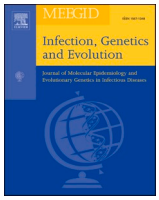
Infection, Genetics and Evolution 103 (2022) 105327

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid



Serological and molecular detection of *Leishmania species* in dog peripheral blood from Bobo-Dioulasso city, a confirmation of canine leishmaniasis enzootic area for Burkina Faso

Arthur D. Djibougou [a,b,c], Achille S. Nikièma [b], Aristide S. Hien [b], Ibrahim Sangaré [c], Bienvenue K. Yameogo [b], Lassane Koala [b], Ali Ouari [a], Serge P. Diagbouga [b], Abdoulaye Diabaté [b], Helen Price [d], Florence Fournet [e], Roch K. Dabiré [b,*]

[a] Centre MURAZ/Institut National de Santé Publique (INSP), 01 BP 390 Bobo-Dioulasso 01, Burkina Faso
[b] Institut de Recherche en Sciences de la Santé (IRSS), Direction Régionale de l'Ouest, 01 BP 545 Bobo-Dioulasso 01, Burkina Faso
[c] Université Nazi BONI, Bobo-Dioulasso, 01 BP 1091 Bobo-Dioulasso 01, Burkina Faso
[d] School of Life Sciences, Keele University, UK
[e] UMR-MIVEGEC-IRD, 34394 Montpellier Cedex 5, France

ARTICLE INFO

Keywords:
Leishmania spp
Zoonosis
Diagnostic
Incidence
Bobo-Dioulasso
Burkina Faso

ABSTRACT

Canine leishmaniasis is increasingly reported worldwide and represent a threat to both animal and human health. In a previous pilot study conducted in Bobo-Dioulasso, the second town of Burkina Faso, we reported five cases of canine leishmaniasis. With the perspective of a One Health action plan, and in the context of increasing urbanization, this study aimed to provide new information on *Leishmania spp* in dogs in this city.

A cross-sectional survey was carried out from May to August 2018 in six districts of the city in order to record clinical and biological data from domestic dogs randomly selected per district. Blood samples were collected into EDTA tubes (4-5 mL), treated and stored at −20 °C until further analyses. The infection status of the dogs was performed by serological tests using plasma, and real time-PCR (RT-PCR) to detect *Leishmania* parasites using buffy coats. Nested PCR was used for typing the *Leishmania* species in dogs which were found to be RT-PCR positive.

A total of 147 dogs were examined clinically and sampled for blood collection, including 53.7% females and 46.3% of males with a median age of 3 years. The seroincidence of *Leishmania* parasites within this dog population was 4.76% (95% CI:2.26–9.72). The incidence of *Leishmania* was 10.88% (95% CI: 6.73–17.11) by RT-PCR which was significantly more sensitive ($p = 0{,}047$) and a fair concordance was observed between both tests (Kappa = 0.39, $p < 0.001$). The characterization of *Leishmania* species revealed that *L. major* was circulating in this domestic dog population.

Our results confirmed the persistence of zoonotic circulation of *Leishmania* parasites such *as L. major* currently in Bobo-Dioulasso city and highlight the need for targeted interventions in order to control transmission of leishmaniasis in this region.

1. Introduction

Leishmaniasis is a vector-borne disease caused by infection with intracellular parasitic protozoans of the genus *Leishmania,* and associated with high mortality and morbidity particularly in low and middle income countries (LMICs) (Okwor and Uzonna, 2016). The *Leishmania* parasites are transmitted through the bite of an infected female sandfly (*Diptera*: *Psychodidae*) of the genus *Phlebotomus* in the Old World and *Lutzomyia* in the New World. It is estimated that 350 million people are at risk of acquiring leishmaniasis, with 70,000 deaths annually and around 2 million new cases per year statistic instead of this one (Torres-Guerrero et al., 2017). Around 70 animal species including humans and dogs have been found as natural reservoir hosts of *Leishmania* parasites (Esteva et al., 2017).

Canine leishmaniasis (CanL) is a major veterinary issue and also a public health challenge due to its potential for zoonotic transmission to

* Corresponding author.
E-mail address: dabireroch@gmail.com (R.K. Dabiré).

https://doi.org/10.1016/j.meegid.2022.105327
Received 17 January 2022; Received in revised form 23 June 2022; Accepted 26 June 2022
Available online 8 July 2022

humans (Santarém et al., 2020). CanL is endemic in >70 countries in the world and domestic dogs (*Canis familaris*) are well known to contribute substantially to the transmission of *Leishmania (L.) infantum* to humans (Solano-Gallego et al., 2011).

The development of *Leishmania* infection varies between individual dogs and it is characterized by either asymptomatic infections (over 80% of cases), spontaneous cure or acute signs that lead to death without treatment (Ribeiro et al., 2018). The most common clinical manifestations found in CanL are cutaneous lesions (exfoliative dermatitis), weight loss, anorexia, auricular skin lesions, lymphadenopathy, ocular lesions, nasal bleeding, locomotary problem and muscular atrophy onychogryposis (Baneth et al., 2017; Sasani et al., 2016; Solano-Gallego et al., 2011). Diagnosis of the disease is complex due to the variable clinical manifestations which may be confused with many other conditions (Solano-Gallego et al., 2017). However, definitive diagnosis of CanL is possible using serological tests and molecular methods to detect and identify the *Leishmania* parasite (Solano-Gallego et al., 2017). Serological tests appear to be the first choice for diagnosis of CanL because of high sensitivity and specificity and simplicity to perform the test (Sundar and Singh, 2018). However these tests are limited by cross reactions with *Trypanosoma cruzi* where this is present, and issues in immunocompromised hosts (Carvalho et al., 1987; Lévêque et al., 2020). Molecular methods are the most accurate methods but they remain expensive as routine diagnostics.

