmission of the parasite. Additionally, the state is characterized by poverty, malnutrition, and poor

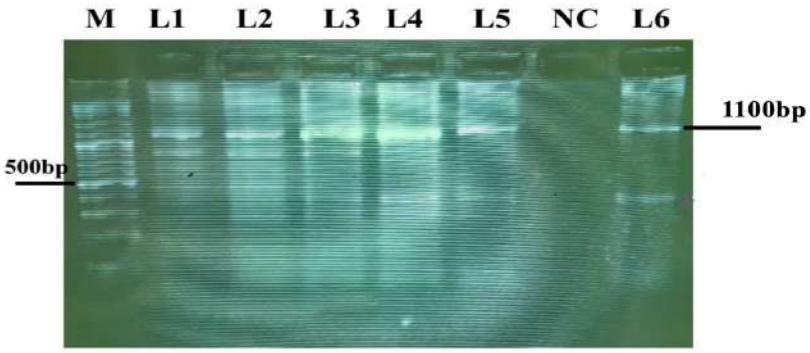


Figure 3: Agarose gel (0.4) of 18S LEISH primer of *L. donovani* by PCR Lane (M): molecular ladder; Lane (1–3): dog positive samples; lane (4–6) human positive samples; lane (NC): negative control.

sanitation, all of which are known risk factors for Leishmania infection. The fact that the study areas are agricultural areas and that the people in these areas have certain lifestyles and traditions, such as seasonal work in the agricultural sector, raising livestock, and the use of domestic dogs for social purposes and companionship, further increases their risk of getting infected with leishmaniasis [23].

The current study found that the prevalence of Leishmania infection was higher in humans than in dogs. This is likely due to a combination of factors. Studies showed that there are differences in host susceptibility and immune response for *L. donovani* between humans and dogs, in which humans are the definitive host and dogs are reservoir hosts [20, 22, 35, 36]. This means that humans are more likely to develop disease after infection, and their immune systems may be less effective at controlling the parasite. In addition, the differences in diagnostic sensitivity could be another possible reason for this difference. Hence, the serological methods used in the study may be less sensitive for detecting infection in dogs.

It is important to note that the human samples were taken for patients attending healthcare facilities, and the dog samples were taken from the general population, so this can be another possible reason for the difference in prevalence between humans and domestic dogs. Further research is needed to better understand the factors that contribute to this difference.

The prevalence of Leishmaniasis in the southern part of Gadarif State, Sudan, is alarmingly high, and the potential role of dogs as reservoir hosts of the parasite is concerning. These findings suggest that Leishmaniasis is a major public health problem in Gadarif State and that further research is urgently needed to better understand the epidemiology and transmission dynamics of Leishmaniasis in Sudan. This research will inform the development of more effective control and prevention strategies.

Furthermore, the study also showed that different diagnostic methods have varying sensitivities and specificities for detecting Leishmania infection in humans and dogs, whereby, the parasitological method based on microscopy of blood and lymph node samples was the least sensitive method for diagnosing Leishmania infection in humans and dogs. None of the blood samples from humans and dogs were positive for Leishmania amastigotes by microscopy, which is consistent with previous studies that reported a low sensitivity of direct

microscopy in peripheral blood [12, 16, 23]. The lymph node samples from humans and dogs were more sensitive than the blood samples for detecting Leishmania amastigotes by microscopy, however, the sensitivity varied among different studies [12, 23]. This is likely due to the fact that Leishmania amastigotes are often difficult to detect in blood and lymph node samples of humans and dogs, especially in the early stages of infection [37, 38].

The immunological method was based on the IT Leish kit test which has been validated and recommended by the WHO as a reliable and simple tool for the diagnosis of leishmaniasis in endemic areas [13]. The current study found that the IT Leish kit test was the most sensitive and specific method for diagnosing Leishmania infection in both humans and dogs. However, the prevalence of Leishmania infection in humans by the IT Leish kit test was slightly lower than what was reported by Boelaert *et al.* [13], who found 91.9% positivity for humans in Sudan using the same test.

Additionally, the prevalence of Leishmania infection in dogs by the IT Leish kit test was much higher than what was reported by Hassan *et al.* [20], who found 6.9% positive for dogs using the direct agglutination test (DAT) in 10 villages along the Rahad River, and Dereure *et al.* [22], who found 29.1% positive for dogs using the indirect fluorescent antibody test (IFAT) and culture in Barbar El Fugara village in Al Gadarif State. The IT Leish kit test is generally considered to be more sensitive than the DAT and IFAT [38], so this is likely one reason for the difference in prevalence rates observed. Additionally, the prevalence of Leishmania infection in dogs may have increased in the Gadarif State region of Sudan since the studies by Hassan *et al.* and Dereure *et al.* were conducted.

