Prevalence and risk factors of canine leishmaniasis in Morocco: a systematic review and meta-analysis

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Abstract Human visceral leishmaniasis has long been associated with canine leishmaniasis (CL). However, to date, there is no clear information on the status of the disease in dogs in Morocco that could be used by policymakers for the prevention of human cases. This study aims to assess the status of CL in Morocco and its risk factors through an exhaustive literature search. The meta-analysis was performed using RevMan 5.4. The main results showed that the overall prevalence of CL in Morocco is 17% (95% CI: 0.12–0.22), caused by two strains of Leishmania parasite: Leishmania tropica and L. infantum. According to the region, the maximum prevalence was reported in the coastal provinces and in the central part of the country; while, the CL risk was higher in rural area (18% [95% CI: 0.14–0.23]) and at altitude above 1000 m (23% [95% CI: − 0.08–0.53]). Regarding the intrinsic factors, the prevalence of the disease increased with the age of the dog, (30% [95% CI − 0.09–0.68]) and the risk was very high in clinically asymptomatic dogs (RR = 2.08 [95% CI: 1.15–3.76]). This study is the first in Morocco indicating the CL prevalence, its geographical distribution and detailing its risk factors. These results are needed to improve management strategies for the canine reservoir of leishmaniasis in Morocco and interrupt the local transmission cycle to humans.

Keywords Canine leishmaniasis · Prevalence · Risk factors · Systematic review · Meta-analysis · Morocco

Introduction

Human visceral leishmaniasis is the most severe form of leishmaniases. It is fatal if left untreated and it is transmitted to humans by the bite of an infected female sand fly (Diptera: Psychodidae) (WHO 2015). It is a zoonosis of parasitic origin caused by Leishmania infantum (L. infantum) in the Mediterranean basin and the reservoir is a canine, mainly the dog Canis lupus (WHO 2011).

In Morocco, human visceral leishmaniasis was classically known to be endemic in the northern region where active outbreaks of canine leishmaniasis (CL) were reported (Guessed-Idrissi et al. 1997a, b; Nejjar et al. 1998; Sahibi et al. 2001; Lemrani et al. 2002). Prior identification of parasitized dogs can help prevent disease in humans, even in non-endemic area of the country (Sevá et al. 2021). On this basis, the Moroccan Ministry of Health and Social Protection (MMH) granted, within the framework of its national leishmaniasis control program, a strategic focus on the control of the canine reservoir which included, among
others, measures for the elimination of sick stray dogs (MMH 2010). Although the culling of stray dogs and clinically suspect’s dogs is done periodically as part of the national rabies control program, the morbidity and mortality rate of human visceral leishmaniasis have not been reduced (MMH 2018). In addition, a recent study has shown that the disease has gradually spread to the urban environment (Kahime et al. 2017). This urbanization of human visceral leishmaniasis was attributed to the movement of dogs as reservoirs of the disease (Kahime et al. 2017). Thus, vectorial and reservoir control strategies for human visceral leishmaniasis are needed to reduce their burden (Rodrigues et al. 2020).

The National Leishmaniasis Control Program (NLCP) focuses its canine reservoir control activities on the dog as the main reservoir of the visceral form of human leishmaniasis (MMH 2010). Although human visceral leishmaniasis has long been known to be associated with CL, however, until today there is no clear information on the status of Leishmania infection in dogs, nor on the overall prevalence estimate of CL in Morocco. That could be used by policymakers for effective management of the canine reservoir and prevention of human cases of the visceral form of the disease. Therefore, this study aims to provide combined data summarizing the prevalence of CL and its geographical distribution. In addition, these systematic review and meta-analysis are scientific evidence related to the issue of control of the dog as a reservoir host of human visceral leishmaniasis, by exploring the active foci and risk factors associated with Leishmania sp in dogs in Morocco.

**Methods**

**Study area**

Morocco is geographically located in the northwest of Africa. It is bounded by the Strait of Gibraltar and the Mediterranean Sea to the North, Algeria to the East, Mauritania to the South, and the Atlantic Ocean to the West.