It is well known that human leishmaniasis cases in some regions are closely associated with the presence of domestic infected dogs which allow sandflies to be infected, thus risking transmission of the *Leishmania* parasite to other dogs or human hosts (Abbehusen et al., 2017; Faye et al., 2010; Reithinger et al., 2003). In Burkina Faso, human cutaneous leishmaniasis (HCL) was reported as endemic since the independence's in 1960 (Oddou, 1960). Many others authors reported also from 1996 to 2012, in the city of Ouagadougou some HCL cases with *L. major* being responsible for the disease (Traoré et al., 2001; Guiguemdé et al., 2003; Bamba et al., 2017). More recently, over 100 cases of HCL due to *L. major* were reported in the rural village of Larama, a locality close to Bobo-Dioulasso city (Konate et al., 2020). In addition, clinical signs were found in dogs from this city which prompted work to investigate CanL in the area. The results, although very limited, showed the presence of infected dogs with *L. infantum* (Sangaré et al., 2016), indicating a zoonotic focus of CanL although potentially infected vectors were not identified. The study involved serological testing of 89 dogs, with 5 positive cases found, 3 of which were confirmed by PCR albeit with a very low yield. With the exception of this study, the issue of CanL remains neglected in Burkina Faso because there are no updated or permanent data available in the veterinary health services, nor in the research centers of the country.

In the context of rapidly spreading urbanization having modified the features of the city and in view of a One Health action plan, we performed the current study to provide updated information on CanL using a representative number of samples and multiple and diversified diagnostic resources.

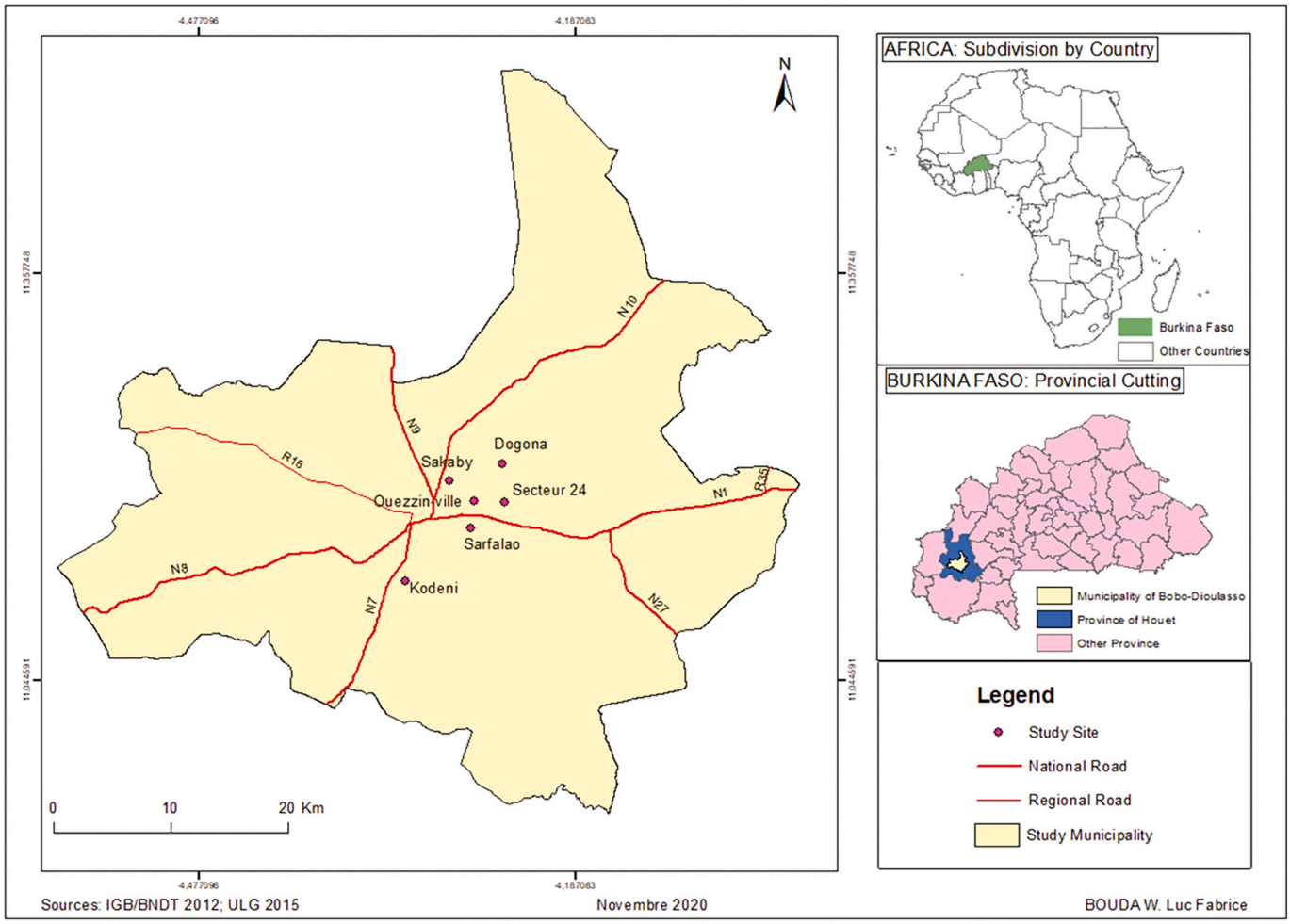


Fig. 1. Maps showing location of the study sites in Burkina Faso.

2. Material and methods

2.1. Study sites

A cross-sectional study was conducted from May to August 2018 in the municipality of Bobo-Dioulasso (11°10′42"N; 4°17′35"W), capital of the province of Houet, a crossroads town in the west of Burkina Faso. The annual average rainfall is 1000 to 1300 mm, with an annual average temperature of 28.2 °C which varies from 16 to 45 °C. The study collection sites were Kodéni, Sarfalao, Sakaby, Secteur 24, Ouezzin-Ville, Dogona which are all from the municipality of Bobo-Dioulasso (Fig. 1). The selection of these areas was justified by the extension of collection sites according to the recommendations of the pilot study conducted in 2013 (Sangaré et al., 2016).

2.2. Dog samples' collections and preparing for serological and molecular analysis

2.2.1. Population study, sample size and sampling

Population study consisted to domestic dogs and the sampling was done according to a prospective chronology in the households. As a cross-sectional study, the formula used to calculate the sample size was that of Daniel Schwartz which follows: $N = (z^2.p.\ q) / (d^2)$.

