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Molecular detection of Leishmania in cutaneous scrapping and blood samples of dogs in Mosul city

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Abstract

This study included 92 dogs of both sexes with various breeds, health statuses, and ages ranging from 2 months to 4 years old. Samples, including cutaneous lesions or scars and blood, were collected via the cephalic vein into EDTA tubes. The buffy coat was separated from the blood using lymph prep, and DNA was extracted from both blood and scars using a commercial kit. Polymerase chain reaction (PCR) was attempted to investigate the DNA of. Leishmania, using LITSR and L5.8S primers to detect the ITS1 gene of Leishmania. The results showed that 22 animals were infected with leishmaniasis, with the highest positive results recorded in buffy coat samples compared to skin biopsy samples. According to the clinical signs, animals suffering from skin lesions recorded higher positive outcomes than those with other clinical manifestations. There was a higher positivity rate in male and Rottweiler dogs compared to different groups of dogs. Older dogs up to 4 years old showed a lower infection prevalence than dogs younger than one year old, which recorded a higher positivity rate with significant variability in the age of dogs.

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Introduction

Anthropozoonotic disease, known as leishmaniasis, is caused by parasitic protozoa of the genus Leishmania and is endemic in tropical and subtropical regions of the world. Visceral leishmaniasis (VL) affects an estimated 12 million people globally, with an incidence of 0.2-0.4% (1). According to Mauricio *et al.* (2), the species that cause VL are *Leishmania infantum* (syn. *chagasi*) (New World), *L. infantum*, and *L. donovani* (Old World). *L. infantum* is endemic for humans and dogs in many parts of Brazil and is widely distributed throughout the Americas (3). The parasite is transmitted via infected blood-sucking Phlebotominae sand flies from the vertebrate host to other vertebrate animals of the *Lutzomyia longipalpis* (Lu. longipalpis) species, which is the primary vector of VL in Brazil (4). Dogs in Mediterranean countries such as Cyprus, Greece, Albania, Croatia, Italy, Malta, France, Spain, and Portugal are also susceptible to VL. Due to dogs increasing mobility and other drivers of global change, such as climate changes and international travel, the average seroprevalence in domestic dogs is up to 25%, and it has spread to new regions in Europe. Estimates suggest that approximately 700 autochthonous VL human cases caused by *L. infantum* occur every year (5). In addition, seropositive canines have a high prevalence in the main foci of human VL (6). Dogs are considered the primary reservoir in urban settings, while wild carnivores are the most significant reservoirs in rural areas (7). Direct parasitological examination and serological procedures, which are time-consuming and may not be accurate, are used for the laboratory diagnosis of cutaneous leishmania. Since clinically suspected and seemingly healthy dogs can transmit the parasite to phlebotomine vectors, it is crucial to use more precise and sensitive techniques, including genetic diagnostic tools, to identify Leishmania infection (8). A recent meta-analysis on cutaneous leishmania conducted in Iran found that most infected canines showed no clinical symptoms (9). Nowadays,

polymerase chain reaction can detect Leishmania spp. in various clinical samples and determine the parasite species, strains, and genotypes (10). Numerous studies use quick testing on dogs to detect the parasite and its specific antibodies, as more precise and sensitive tests are unavailable (11). At the beginning of military operations to retake Mosul, thousands of civilians migrated to camps and began living under challenging conditions, the development of diseases, such as Leishmaniasis, where cases were reported, exacerbated the already poor health and service conditions, creating a favorable environment. According to the World Health Organizations report for the year 2017, there were several infections with this parasite in field hospitals, and it ranked high in the list of reported diseases (12).

Dogs play a crucial role and act as a reservoir for the parasite, hence the need for a method to estimate the parasite infection rates in dogs. This study aims to estimate the parasite infection rates in dogs using a polymerase chain reaction.

Materials and methods

Ethical approve

This study obtained approval from the scientific board, College of veterinary medicine, Mosul University, Mosul, Iraq, the approval issue UM.VET.2021.078.

Dog population and sampling

From 2021 to 2022, 92 dogs were checked by house-to-house visits or during vaccination routine programs (from both sexes, breeds, health statuses, and ages between 2 months to 4 years old). Dogs were examined for clinical signs, and samples were collected, which included cutaneous lesions or scars. Blood was collected via a cephalic vein to EDTA tubes, and the buffy coat was separated from blood using lymph prep (Sigma) (13,14).