The administrative division of 12 regions is adopted in Morocco since 2015 (HCP 2014). The total area of Morocco is 710,850 km² and its population is estimated at 33.8 million inhabitants, 40% of whom live in rural areas (HCP 2014). The total area of Morocco is 710,850 km² and its population is estimated at 33.8 million inhabitants, 40% of whom live in rural areas (HCP 2014). The total area of Morocco is 710,850 km² and its population is estimated at 33.8 million inhabitants, 40% of whom live in rural areas (HCP 2014). With its two maritime facades: Atlantic ocean and the Mediterranean Sea and its temperate continental climate, in summer, dry seasons especially in its arid and semi-arid areas, while in winter, cold seasons in its humid and sub-humid areas (Driouech 2010). The average annual rainfall varies from 500 to 2000 mm in the northern region, from 200 to 1000 mm in the western and central region, from 100 to 200 mm in the eastern region, and less than 100 mm in the south of the country (Masen 2018).

**Design and data collection**

This systematic review and meta-analysis considered all original studies including cohort, cross-sectional, and retrospective studies, conducted in all regions of Morocco on the prevalence of CL and the risk factors associated with Leishmania sp infection in dogs. In fact, a comprehensive literature search was performed to identify all studies reporting data on CL in Morocco.

In accordance with the Recommended Reporting Items for Systematic Review and Meta-analysis (PRISMA) guidelines (Page et al. 2021), an extensive search was conducted between August 2020 and March 2021 in digital databases: PubMed, Web of Science, Science Direct, Scopus, Springer Link, Google Scholar, Othohati, using the keywords: “dog”, “leishmaniasis”, “Leishmania”, “canine”, “Canidae”, “canine leishmaniasis”, “prevalence”, “incidence”, “Morocco” and “epidemiology” alone or in combination, in French and English.

In addition, consultation of the reference section of these studies allowed us to direct our search to other basic works not available in the databases and to contact their authors.

**Eligibility criteria and data extraction**

Selection criteria were defined based on the relevance of the studies. Thus, all publications online until March 31, 2021, were extracted and added automatically to the “Zotero” reference manager, while non-numerical works were added manually. Duplicate articles were carefully checked and eliminated. Titles were double-checked.

To avoid subjectivity in study recruitment, this step was performed independently by the authors concerning the four study selection criteria: (a) disease: leishmaniasis; (b) target population: dogs; (c) study location: Morocco; (d) judgment criteria: prevalence/incidence and Leishmania strain.

Indeed, the eligibility criteria for inclusion in our meta-analysis had to meet the following criteria: (a) cross-sectional and retrospective studies on the prevalence of CL; (b) the study was conducted in Morocco; (c) the number of dogs examined and infected with Leishmania was indicated; (d) the Leishmania parasite was identified. However, the exclusion criteria are: (a) studies reporting prevalence/incidence of leishmaniasis in humans; (b) studies on CL without data on prevalence or number of positive cases.

Finally, the relevant publications were analyzed. Data from the retained studies included author’s name, the publication date, the title,, the study area, the sample size, the number of positive cases, the strain of Leishmania.
identified, diagnostic method, the lifestyle of the dog (stray, semi-stray, or chained), the health status (veterinary follow-up/non-follow-up), the age of the dog, the environment (urban/rural), the clinical status (symptomatic/asymptomatic) were extracted and organized in an Excel file.

Statistical processing and analysis

The relevant data were processed using R software. The Review Manager (RevMan) Version 5.4.1 [The Cochrane Collaboration, 2020] was used to perform the meta-analysis including the Forest plots and funnel graphs. The meta-analysis was performed for CL prevalence and its risk factors using their standard deviation, standard error estimates with 95% confidence interval. The random-effect analysis model with the generic inverse variance method was adopted, which allowed the study weight to be adjusted for variation or heterogeneity (Higgins and Thompson 2002). Indeed, the heterogeneity between studies was assessed using the Cochrane Q test and \( I^2 \) test statistics, whereas publication bias was assessed using the Egger test.

Thus, a critical appraisal of the quality of included studies was performed using the Joanna Briggs Institute (JBI) critical appraisal checklist for studies reporting prevalence data (Joanna Briggs Institute 2020) and the STROBE checklist (Déclaration STROBE 2007) for systematic review and meta-analysis studies. A checklist with 22 items was adopted to report on included studies. These items included Title, Abstract, Introduction, Methods, Results, Discussion and Funding of the study. A score of < 7.75 was considered as very low quality, a score between [7.76 and 15.5] was considered low, a score between [15.6 and 23.5] was considered medium, and a score of > 23.6 was considered as a high quality (Von Elm et al. 2007).