N = sample size required, Z = 1.96 at the significance level of 5% of the value of α, p = assumed rate = 5.61% (data from 2013 in Sangare et al. 2017), q = 1-p = (1–0.0561) =0.944, d = desired precision = 4,

$N = 1.96 \times 1.96 \times 0.0561 \times 0.944 / (0.04)^2 = 127$. We reached 147 samples of dogs in this study.

Domestic dogs were randomly selected in the different study sites. Was include, any dog presenting at least one clinical sign or not, and whose owner gave his consent. Stray and untamed dogs as well as dogs whose owners have declined the questionnaire were excluded from the study. Once consent had been obtained from owners, dogs were restrained with a muzzle and immobilized before performing blood collection (4 mL) by venipuncture into ethylenediaminetetraacetic acid (EDTA) tubes for serological and PCR tests. The dogs were also examined for visual clinical signs as follows: i) weight loss, ii) exfoliative dermatitis, iii) alopecia, iv) auricular skin lesions, v) onychogriphosis. After the blood sampling, information was collected on the dog from the owner following a structured questionnaire including the age, gender and clinical status of the animal. Collected blood samples were transferred to the Laboratory of Parasitology of Centre MURAZ for analyses. Blood samples were centrifuged at 1800 rpm during 20 min for allowing plasma and buffy coats separation. The buffy coats and the plasma were stored into 1.8 mL microtube separately at −20 °C until serological and PCR analyses were performed.

2.2.2. Serological assays

The DiaMed-IT LEISH® kit reagent (Bio-Rad, France catalog 710,124) which is an immuno-chromatographic test (ICT) based on the rK39 antigen was used to detect the presence of the antibodies against *Leishmania spp* in plasma, according to the manufacturer's instructions.

2.2.3. Molecular screening for Leishmania spp. infection in dogs by RT-PCR

Buffy coats were used for DNA extraction using the DNeasy Blood and Tissue kit® (Qiagen, Valencia, CA) according to the manufacturer's instructions.

The STAT-NAT *Leishmania spp* kit (Sentinel Diagnostic, Milano-ITALY), a lyophilized RT-PCR mixture was used for amplification of the kinetoplast DNA (kDNA) minicircles in order to detect *Leishmania spp*. Amplification was performed on the ABI 7500 RT-PCR system platform and consisted of: 1 cycle at 95 °C for 10 min, followed by 45 cycles at 95 °C for 15 s and 45 cycles at 60 °C for 60 s ("STAT-NAT® Leishmania spp. - PCR kit for Leishmaniasis diagnosis,", 2021). The *Leishmania spp* (FAM) and the internal control (IC) (JOE) probes were analysed individually at the end of the amplification. The cycle threshold (CT) of the amplified fragments were normalised using the Internal Control (IC) and ranged from 15 to 38 for positive results, whereas normalised CT > 38 was considered to be negative.

2.2.4. Leishmania species identification by nested PCR

The positive samples for *Leishmania spp* obtained with RT-PCR were analysed to identify the species using a two-step protocol as described previously (Noyes et al., 1998). By following the protocol of Noyes et al. (1998) little modified after optimization, the kDNA was then consisted to two PCR reactions (PCR1 and PCR2). The first PCR (PCR1) reaction has been made in a total volume of 30 μL, containing 6 μL of 5× FIRE-Pol® Master Mix (Solis BioDyne; Tartu-ESTONIA), 1 μL of CSB2XF10μM primers (5′-ATTTTTCGCGATTTTCGCAGAAACG-3), 1 μL of CSB1XR10μM (5′- CGAGTAGCAGAAACTCCCGTTCA-3′), 20.5 μL of PCR grade water and 1.5 μL of DNA. The reaction were performed in the thermal cycler (S1000TM Thermal cycler, Bio-Rad, France) and consisted of the following cycle programme: 94 °C for 4 min, followed by 45 cycles of denaturation at 94 °C for 30 s, annealing at 72 °C for 60 s, and extension at 72 °C for 10 min. The second PCR (PCR2) were made at the same volume (30 μL) like the first PCR, by using 1 μL of PCR1 amplicons diluted at a half (1:2), 6 μL of 5× FIREPol® Master Mix,1 μL of 13Z primers (5′-ACTGGGGGTTGGTGTAAAATAG-3′), 1 μL of LiR (5′-TCGCAGAACGCCCCT-3′) and 21 μL of PCR grade water. The amplification schedule was the same as that of PCR1. Samples were considered as positive when a PCR2 product of 680 bp and/or 560 bp was detected. Positive controls containing DNA of *L. infantum* (680 bp) and *L. major* (560 bp) and a negative control, sample without template DNA, were included. 7 μL of amplified products (PCR2) were separated by 2% agarose gel electrophoresis stained with ethidium bromide and visualized by ultraviolet light. A 100 base pair (bp) DNA ladder (Solis BioDyne; Tartu-ESTONIA) was used as a molecular weight marker. The Amplirun® *Leishmania infantum* and *Leishmania major* DNA control (Vircell; Granada-SPAIN) were used as positive control.

2.3. Data analysis

Data were entered into Sphinx Plus^2 software (Version 5) and exported to Stata MP 14 software for statistical analysis. Animal age distribution was expressed by the mean and standard deviation. The Chi-square or Fisher's exact tests were used for bivariates analyses. The comparative analyses of the diagnostic tests were performed by the Cohen Kappa of concordance, where a value of 0.21–0.60 represents fair to moderate agreement, > 0.60–0.80 represents substantial agreement, > 0.80 represents almost perfect agreement (Altman, 1990). A probability value of 0.05 or less was considered statistically significant.

2.4. Ethical considerations

This study received previously authorization from the local authorities of the Animal.

Resources under N°: 2013–230/MRAH/RHBS/DRRAH/DPRAH-HUE. During the survey on the field, oral informed consent was obtained from dog owners.

3. Results

3.1. Distribution of dog selected in study sites

A total of 147 domestic dogs were selected and sampled. The median age was 3 years interquartile range, 1–4 years with a gender ratio of 0.86 (68/79). The characteristics of dogs selected are summarised in the Table 1.

Table 1
Distribution of dogs sampled for the study, according to the site of recruitment, gender, age group and clinical status.