Overall, the results of the current study suggest that the IT Leish kit test is a reliable and sensitive tool for diagnosing Leishmania infection in both humans and dogs in Gadarif State, Sudan. However, further research is needed to better understand the reasons for the differences in prevalence rates observed in the current study compared to previous studies.

The molecular method based on PCR was used as a confirmatory test for the positive samples by microscopy and the IT Leish kit test. The PCR was performed on DNA extracted from blood and lymph node samples from humans and dogs using the LEISH primer pair that targets a 357 bp fragment of the 18S rRNA gene of *L. donovani*. This primer pair has been reported to have a high sensitivity and specificity for detecting and identifying Leishmania species in clinical samples from humans and animals [39]. The results showed that all the positive samples by microscopy and the IT Leish kit test were confirmed as positive by PCR, indicating that there was no false positive result by these methods. The PCR results also confirmed that *L. donovani* was the causative agent of VL in humans and dogs in the study areas, which is consistent with previous studies that reported *L. donovani* as the predominant species of Leishmania causing VL in Sudan [39]. The results also showed that dogs were infected with *L. donovani*, suggesting that they may act as reservoir hosts or sources of infection for humans.

The findings of the current study highlight the importance of using a combination of diagnostic methods to confirm a diagnosis of leishmaniasis. PCR is a highly sensitive and specific method for detecting Leishmania DNA, but it is not always accessible in endemic areas. Microscopy and the IT Leish kit test are more accessible and affordable diagnostic methods, but they are not

as sensitive as PCR. By using a combination of these methods, clinicians can more accurately diagnose leishmaniasis and ensure that patients receive the appropriate treatment. This study provided up-to-date epidemiological information about Leishmania infection in human patients and domestic dogs in the southern region of Gadarif State, eastern Sudan. This information is essential for designing and implementing effective control measures for leishmaniasis in the region that target both humans and dogs, in accordance with the One Health concept.

5. Conclusion

The study showed that leishmaniasis caused by *L. donovani* is prevalent in human patients and domestic dogs in the study areas. The different diagnostic methods had different sensitivities and specificities for detecting Leishmania infection in humans and dogs. The IT Leish kit test was the most sensitive and specific method, while microscopy of blood and lymph node smears was the least sensitive method. PCR was used as a confirmatory test for the positive samples and showed 100% positivity. The study suggests that the IT Leish kit test is a reliable and simple tool for the diagnosis of leishmaniasis in endemic areas and that PCR is useful for confirming and identifying Leishmania species. Further research is needed to develop more effective and affordable diagnostic tools and treatments for Leishmania infection. The study also implies that dogs may act as reservoir hosts or sources of infection for humans and that control measures should be taken to prevent and treat leishmaniasis in humans and dogs. Additionally, more research is needed to understand the role of dogs in the transmission of Leishmania infection in Sudan.

6. Limitations

The study had some limitations that should be considered. The study was cross-sectional and could not capture the temporal and spatial variations of Leishmania infection. Additionally, the sample size was relatively small, which limits the generalizability of the findings. The study only included hospital patients and domestic dogs and could not represent the whole population or other potential reservoirs or hosts. The study only used one primer pair for PCR and could not identify *L. donovani* at the strain or sub-species level. Despite these limitations, the study provides valuable information on the prevalence and characterization of *L. donovani* in humans and dogs in Gadarif State, Sudan. The study also highlights the importance of using a combination of diagnostic tests to confirm a diagnosis of Leishmaniasis.

Acknowledgements

None.

Ethical Considerations

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and the national guidelines for research involving human and animal subjects. The study protocol was reviewed and approved by the Ethics Review Committee, Faculty of Medicine and Health Sciences, University of Gadarif (Ref. No; GU/FM/REC.Q1.3.22.1). The purpose and procedures of the study were explained to the human participants and the dog owners before the sample collection. The human participants gave their written informed consent or assent from their guardians if they were minors. The dog owners gave their verbal consent after being assured that

the sample collection would not harm their dogs or affect their health. The participants and the owners were free to withdraw from the study at any time without any consequences. The confidentiality and anonymity of the participants and the owners were maintained throughout the study. The samples were used only for the purposes of this study and were disposed of properly after the analysis.

Competing Interests

Authors declare no conflict of interest.

Availability of Data and Material

Data are available within the submitted article.

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