Results

Results of the literature search

A total of 168 articles were collected through literature searche of online electronic databases (163 studies) and by contacting the authors (5 studies). Because of duplication, 85 articles were eliminated. Out of the remaining 83 articles, 65 were excluded after evaluation of their title and abstract. The remaining 18 articles were qualified as eligible for full-text review. Out of these 18 eligible studies, 3 studies were removed based on specific exclusion criteria (two experimental studies evaluating the efficacy of anti-Leishmania treatments and one observational study reporting only the evolution of clinical signs of the disease) (Fig. 1). Finally, 15 full-text studies were reviewed qualitatively and 11 studies were reviewed quantitatively using meta-analysis (Table 1).

Characteristics and quality of eligible studies

The studies included in this review were conducted in 8 administrative regions of Morocco. Indeed, the region that benefited from the greatest number of studies on CL was the region of Fez-Meknes (33%); followed by the region of Rabat-Sale-Kenitra (13%); the region of Marrakech-Safi (13%); Casablanca-Settat (7%); the region of Tangier-Tetouan-Al Hoceima (7%); the region of Oriental (7%); the region of Beni Mellal, Khenifra (7%); and the region of Souss-Massa (7%). However, no study was conducted in the other administrative regions namely, Drâa-Tafilalet; Guelmim-Oued Noun; Laâyoune-Sakia El Hamra; Dakhla-Oued Ed Dahab. Thus, the representativeness rate of the included studies was 66.6% of the whole country territory.

According to the date of the publication, this review covers a period of 90 years, including the eligible studies on CL in Morocco published between 1932 and March 2021. In addition, the descriptive analysis revealed that 13% of these studies were conducted during the first 20-year period (1932–1951). While, no publications were found during the next 30 years (1952–1981), while 47% of these studies were conducted in the period from 1982 to 2001 and the remaining studies (40%) were conducted during the period from 2002 to 2021.

11 (out of 15) studies reported prevalence data (Table 1) with the sample size ranged from 61 to 1362 (Sahibi et al. 2001; Fellah et al. 2014). Nevertheless, two publications have used the same data (Natami et al. 2000; Sahibi et al. 2001). Therefore, we included both publications with a single citation of the results.

The quality assessment of all eligible studies in our meta-analysis was performed based on the STROBE checklist; the mean score for the 15 analyzed articles was obtained at 21.06, indicating moderate quality. In addition, the critical appraisal of the 11 studies reporting prevalence data based on the checklist of prevalence studies as determined by the Joanna Briggs Institute (JBI) (Joanna Briggs Institute 2020) showed that the studies included in this search were of good quality with a low risk of bias (Table 1, Figs. 2 and 3).

Assessment of publication bias

Testing for publication bias between studies was performed using the funnel plot (Fig. 4). Inspection of the visual representation of publication bias across the eleven studies showed there was a significant level of symmetry. This indicates that there could be no significant publication bias
among the studies included in the meta-analysis of the clustered prevalence of CL in Morocco.

**Descriptive analysis**

Descriptive statistics were performed to determine the total number of the sampled dog population and the range of prevalence estimates in different regions of Morocco. A
A total of 3900 dogs were tested for *Leishmania* sp. infection, of which 580 were positive. The apparent prevalence of CL in Morocco was 14.87%, ranging from 2.1 to 42%. The maximum prevalence was 42.0% recorded in Rabat (Rami et al. 2005), followed by a prevalence of 28.1% in Sefrou (Nejjar et al. 2000); while, the minimum prevalence was 2.1% recorded in Al-Haouz province (Dereure et al. 1986).

Two major forms of leishmaniasis have been reported in dogs caused by two *Leishmania* species namely *L. tropica* and *L. infantum*. *L. infantum* is the most common *Leishmania* species and it was isolated from both the skin and viscera of the same dog as *L. tropica*. In addition, five pathogenic strains of *Leishmania* sp were identified: three strains belonging to *L. tropica* zymodemes (MON-102,
MON-113 and MON-279), and two strains belonging to *L. infantum* zymodemes (MON-24 and MON-1). Zymodeme MON-1 was described as the most common strain (Table 1).