Variables	Number	Proportion (%)
Recruitment sites in Bobo-Dioulasso		
Dogona	29	19.73
Kodeni	29	19.73
Ouezzin ville	11	7.48
Sakaby	36	24.49
Sarfalao	21	14.29
Secteur 24	21	14.29
Gender		
Male	68	46.26
Female	79	53.74
Age (years)		
<3	66	44.90
3–5	45	30.61
≥5	36	24.49
Clinical status		
Asymptomatic	73	49.66
Weight loss	1	0.68
Exfoliative dermatitis	17	11.56
Auricular skin lesions	56	38.10
Total	147	100.00

3.2. Clinical signs recorded from dogs involved in the study

In this study, the proportion of dogs presenting with clinical signs was 50.34% (74/147). The main clinical signs included auricular skin lesions 38.09% (56/147), exfoliative dermatitis 11.56% (17/147) and weight loss 0.68% (1/147).

3.3. Seroprevalence of Leishmania spp. within dog populations

A seroprevalence of 4.76% (95%CI: 2.26–9.72) was found using the rK39-ICT (Table 2). The majority of cases were females (6/7 or 85.71%), however there was no statistically significant link between positive serology and gender ($p = 0.124$). In addition, a statistical bivariate did not show a link between positive serology and age ($p = 0.340$). Among the dogs tested, only 2 out of 7 seropositive dogs (29%) presented typical clinical signs for leishmaniasis, which were exfoliative dermatitis and auricular skin lesions.

3.4. Incidence of Leishmania spp. revealed by RT-PCR

The incidence of *Leishmania spp* in dogs tested by RT-PCR was 10.88% (95%CI: 6.73–17.11) (Tables 2 & 3). This incidence was significantly higher than that obtained by serology ($p = 0.045$) and we found a fair kappa coefficient of concordance between both tests k = 0.390, $p < 0.001$ (Table 3). At least one dog tested positive for *Leishmania spp* per field site (Table 2). There were no statistically significant differences regarding gender and age group ($p > 0.05$). Regarding signs in the RT-PCR positive cases, as for serology, we did not observe a relationship between CanL and RT-PCR results ($p > 0.05$). Out of 16 positive dogs, 10 (62.5%) presented clinical signs which were exfoliative dermatitis and auricular skin lesions.

3.5. Pattern of Leishmania species of CanL and clinical status occured

For the 16 positive cases of *Leishmania spp* revealed by RT-PCR, species identification was then performed. Eight (50%) of the animals were infected with *Leishmania major* (Fig. 2A). The remaining eight (08) samples could not be identified using the nested PCR (Fig. 2B). Five of the *L. major* were found to be dogs showing typical leishmaniasis signs. We did not find an association between the parasite and the clinical status (χ^2=1.11; $p = 0.574$), however we found that *L. major* was associated with the sign ear skin lesions in infected dogs (Table 4).

4. Discussion

4.1. Incidence of the canine leishmaniasis (CanL)

In this survey the percentage of seroincidence of dogs to *Leishmania spp* as revealed by serology test was relatively low (4.76%) compared to the previous study in 2013 in the same city where a seropositivity rate of 5.61% was recorded using the same tools (Sangaré et al., 2016). The difference of rates could be due to the fact that we assessed more dogs ($n = 147$) in the current study. However, the use of real-time PCR in the current study reported a relatively higher rate of positive cases of 10.88%. There was at least one infected dog in each of the collection

Table 3
Comparison between serological and molecular techniques.

DiaMed-IT LEISH results	RT-PCR results		
	Positive n (%)	Negative n (%)	Total (%)
Positive	5(71.43)	2(28.57)	7(4.76)
Negative	11(7.86)	129(92.14)	140(95.24)
Total	16(10.88)	131(89.12)	147(100)

Kappa Cohen test k = 0.390; $p < 0.001$.

Table 2
: Bivariate analysis of CanL according to the results of RT-PCR and serology (rk39 ICT).

Variables	RT-PCR + No (%)	RT-PCR - No (%)	*p-value*; χ^2	rk39 ICT+ No (%)	rk39 ICT - No (%)	*p-value*; χ^2
Recruitment sites: n = 147			0.97; 1.05			
Dogona	2 (6.90)	27 (93.10)		0 (0.00)	29 (100.00)	
Kodenie	4 (13.79)	25 (86.21)		1 (3.45)	28 (96.55)	
Ouezzin ville	1 (9.09)	10 (90.91)		0 (0.00)	11 (100.00)	0.562; 4.17
Sakaby	4 (11.11)	32 (88.89)		3 (8.33)	33 (91.67)	
Sarfalao	3 (14.29)	18 (85.71)		2 (9.52)	19 (90.48)	
Secteur 24	2 (9.52)	19 (90.48)		1 (4.76)	20 (95.24)	
Gender (n = 147)			0.071; 3.263			
Female	12 (15.19)	67 (84.81)		6 (7.59)	73 (92.41)	0.124; 3.02
Male	4 (5.88)	64 (94.12)		1 (1.47)	67 (98.53)	
Age in year						
<3	8 (12.12)	58 (87.88)	0.865; 0.290	8(28.57)	20 (71.43)	0.340; 2.40
3–5	4 (8.89)	41 (91.11)		5 (10.87)	41 (89.13)	
≥5	4 (11.11)	32 (88.89)		3 (11.12)	24(88.88)	
Clinical status of dog (n = 147)						
Asymptomatic	6 (8.22)	67 (91.78)	0.40; 2.650	4 (4.44)	86 (95.56)	0.60; 1.90
Weight loss	0 (0.00)	1(100.00)		3 (8.82)	31 (91.18)	
Exfoliative dermatitis	1(5.88)	16(94.12)		0 (0.00)	23(100.0)	