DNA extraction

DNA extraction was performed using a commercial kit (Roche, Germany). Polymerase Chain Reaction (15). The primers used in this reaction were LITSR: CTGGATCATTTTCCGATG and L5.8S: TGATACCACTTATCGCACTT to detect the ITS1 gene of Leishmania at 311 bp. The PCR conditions were two µl DNA, 1.5 mM MgCl2, 0.2 mM dNTPs, 25 pmol primers, 1U Taq, and 1× buffer (Promega). The amplification steps included denaturation at 95°C (16-20), annealing at 53°C (20), and extension at 72°C (20) for 32 cycles. The final products were detected by electrophoresis using 2% agarose.

Results

This study showed the presence of 22 animals infected with leishmaniasis through the investigation of DNA for the parasite (Figure 1). The high positivity rate was recorded in buffy coat samples compared to skin biopsy samples without significant variance (Table 1). According to the clinical signs, the animals that suffer from skin lesions recorded high positive results compared with other clinical manifestations (Table 2). Males recorded a higher infection prevalence than females, without significant variance (Table 3). The German Shepherd breed showed a higher positivity rate with leishmaniasis, while the Lolo Fox breed recorded a lower prevalence of infection (Table 4). Older animals, up to 4 years old, showed a lower infection prevalence than animals younger than one year old, who recorded a high positivity rate. There was significant variability in the age of dogs (Table 5).

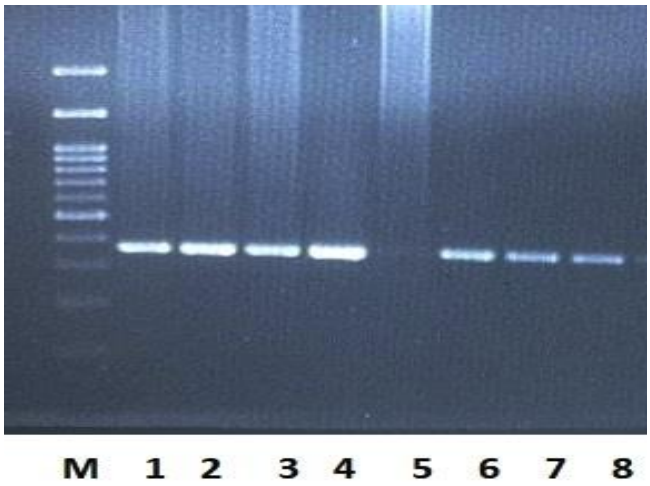

Figure 1: Shows the amplification of the ITS1 gene, The markers are 1-4,6-8 (311 bp), which are positive for Leishmania, and 5 is negative.

Table 1: Percentage of positivity of Leishmania according to the type of sample

Type of sample	Total examined (n)	Positive (n)	Positive (%)
Buffy coat	92	22	23.9
Tissue biopsy	92	15	16.3

Table 2: Percentage of positivity of Leishmania according to type of clinical manifestations in dogs

Clinical manifestations	Total examined (n)	Positive (n)	Positive (%)
Bloody diarrhea	43	8	18.6
Weakness	29	5	17.2
Crackles in paw	18	11	61.2
Skin lesions	56	20	35.7

Table 3: Percentage of positivity of Leishmania according to the gender of dogs

Sex	Total examined (n)	Positive (n)	Positive (%)
Male	54	14	25.9
Female	38	8	21.0

Table 4: Percentage of positivity of Leishmania according to the breed of dogs

Breed	Total examined (n)	Positive (n)	Positive (%)
Lolo fox	21	1	4.7
Terrier	10	2	20.0
Sebrina Huskey	13	3	23.0
Doberman	7	2	28.5
German shepherd	36	11	30.5
Rot Weller	5	3	60.0

Table 5: Percentage of positivity of Leishmania according to the age of dogs

Age	Total examined (n)	Positive (n)	Positive (%)
> 1 year	24	4	16.7
< 2 years	31	3	9.6
2-4 years	21	5	23.8
< 4 years	16	10	62.5

Discussion

Numerous studies have demonstrated the utility of PCR in detecting CVL (16). However, each investigation utilized a single PCR technique and a variety of sample types. To our knowledge, only one comparative PCR study for *L. infantum* (17) has been conducted, making selecting an appropriate assay challenging. PCR diagnosis is still problematic due to a variety of technical factors. The main goal of this study was to establish a reliable PCR technique that could identify Leishmania in dogs using peripheral blood.