To confirm the diagnosis of *Leishmania* parasite, 93% (14/15) of the included studies were based on serological methods; 60% (9/15) of the studies incorporated biochemical methods, and 33% (5/15) were referred to molecular methods. On the other hand, the combination of the three diagnostic methods was observed in 27% (4/15) of the studies and the combination of two methods: serological and biochemical was reported in 53% (8/15) of the studies. Thus, the most used tests were: NNN (47%), ELISA (47%), IFT (47%), and PCR (33%) (Table 1).

**Fig. 2** Risk of bias summary: review authors’ judgements about each risk of bias item for each included study

### Meta-analysis and subgroup analysis

Given the heterogeneity found between studies, a random-effects model was adopted in this meta-analysis. As result, the inter-study variability was moderate ($\tau^2 = 0.00$, heterogeneity $I^2 = 60\%$ with $\chi^2 = 25.05$, degree of freedom $df = 10$) and statistically significant ($p < 0.00001$). Study weights ranged from 4.1% to 14.1%. The pooled overall prevalence estimates for CL were 17% (95% CI [0.14–0.23]) (Fig. 5). A subgroup meta-analysis was performed to compare the prevalence of CL in the different regions and provinces of Morocco and to determine the temporal evolution of this prevalence and the risk factors associated with this disease (Fig. 5).
Spatial evolution of canine leishmaniasis in Morocco

The maximum pooled prevalence estimates were recorded in the region of Rabat-Sale-Kenitra with a pooled prevalence of 29% ([95% CI: 0.04–0.053]; \( p = 0.02 \)) and the Oriental region with a pooled prevalence of 21% ([95% CI: 0.10–0.32]; \( p = 0.0002 \)), followed by the central region of Fez-Meknes with a pooled prevalence of 20% ([95% CI: 0.13–0.26]; \( p < 0.00001 \)). While the minimum prevalence was recorded in the southern region in Sous-Massa with a rate of 1% ([95% CI: – 0.09–0.21]; \( p = 0.92 \)). The meta-analysis indicated that there was moderate heterogeneity \( (\tau^2 = 0.00, \text{ heterogeneity } I^2 = 47\% \text{ with } \text{Chi}^2 = 24.63, \text{ degree of freedom } df = 13; \ p < 0.00001) \) (Fig. 6; Online Appendix A). While, the results of the meta-analysis of prevalence by province revealed that the maximum values were recorded successively in the provinces of Rabat (42% [95% CI: 0.25–0.59]), Chichaoua (33% [95% CI: – 0.05–0.71]), Sefrou (28% [95% CI: 0.17–0.39]), Moulay-Yacoub (27% [95% CI: 0.10–0.44]) and Nador (21% [95% CI: 0.10–0.32]). While the minimum value was recorded in the province of Taroudante (2% [95% CI: – 0.22–0.26]). Weak heterogeneity was observed in the calculation of interprovincial prevalence estimates \( (\tau^2 = 0.00, \text{ heterogeneity } I^2 = 39\% \text{ with } \text{Chi}^2 = 31.14, \text{ degree of freedom } df = 19; \ p < 0.00001) \) (Fig. 7; Online Appendix B).
Temporal evolution of canine leishmaniasis in Morocco

The results of the meta-analysis of the prevalence, according to period, show that the prevalence rate during the first period (1932–1951) was 13% (95% CI [0.03–0.23]; \( p = 0.01 \)); while, the prevalence during the second period (1952–1981) could not be estimated due to the absence of studies. In the third period (1982–2001), the estimated prevalence was 15% (95% CI [0.10–0.16]; \( p < 0.00001 \)) and 24% (95% CI [0.10–0.25]; \( p = 0.0001 \)) in the last period (2002–2021). Thus, the heterogeneity was moderate and inter-study variability was absent (\( \tau^2 = 0.00 \), heterogeneity \( I^2 = 60\% \) with \( \text{Chi}^2 = 25.05 \), degree of freedom \( df = 10 \), and a \( p \) value < 0.00001) (Fig. 8).