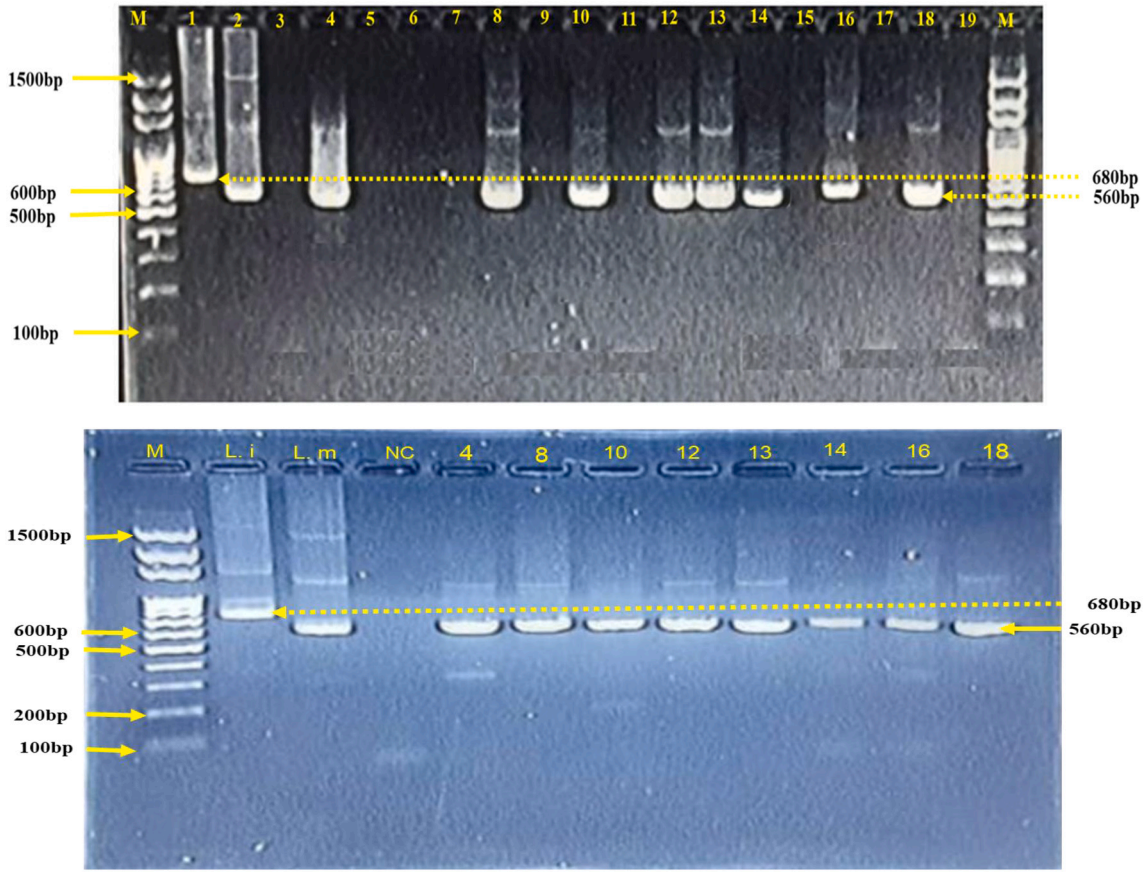


Fig. 2. A: Agarose gel electrophoresis (2%) image showing the PCR product analysis of kDNA from 16 dogs samples being positive at RT-PCR; M = marker (100–1500 bp), Samples 1 = *Leishmania infantum* (positive control); 2 = *L.major* (Positive control),3 = Negative control; 4,8,10,12,13,14,16,18 = positive samples belonging to L. *major* at 560 bp; 5,6,7,9,11,1517,19 = negative samples but positive at the RT-PCR. B. Agarose gel electrophoresis (2%) image that show nested PCR product analysis of kDNA in 8 Leishmania positive samples (4,8,10,12,13,14,16,18). M = marker (100-1500 bp), *L. i* = *Leishmania infantum* (680 bp), *L. m* = *L. major* (560 bp), NC=Negative control.

Table 4
Leishmania species according to the clinical status.

Clinical status	PCR-species			
	L. major n (%)	Undetermined species n(%)	Total n (%)	*p-value*; χ^2
Asymptomatic	3(50)	3(50)	6(37.5)	
Exfoliative dermatitis	0(0)	1(100)	1(6.25)	0.574;
Auricular lesions	5(55,56)	4(44.44)	9(56.25)	1.11
Total	8(50)	8(50)	16(100)	

Undetermined species: RT-PCR positive but negative by nested PCR.

sites, suggesting a wide distribution of the disease in this region. This would therefore be scientific evidence that the city of Bobo-Dioulasso constitutes an enzootic area for leishmaniasis in the country. Other authors in Africa found higher rates compared to ours. Indeed in Senegal, Faye et al. found that even higher at 46.3% ($n = 160$) (Faye et al., 2010), in Tunisia, Bouratbine et al. estimated that at 93% ($n = 40$) (Bouratbine et al., 2005) Similar rate of 10% ($n = 80$) in Iran by Moshfe et al. (2012) (Moshfe et al., 2012). The probable explanation in this disparity of rate of CanL, would be the difference in performance of the methods used and different levels of infection according to the locality. In the context of the risk of spreading diseases to human, the prevelance of CanL in our study suggests that leishmaniasis should not be neglected in this city.

We noticed a good diagnostic performance of the RT-PCR in term of sensitivity compared to the rK39-ICT (10.88 versus 4.76, $p = 0.045$) for CanL. Indeed it was significantly higher than that obtained by serology ($p = 0.045$) and the test of concordance kappa (k) between both assays remained fair k = 0.39, $p < 0.001$. This could be explained by the fact that the molecular test is more sensitive and able to detect fewer parasites (Briffod, 2011). Indeed, despite a very low parasite load (2–5parasites/μL), PCR remains sensitive (Mesa et al., 2020). Also we used the minicircles kDNA which allows a good yield for detecting parasite because of their high copy numbers (Mesa et al., 2020; Moshfe et al., 2012). However for serological tests, although very useful in field epidemiology studies, their diagnostic performance are limited by factors such as cross-reactions with other infections (Carvalho et al., 1987).