The current investigation found that the seroprevalence of canine leishmaniasis was generally high 22/92 (23.9%). Weliso had the highest seroprevalence 137/146 (93.84%), followed by Ambo 86/109 (76.11%) and Ejaji town 87/109 (79.82%). The results of the present study were markedly higher than the seroprevalences of canine leishmaniasis of 13.9% recorded in the Benishangul Gumuz Regional State, Ethiopia (18), 40% in dogs in Northwest Ethiopia (19), 6.9% in domestic dogs in Eastern Sudan (20), 11.7% in stray dogs, 9.7% in National Guard canines, and 5.9% in agricultural dogs in Algeria (20). Previous studies have also found significantly lower Seroprevalence of CLI than the current study, such as 6.5% in an endemic focus of the Satluj River valley in Himachal Pradesh (India) (21) and 35% VL among canines in VL endemic areas of Mymensingh. The study showed that the positivity rate was higher in buffy coat samples compared to skin biopsy samples (22).

In contrast to the advantages of utilizing peripheral blood for the PCR detection of CVL over lymph node biopsy, aspirate, or bone marrow aspiration, which are as follows: I) dogs can have circulating parasites; II) it is easy and less invasive; III) it has high sensitivity compared to traditional procedures (serological and parasitological); and IV) it enables the identification of dogs that are not showing any symptoms (and occasionally seronegative animals). The limitation of PCR is that a positive result only confirms Leishmania infection, not an illness. Therefore, it cannot be utilized as a unique diagnostic tool for verifying disease. Despite negative serology and positive PCR data, leishmaniasis might be established by clinical solid suspicion (23).

High positivity samples in animals that showed crackles in their paw (24) indicated that quantitative PCR values in sick dogs were significantly more significant compared to infected dogs. The results showed differences in positivity between male and female dogs, with higher positivity in males than females. 33 intact females and 36 intact males. The relationship between the breed of dogs and positivity to Leishmania infection showed variation in infectivity, with higher positivity in Rottweiler dogs and lower positivity in Lolo Fox dogs (25). We can attribute this variation to management types according to the breed of dogs (26,27). On the other hand, the wet season and mixed living environment were significantly associated with CLI seropositivity ($P = 0.001$ and $P = 0.025$, respectively) (28). This may be due to the favorable environmental conditions for sandfly breeding in these regions and the increased exposure of dogs to sandfly bites. The lower risk of exposure to sandfly bites may cause lower illness in domestic dogs (29).

A high positivity rate was recorded in older dogs compared to younger ones (30). This study documented that older dog had significantly higher infection rates than younger dogs, stray dogs had higher infection rates than owned dogs, and rural dogs had higher infection rates than urban dogs (31). In Brazil, where the re-emergence of the disease appears to be a result of the termination of control programs, a high positivity rate was also observed (32). Similarly, in the central region of Colombia, owned dogs had significantly higher infection rates than stray dogs (33,34).

Conclusions

Dogs affected Leishmania, and there are more dominant parasites in dogs in Nineveh province.

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Conflict of interest

The authors declare that there is no conflict of interest in the manuscript.

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الكشف الجزيئي عن طفيلي الليشمانيا في القشطات الجلدية وعينات دم الكلاب في مدينة الموصل

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الخلاصة

شملت هذه الدراسة ٩٢ كلبا من مختلف، السلالات، والحالة الصحية، والأعمار التي تراوحت من شهرين إلى ٤ سنوات. تم جمع العينات والتي شملت الآفات الجلدية والدم، تم جمع الدم من الوريد الراسي ووضع في أنابيب مانع تخثر ومن ثم فصلت طبقة الخلايا اللمفية باستخدام الفايكول ومن ثم تم استخلاص الدنا من القشطات الجلدية والدم باستخدام العدة التشخيصية الخاصة بذلك والمتوفرة تجاريا ومن ثم تم إجراء اختبار تفاعل البلمرة المتسلسل للكشف عن الدنا لطفيلي الليشمانيا وباستخدام زوج من البادئات المتخصصة. بينت النتائج وجود ٢٢ حيوانا إيجابيا لطفيلي الليشمانيا مع تسجيل اعلى نسبة لتواجد الدنا للطفيلي في عينات طبقة الخلايا اللمفية مقارنة بالقشطات الجلدية، سجلت الحيوانات التي تعاني من تواجد الآفات الجلدية اعلى نسبة إصابة مقارنة بالحيوانات التي تعاني من أعراضا سريرية أخرى، كما سجلت الذكور اعلى نسبة إصابة فيما وجدت اعلى نسبة إصابة في الكلاب من سلالة الروت ويلر مقارنة بالسلالات الأخرى من الكلاب، أما الكلاب الأكبر من ٤ سنوات فقد سجلت اقل نسبة إصابة مقارنة بالكلاب الأصغر سنا الأقل من سنة واحدة والتي سجلت اعلى نسبة إصابة وبفروق معنوية واضحة.

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