Risk factors associated with canine leishmaniasis in Morocco

We studied eight variables that we classified into intrinsic factors (sex, age category, presence of clinical signs of disease, and breed) and extrinsic factors (altitude, location, lifestyle, and health status). The meta-analysis of all these variables was carried out by the random-effects method.

Sex of the dog

The association between the sex of the dog and the occurrence of CL was investigated. The results show that this variable may not be a risk factor of CL in Morocco (RR = 1.14 [95% CI: 0.75–1.73]) as confirmed statistically (\( p = 0.55 \)) (Fig. 9).
Clinical status of the dog

The clinical status of the dog refers to the clinical manifestation of the disease. Thus, a symptomatic dog is a dog showing signs of leishmaniasis as described (Oliva et al. 2006), while asymptomatic dog is dog without leishmaniasis. The study of this variable was based on risk assessment relative to the presence of clinical signs of the disease and the comparison of seropositivity in symptomatic dogs with those asymptomatic.

Our results show that the risk of *Leishmania* was twice as high in healthy dogs with asymptomatic clinical status as in symptomatic dogs (RR = 2.08 [95% CI: 1.15–3.76]; *p* = 0.02) (Fig. 10). In fact, the prevalence in asymptomatic dogs was 9% (95% CI [0.06–0.13]; *p* < 0.00001)
and in symptomatic dogs was 5% (95% CI [0.02–0.09]; \( p = 0.003 \)) (Fig. 11).

**Age of the dog**

The association between dog age and the occurrence of leishmaniasis was investigated. Although the studies reported this variable differently, we have grouped the ages into three classes for analysis: the first is the age class of less than two years, the second is the age between two and four years, and the third is beyond four years (Fig. 12). The comparison between these three age classes showed that the combined *Leishmania* prevalence increased with the age of the dog, it was higher in adult dogs older than four years compared to younger dogs less than two years. Thus, it is 30% (95% CI [−0.09–0.68]; \( p = 0.13 \)) for youngest.
### Fig. 8
Forest plot of canine leishmaniasis prevalence estimates in Morocco by time period

| Study or Subgroup | Prevalence | SE   | Weight | Prevalence IV, Random, 95% CI | Prevalence IV, Random, 95% CI |
|-------------------|------------|------|--------|------------------------------|------------------------------|
| **6.1.1 Period: 1932-1951** | | | | | |
| Velu et al. 1932 | 0.12903226 | 0.05300541 | 6.1% | 0.02 [0.01, 0.03] | 0.13 [0.03, 0.23] |
| **Subtotal (95% CI)** | | | | 9.9% | 0.13 [0.03, 0.23] |
| **6.1.2 Period: 1952-1981** | | | | | |
| Subtotal (95% CI) | | | | Not estimable | |
| **6.1.3 Period: 1982-2001** | | | | | |
| Dereure et al. 1986 | 0.21126767 | 0.08302604 | 8.1% | 0.16 [0.04, 0.29] | 0.16 [0.04, 0.29] |
| Guessous et al. 1997 | 0.16256158 | 0.0642266 | 8.1% | 0.16 [0.04, 0.29] | 0.16 [0.04, 0.29] |
| Natami et al. 2000 | 0.06586351 | 0.03003873 | 13.8% | 0.09 [0.03, 0.14] | 0.09 [0.03, 0.14] |
| Nejar et al. 1998 | 0.28070175 | 0.05616776 | 9.4% | 0.20 [0.17, 0.23] | 0.20 [0.17, 0.23] |
| Nejar et al. 2000 | 0.16716266 | 0.05077777 | 10.2% | 0.17 [0.07, 0.27] | 0.17 [0.07, 0.27] |
| Sahibi et al. 2001 | 0.16073147 | 0.02842122 | 14.1% | 0.16 [0.11, 0.22] | 0.16 [0.11, 0.22] |
| **Subtotal (95% CI)** | | | | 61.8% | 0.15 [0.09, 0.21] |
| **6.1.4 Period: 2002-2021** | | | | | |
| Boussaa et al. 2014 | 0.21011673 | 0.05654394 | 9.5% | 0.21 [0.10, 0.32] | 0.21 [0.10, 0.32] |
| Fellah et al. 2014 | 0.41975309 | 0.08463771 | 6.0% | 0.42 [0.25, 0.59] | 0.42 [0.25, 0.59] |
| Rami et al. 2003 | 0.11934156 | 0.06020057 | 8.8% | 0.12 [0.00, 0.24] | 0.12 [0.00, 0.24] |
| Rami et al. 2005 | 0.24590164 | 0.11118574 | 4.1% | 0.25 [0.03, 0.46] | 0.25 [0.03, 0.46] |
| **Subtotal (95% CI)** | | | | 28.3% | 0.24 [0.12, 0.36] |
| **Total (95% CI)** | | | | 100.0% | 0.17 [0.12, 0.22] |