No statistical differences were found between male and female dogs for serology ($p = 0.124$) or for RT-PCR ($p = 0.450$) suggesting that they are equally exposed to infection and that the infected female sandfly would not have a trophic preference according to the gender. Similar findings were recorded by Moshfe et al. in Iran (Moshfe et al., 2012). In the current study the parasite was found in dogs of all age ranges, mainly by RT-PCR technique. No statistical differences were observed between the age and the positivity of both techniques ($p = 0.340$; $p = 0.865$) suggesting that the dog's age does not influence the risk of infection. Similar results ($p > 0.05$) were found in Brazil and in Iran as well (de Almeida et al., 2012; Mahshid et al., 2014). This would be linked to the wandering of dogs and their environment which makes them more exposed to bites from infected sandflies.

In the present study, 2 out of 7 of seropositive dogs (29%) and 10 out of 16 of positive dogs by RT-PCR (62.5%) showed clinical signs characteristic of CanL. Using serological tests, studies in Senegal and Iran showed that 18.2% and 16% of positive dogs, respectively, showed

clinical signs (Faye et al., 2010; Haddadzade et al., 2013). In this study, although there is no statistical difference regarding sign and infection, 5 (71%) were asymptomatic seropositive dogs and 37.5% asymptomatic dogs at the RT-PCR; although there is no statistical difference regarding signs and infection. These findings have important implications regarding the epidemiology of CanL and the transmission of the disease to human beings, dogs with clinical signs as well as asymptomatic positive dogs have capacity to transmit the disease to human beings in presence vectors susceptible to *Leishmania* species or any favorable conditions. Any public action must take into account the long incubation period after transmission of the parasite to a new canine host by an infected sand fly. Indeed, the incubation period can extend from 3 months to 7 years (Salant et al., 2021), suggesting the importance of having high-performance tools for an early diagnosis capacity.

4.2. *Leishmania species typing in infected dogs and observed clinical signs*

Of the 16 positive dogs by RT-PCR, parasite typing by nested PCR revealed 8 cases were infected with *L. major*. This could be explained by the origin of sample where the DNA was extracted. Indeed, our DNA was extracted from blood where the parasite load is generally low compared to other compartments of the body such as the liver, spleen, skin (Reithinger and Dujardin, 2007). Also it is known that RT-PCR is more sensitive than conventional PCR and when the parasite load is very low, it would be possible to have discordant results.

Two species of *Leishmania* are now believed to be present in dogs population in this urban area of Bobo-Dioulasso. In fact during this study, we identified *L. major* and the previous study conducted in this area had reported the presence of *L. infantum* in dogs (Sangaré et al., 2016). Although the sampling was done at almost the same site, this dichotomy of results is possible. Indeed, it is known that *Phlebotomus dubosqi (Phle. dubosqi)* is the main vector of *L. major* in West Africa including Burkina Faso (Maroli et al., 1986). Previous studies have shown the occurrence of colonies of vectors susceptible to *L. major* and *L. infantum*, which are zoo-anthropophilic such as *Phle. dubosqi*, *Phle. longicuspis* in known foci of leishmaniasis in Burkina Faso (Depaquit et al., 2005; Maroli et al., 1986). These vectors could potentially participate in the dynamics of transmission of *Leishmania species* to specific host species.

L. major has been known to be endemic in Burkina Faso since 1960, and has reservoir hosts including, rodents such as *Mastomys (M.) erythroleucus*, *M. natalensis* (Zida et al., 2020). In the current study we have identified *L. major* in dogs for the first time, suggesting that this animal could also constitute an important reservoir of the parasite in Burkina Faso, as previously reported in dogs in several other countries (Baneth et al., 2016). Determinants such as urbanization of the city by the development of peripheral areas may have contributed to the destruction of rodents natural habitats (Kahime et al., 2017), increased interactions between sandflies and domestic dog populations and changes in the blood meal preference of sandflies in the city. This issue is important in relation to epidemiological aspects and the transmission of *Leishmania spp* to humans (Haddadzade et al., 2013) and suggests the need to develop a strategy of surveillance to prevent outbreaks of human leishmaniasis in this city.

5. Conclusions

Our study reported a seroincidence of 4.76% and a high molecular incidence of 10.88% 10.88% in dogs in the city of Bobo-Dioulasso. Our findings emphasize that using more than one technique is necessary to accurately detect *Leishmania spp* infection (Fernandes et al., 2019). Our results also show the presence of *L. major*-infected dogs in an area where *L. infantum* was previously found in dogs, suggesting the circulating of two zoonotic *Leishmania species.* This work provides for the first time a body of scientific evidence that Bobo-Dioulasso is an enzootic area of CanL, calling for the need for multidisciplinary actions through an integrated One Health approach to better control the disease in dogs before this emerges in human.

Funding

This study was funded by Project « Programme d'Appui et de Developpement des Centres d'Excellence Régionaux de l'UEMOA; Phase Interimaire », the French Embassy in Burkina Faso and the International Laboratory of Vector borne Diseases (LAMIVECT).

Authors' contributions

Conceptualization: RKD, DAD
Methodology: RKD, DAD, ASN, BKY
Software: DAD
Validation: DAD, HP, AD, RKD
Formal Analysis: DAD, RKD
Investigation: DAD, AO, ASN
Resources: DAD, RKD
Data curation: DAD, ASN, RKD
Supervision: DAD, HP, RKD
Writing-original draft: DAD, RKD
Writing-review and editing: DAD, ASN, ASH, IS, BKY, LK, AO, SPD, HP, AD, FF, RKD
Visualization: DAD, RKD
Project administration: RKD
Funding acquisition: RKD

Declaration of Competing Interest

The authors declare that there is no conflict of interests concerning the publication of the present article.

Acknowledgements

The authors thank the dog owners and the Regional Director of animal resources of Bobo-Dioulasso for their collaboration.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2022.105327.

References

Abbehusen, M.M.C., Almeida, V. Dos A., Solcà, M. Da S., Pereira, L. Da S., Costa, D.J., Gil-Santana, L., Bozza, P.T., Fraga, D.B.M., Veras, P.S.T., Dos-Santos, W.L.C., Andrade, B.B., Brodskyn, C.I., 2017. Clinical and immunopathological findings during long term follow-up in Leishmania infantum experimentally infected dogs. Sci. Rep. 7, 15914. https://doi.org/10.1038/s41598-017-15651-8.

Altman, D.G., 1990. Practical Statistics for Medical Research. CRC press.