Heterogeneity: Tau² = 0.00; Chi² = 12.49, df = 5 (P = 0.03); I² = 60%
Test for overall effect: Z = 4.99 (P < 0.0001)

### Fig. 9
Forest plot represents relative risk of Leishmania infection by sex of dog

| Study or Subgroup | Male Events | Female Events | Total Weight | Risk Ratio M-H, Random, 95% CI | Risk Ratio M-H, Random, 95% CI |
|-------------------|-------------|--------------|-------------|-------------------------------|-------------------------------|
| Fellah et al. 2014 | 8 28 | 7 33 | 22.5% | 1.35 [0.68, 2.68] | 1.35 [0.68, 2.68] |
| Rami et al. 2003 | 25 114 | 29 143 | 77.5% | 1.08 [0.67, 1.74] | 1.08 [0.67, 1.74] |
| **Total (95% CI)** | 33 36 | | 100.0% | 1.14 [0.75, 1.73] | 1.14 [0.75, 1.73] |

Heterogeneity: Tau² = 0.00; Chi² = 0.19, df = 1 (P = 0.67); I² = 0%
Test for overall effect: Z = 0.80 (P = 0.60)
**Fig. 10** Forest plot represents the relative risk of *Leishmania* infection according to the clinical status of the dog.

**Fig. 11** Forest plot on prevalence estimates of canine leishmaniasis in Morocco using subgroup analysis by clinical status.
dogs, rising to 58% (95% CI [0.46–0.71]; \( p < 0.00001 \)) in the middle class and peaking with a rate of 64% (95% CI [0.15–1.43]; \( p = 0.11 \)) for the older dogs.

**Breed of dog**

The association between the breed of dog and the presence of the disease was analyzed. In fact, the results showed that the prevalence was 36% (95% CI [0.06–0.65]; \( p = 0.02 \)) in sheepdogs and 13% (95% CI [0.25–0.50]; \( p = 0.51 \)) in crossbred dogs (Fig. 13).

**Altitude**

The results of our analysis showed that CL in Morocco was present in all altitude but with a high prevalence in high altitudes. This variable was analyzed at four levels: very low altitudes at ground level (0 m), low altitudes (<500 m), medium altitudes (500–1000 m), and high altitudes (>1000 m) (Fig. 14).

**Life style of the dog**

Dog lifestyle is a variable related to three states: chained dog, stray dog, or semi-stray dog. Out of a total of 1882,

![Forest plot on prevalence estimates of canine leishmaniasis in Morocco by subgroup analysis according to dog age category](image-url)
75.8% of the dogs were living in a chained state, 2% of the dogs were strays and 22.2% were living in a semi-stray state. Unfortunately, none of the studies reported data on the seropositivity of these dogs according to their lifestyle. Therefore, it was not possible to estimate the pooled prevalence for these categories of dogs or to establish a risk ratio (Table 1).

**Living environment of dogs**

The living environment of the sampled dogs refers to rural and urban environment. Comparison of the combined prevalence in the two settings was very high in rural area (18% [95% CI: 0.14–0.23]; \( p < 0.00001 \)) compared to urban area (15% [95% CI: 0.01–0.29]; \( p = 0.04 \)) (Fig. 15).

**Health status of the dog**

Health status is a variable related to whether or not the dog is followed by a veterinarian. Indeed, out of a total of 566 dogs, 8.1% were revealed to be under veterinary care, while 91.9% were found to be without veterinary care. Unfortunately, neither of these two studies reported seropositivity data concerning this variable. Therefore, it
was not possible to estimate the pooled prevalence of these two categories of dogs or to establish a risk ratio.

**Discussion**

To our knowledge, this is the first systematic review and meta-analysis on the status of CL in Morocco. The results presented in this report are based on the analysis of data obtained from a systematic review of scientific studies on the prevalence of CL in Morocco between 1932 and 2021. The majority of the studies were carried out from 1982 onwards and more than half of them came from the central regions of the country. It is within this framework that the present review attempts to assess the status of the canine *Leishmania* reservoir in Morocco. However, it should be noted that although the term CL refers to animals belonging to the canidae, in Morocco this term is often used to designate leishmaniasis affecting dogs whether it is the bladder or skin form. Therefore, we will use the term “canine leishmaniasis” instead of dog leishmaniasis throughout the discussion of our results.

In Morocco, the dog is considered the main reservoir host of *Leishmania infantum* and the source of infection because it plays an essential role in maintaining the chain of transmission to humans (MMH 2010). The NPCL established on 1997 and revised on 2010 states that control of the severe visceral form of *L. infantum* leishmaniasis requires control of the canine reservoir and advocates a strategic focus on eliminating this disease by 2030 (MMH 2016; El-Mouhdi et al. 2020a, b, c). Unfortunately, there is no documented data on the global estimate of the sero-prevalence status of the canine reservoir in Morocco. The existence of such information allows policymakers to...
further focus their control efforts to target at-risk areas where canine reservoir foci are active. Indeed, leishmaniasis in the canine population is a very important indicator to be taken into consideration to prevent the disease in humans.

To date, the status of canine reservoir infection with *Leishmania* is unclear and the overall estimate of leishmaniasis prevalence in dogs is unknown. It is in this perspective that the present review was conducted to know the status of CL in Morocco and the risk factors associated with the disease to provide officials with an effective reference tool indicating active foci of CL with high prevalence where control actions should be conducted to prevent the risk of occurrence of human visceral leishmaniasis. The present study constitutes the synthesis and meta-analysis of the various individual studies carried out on the seroprevalence and risk factors of CL in Morocco. It was conducted following precise and predetermined steps (Higgins and Green 2008). Strict and rigorous criteria have been set for selection and identification of the publications, object of the present study (Deeks et al. 2008).

Specifically, after an exhaustive literature search, 15 studies were eligible for this review. It included studies examining the seroprevalence and risk factors associated with *Leishmania* infection in the canine population in Morocco. These studies underwent a quality control review primarily concerned with assessing the risk of bias of the included studies and was performed using the assessment tool for studies reporting prevalence data (Joanna Briggs Institute 2020). Our results showed a low risk of bias. Moreover, the majority of the studies were conducted in 8 administrative regions representing 66.6% of the total country territory, which seems fairly representative. This allows us to draw reliable conclusions about the overall prevalence of CL in the country. Eleven studies (Guessous-Idrissi et al. 1997a, b; Nejjar et al. 1998, 2000; Sahibi et al. 982 J Parasit Dis (Oct-Dec 2022) 46(4):967–987

**Fig. 15** Forest plot on prevalence estimates of canine leishmaniasis in Morocco by subgroup analysis according to the living environment of the dog.
2001; Fellah et al. 2014; Natami et al. 2000; Rami et al. 2003, 2005; Dereure et al. 1986; Boussa et al. 2014; Velu et al. 1932) reported prevalence data. In fact, out of a total of 3900 diagnosed dogs 580 were reported positive for Leishmania sp. with an apparent prevalence of 14.87%.

The diagnostic methods that were used ranged from simple biochemical tests to molecular tests. The pathogens incriminated in cutaneous and visceral leishmaniasis in dogs ranged from frequently identified strains (L. infantum zymodeme MON-1) to rare strains (L. tropica MON-102; L. tropica MON-113; L. tropica MON-279 and L. infantum MON-24). This can be explained, on the one hand, by the predominance of the L. infantum strain in the canine population in Morocco as in the rest of the Mediterranean countries (Organization and Mediterranean 2017), and on the other hand, by the virulence of the pathogenic strains circulating in the high prevalence regions, knowing that visceral and cutaneous forms of CL can be caused by strains belonging to L. infantum (Dereure et al. 1991; Haralambous et al. 2007) as well as by L. tropic (Sahibi et al. 2001; Lemrani et al. 2002; Guessous-Idrissi et al. 1997a, b).