Bamba, S., Sawadogo, H., Zoungrana, J., Sondo, A.K., Konsegré, V., Diallo, B., 2017. Cutaneous Leishmaniasis: report of cases from 2006 to 2012 in Ouagadougou, Burkina Faso 7. Ann Clin Cytol Pathol 3, 1052.

Baneth, G., Nachum-Biala, Y., Shabat Simon, M., Brenner, O., Gaier, S., Rojas, A., Yasur-Landau, D., 2016. Leishmania major infection in a dog with cutaneous manifestations. Parasit. Vectors 9. https://doi.org/10.1186/s13071-016-1541-2.

Baneth, G., Yasur-Landau, D., Gilad, M., Nachum-Biala, Y., 2017. Canine leishmaniosis caused by Leishmania major and Leishmania tropica: comparative findings and serology. Parasit. Vectors 10. https://doi.org/10.1186/s13071-017-2050-7.

Bouratbine, A., Aoun, K., Gharbi, M., Haouas, N., Zaroui, J., Harrat, Z., Baba, H., Darghouth, M.A., 2005. Epidemiological, clinical and parasitological data about canine leishmaniasis in Tunisia. Bull. Soc. Pathol. Exot. 1990 (98), 359–362.

Briffod, C., 2011. Revue actuelle de la leishmaniose canine. Thèse d'exercice Médecine vétérinaire Toulouse 3, 101p.

Carvalho, E.M., Reed, S.G., Johnson Jr., W.D., 1987. Cross reactivity between Trypanosoma cruzi and Leishmania antigens in the lymphocyte blastogenesis assay. Trans. R. Soc. Trop. Med. Hyg. 81, 82–84. https://doi.org/10.1016/0035-9203(87)90291-4.

de Almeida, A., Do, B.P.F., Sousa, V.R.F., Da Cruz, F.A.C.S., Dahroug, M.A.A., Figueiredo, F.B., Madeira, M. De F., 2012. Canine visceral leishmaniasis: seroprevalence and risk factors in Cuiabá, Mato Grosso, Brazil. Rev. Bras. Parasitol.

Vet. Braz. J. Vet Parasitol. Orgao Of. Col. Bras. Parasitol. Vet. 21, 359–365. https://doi.org/10.1590/s1984-29612012005000005.

Depaquit, J., Muller, F., Gantier, J.-C., Leger, N., Ferte, H., Ready, P., Niang, A.-A., 2005. Phlebotomine sand flies from Ouagadougou, Burkina Faso: first record of Phlebotomus (Larroussius) longicuspis south of the Sahara. Med. Vet. Entomol. 19, 322–325. https://doi.org/10.1111/j.1365-2915.2005.00561.x.

Esteva, L., Vargas, C., Vargas de León, C., 2017. The role of asymptomatics and dogs on leishmaniasis propagation. Math. Biosci. 293, 46–55. https://doi.org/10.1016/j.mbs.2017.08.006.

Faye, B., Bañuls, A.L., Bucheton, B., Dione, M.M., Bassanganam, O., Hide, M., Dereure, J., Choisy, M., Ndiaye, J.L., Konaté, O., Claire, M., Senghor, M.W., Faye, M.N., Sy, I., Niang, A.A., Molez, J.F., Victoir, K., Marty, P., Delaunay, P., Knecht, R., Mellul, S., Diedhiou, S., Gaye, O., 2010. Canine visceral leishmaniasis caused by Leishmania infantum in Senegal: risk of emergence in humans? Microbes Infect. 12, 1219–1225. https://doi.org/10.1016/j.micinf.2010.09.003.

Fernandes, M.A., Leonel, J.A.F., Isaac, J.A., Benassi, J.C., Silva, D.T., Spada, J.C.P., Pereira, N.W.B., Ferreira, H.L., Keid, L.B., Soares, R.M., Oliveira, De S., 2019. Molecular detection of *Leishmania infantum* DNA according to clinical stages of leishmaniasis in dog. Rev. Bras. Parasitol. Vet. 28, 194–202. https://doi.org/10.1590/S1984-29612019015.

Guiguemdé, R.T., Sawadogo, O.S., Bories, C., Traore, K.L., Nezien, D., Nikiema, L., Pratlong, F., Marty, P., Houin, R., Deniau, M., 2003. Leishmania major and HIV co-infection in Burkina Faso. Trans. R. Soc. Trop. Med. Hyg. 97, 168–169. https://doi.org/10.1016/S0035-9203(03)90109-X.

Haddadzade, H., Fattahi, R., Mohebali, M., Akhoundi, B., Ebrahimzade, E., 2013. Seroepidemiologcal investigation of visceral Leishmaniasis in stray and owned dogs in Alborz Province, Central Iran using direct agglutination test. Iran. J. Parasitol. 8, 152–157.

Kahime, K., Boussaa, S., Nhammi, H., Boumezzough, A., 2017. Urbanization of human visceral leishmaniasis in Morocco. Parasite Epidemiol. Control 2, 1–6. https://doi.org/10.1016/j.parepi.2017.07.001.

Konate, I., Sangare, I., Zoungrana, J., Meda, Z.C., Kafando, C., Sawadogo, Y., Dabiré, R., Meda, N., Diallo, B., Andonaba, J.-B., Barro-Traoré, F., Niamba, P., Traoré, A., 2020. Description d'un nouveau foyer épidémique de leishmaniose cutanée à Leishmania major à l'Ouest du Burkina Faso. Pan Afr. Med. J. 35 https://doi.org/10.11604/pamj.2020.35.65.20825.

Lévêque, M.F., Lachaud, L., Simon, L., Battery, E., Marty, P., Pomares, C., 2020. Place of serology in the diagnosis of zoonotic Leishmaniases with a focus on visceral Leishmaniasis due to Leishmania infantum. Front. Cell. Infect. Microbiol. 10 https://doi.org/10.3389/fcimb.2020.00067.

Mahshid, M., Baharak, A., Iraj, S., Sina, K., Javad, K., Mehdi, B., 2014. Seroprevalence of canine visceral leishmaniasis in southeast of Iran. J. Parasit Dis. Off. Organ Indian Soc. Parasitol. 38, 218–222. https://doi.org/10.1007/s12639-012-0226-9.

Maroli, M., Fausto, A.M., Sabatinelli, G., Majori, G., 1986. Phlebotomines (Diptera, Psychodidae) from Burkina Faso. A note on the sandfly species collected in domestic resting sites. Ann. Parasitol. Hum. Comp. 61, 683–688. https://doi.org/10.1051/parasite/1986616683.

Mesa, L.E., Manrique, R., Muskus, C., Robledo, S.M., 2020. Test accuracy of polymerase chain reaction methods against conventional diagnostic techniques for Cutaneous Leishmaniasis (CL) in patients with clinical or epidemiological suspicion of CL: systematic review and meta-analysis. PLoS Negl. Trop. Dis. 14 https://doi.org/10.1371/journal.pntd.0007981.

Moshfe, A., Mohebali, M., Afshoun, E., Mousavizadeh, A., Zarei, Z., Abidi, H., Akhoundi, B., Barati, V., Joukar, S., 2012. Canine visceral leishmaniasis in boyer ahmad district, kohgiluyeh & boyer ahmad province, southwest of Iran. Iran. J. Parasitol. 7, 75–81.

Noyes, H.A., Reyburn, H., Bailey, J.W., Smith, D., 1998. A nested-PCR-based schizodeme method for identifying Leishmania kinetoplast minicircle classes directly from clinical samples and its application to the study of the epidemiology of Leishmania tropica in Pakistan. J. Clin. Microbiol. 36, 2877–2881. https://doi.org/10.1128/JCM.36.10.2877-2881.1998.

Oddou, A., 1960. Leishmaniose cutanee en Haute Volta. Bull Soc Med Afr 7, 28.

Okwor, I., Uzonna, J., 2016. Social and economic burden of human Leishmaniasis. Am. J. Trop. Med. Hyg. 94, 489–493. https://doi.org/10.4269/ajtmh.15-0408.

Reithinger, R., Dujardin, J.-C., 2007. Molecular diagnosis of leishmaniasis: current status and future applications. J. Clin. Microbiol. 45, 21–25. https://doi.org/10.1128/JCM.02029-06.

Reithinger, R., Espinoza, J.C., Llanos-Cuentas, A., Davies, C.R., 2003. Domestic dog ownership: a risk factor for human infection with Leishmania (viannia) species. Trans. R. Soc. Trop. Med. Hyg. 97, 141–145. https://doi.org/10.1016/S0035-9203(03)90101-5.

Ribeiro, R.R., Michalick, M.S.M., da Silva, M.E., dos Santos, C.C.P., Frézard, F.J.G., da Silva, S.M., 2018. Canine Leishmaniasis: an overview of the current status and strategies for control. Biomed. Res. Int. 2018, e3296893 https://doi.org/10.1155/2018/3296893.

Salant, H., Nachum-Biala, Y., Feinmesser, B., Perelmutter, M., Baneth, G., 2021. Early onset of clinical leishmaniosis in a litter of pups with evidence of in utero transmission. Parasit. Vectors 14, 326. https://doi.org/10.1186/s13071-021-04824-0.

Sangaré, I., Djibougou, A., Yaméogo, B.K., Drabo, F., Diabaté, A., Bañuls, A.-L., Fournet, F., Price, H.G., Guiguemdé, R.T., Dabiré, R.K., 2016. First Detection of Leishmania infantum in Domestic Dogs from Burkina Faso (West Africa). https://doi.org/10.3923/jp.2017.27.32.

Santarém, N., Sousa, S., Amorim, C.G., de Carvalho, N.L., de Carvalho, H.L., Felgueiras, Ó., Brito, M., da Silva, A.C., 2020. Challenges in the serological evaluation of dogs clinically suspect for canine leishmaniasis. Sci. Rep. 10, 3099. https://doi.org/10.1038/s41598-020-60067-6.

Sasani, F., Javanbakht, J., Samani, R., Shirani, D., 2016. Canine cutaneous leishmaniasis. J. Parasit Dis. Off. Organ Indian Soc. Parasitol. 40, 57–60. https://doi.org/10.1007/s12639-014-0444-4.

Solano-Gallego, L., Miró, G., Koutinas, A., Cardoso, L., Pennisi, M.G., Ferrer, L., Bourdeau, P., Oliva, G., Baneth, G., 2011. LeishVet guidelines for the practical management of canine leishmaniosis. Parasit. Vectors 4, 86. https://doi.org/10.1186/1756-3305-4-86.

Solano-Gallego, L., Cardoso, L., Pennisi, M.G., Petersen, C., Bourdeau, P., Oliva, G., Miró, G., Ferrer, L., Baneth, G., 2017. Diagnostic challenges in the era of canine Leishmania infantum vaccines. Trends Parasitol. 33, 706–717. https://doi.org/10.1016/j.pt.2017.06.004.

STAT-NAT® Leishmania spp, 2021. PCR kit for Leishmaniasis diagnosis. Sentin. Diagn. URL. https://www.sentineldiagnostics.com/molecular-diagnostics/stat-nat-leishmania-spp/ (accessed 6.2.21).

Sundar, S., Singh, O.P., 2018. Molecular diagnosis of visceral Leishmaniasis. Mol. Diagn. Ther. 22, 443–457. https://doi.org/10.1007/s40291-018-0343-y.

Torres-Guerrero, E., Quintanilla-Cedillo, M.R., Ruiz-Esmenjaud, J., Arenas, R., 2017. Leishmaniasis: a review. F1000Research 6. https://doi.org/10.12688/f1000research.11120.1.

Traoré, K.S., Sawadogo, N.O., Traoré, A., Ouedraogo, J.B., Traoré, K.L., Guiguemdé, T.R., 2001. Preliminary study of cutaneous leishmaniasis in the town of Ouagadougou from 1996 to 1998. Bull. Soc. Pathol. Exot. 1990 (94), 52–55.

Zida, A., Sangare, I., Nezien, D., Bretagne, S., Bamba, S., Deniau, M., Guiguemde, R.T., 2020. Mastomys natalensis, Cricetomys gambianus and Taterillus sp. were found PCR positive for Leishmania major in Burkina Faso. West Africa. Ann. Parasitol. 66, 251–254. https://doi.org/10.17420/ap6602.262.