Our Meta-analysis showed that the overall combined prevalence of CL in Morocco was 17% (95% CI: 0.12–0.22; p < 0.00001). It was very high in the coastal regions notably in the provinces of Rabat (42% [95% CI: 0.25–0.59]), Nador (21% [95% CI: 0.10–0.32]) and Al Hoceima (15% [95% CI: 0.01–0.29]). And in the central region of the country, specifically in the provinces of Chichaoua (33% [95% CI: −0.05–0.71]); Sefrou (28% [95% CI: 0.17–0.39]) and Moulay-Yacoub (27% [95% CI: 0.10–0.44]). These differences in the distribution of prevalence across Morocco can be attributed to the specific environmental conditions, to biological characteristics of the local dog populations and consequently by the local Leishmania cycle of transmission (Parasite, vector, reservoir host).

On the other hand, a critical analysis of the literature over time showed that the prevalence of CL in the early years of publication (1932–1951) was estimated at 13% (95% CI [0.03–0.23]; p = 0.01). In this period, the main documented canine surveys were carried out in the the El Jadida city (at that time called Mazagan) and in Casablanca city to ascertain the existence of leishmaniasis in dogs (Velu et al. 1932). The results obtained affirmed that CL is exceedingly rare, if not non-existent in Morocco (Velu et al. 1932). A decade later, this finding was confirmed by Zottner (1941) reporting an autochthonous case of symptomatic CL in Morocco. It was considered to be the 3rd case observed in Morocco after the 1st case reported by Jeaume (1932) in a fox dog born and raised in Tangier (northern Morocco) and the 2nd case reported in a rural dog in Fes (central Morocco) (Zottner 1941). These two dogs were found to be carriers of the Leishmania parasite despite being asymptomatic (Zottner 1941). At that time, authors concluded that if leishmaniasis disease exists in the canine population, the percentage of contaminated animals is certainly low and difficult to establish (Velu et al. 1932; Zottner 1941). Therefore, investigations on CL in Morocco were stopped for the next 30 years. This probably explains the absence of publications between the years 1952 and 1981 and the impossibility of estimating the prevalence in this second period.

However, during the third period from 1982 to 2001, there was a strong return to the investigations of CL in Morocco. The publications in this period represent 47% of the total number of publications. This resurgence can be explained by the signaling of the increase of indigenous cases of infantile visceral leishmaniasis (Kirmse et al. 1987). In fact, this period was also marked by the official launch of the NLCP (MMH 1997) and leishmaniasis became a concern for national health authorities. In addition, the publications in this period were very rich in terms of the identification of circulating pathogenic strains and the indigenous zoonotic cycles in the country. They proved the existence of a zoonotic cycle of canine visceral leishmaniasis caused by L. infantum MON-1 in the southern area of Morocco (Dereure et al. 1986) and discovered for the first time, in 1991, the presence of canine cutaneous leishmaniasis caused by L. tropica MON-113 and MON-102 in Azilal and Essaouira provinces (Dereure et al. 1991). Also, for the first time in Morocco, a case of canine visceral leishmaniasis caused by L. tropica was reported in the North of the country (Guessous-Idrissi et al. 1997a, b). The second case was found in the locality of Oulde Hsain in the province of Taounate (Sahibi et al. 2001). Then, endemic foci of CL have been reported in the north and centre of the country (Natami et al. 2000; Sahibi et al. 2001; Nejjar et al. 2000, 1998; Guessous-Idrissi et al. 1997a, b). Thus, the results of our meta-analysis revealed that the combined prevalence in this period was found to be increased with a percentage of 15% (95% CI [0.10–0.16]; p < 0.00001).

The fourth period (2002–2021) was characterized by the continued identification of other active foci of CL in the northern, central and southern regions of the country (Fellah et al. 2014; Rami et al. 2003, 2005; Boussa et al. 2014). In addition, the main result in this period wasthe detection of new zymodemus of the pathogens of the disease: L. tropica MON-279 proved for the first time as the causal agent of the visceral form of CL in the north (Lemrani et al. 2002) and L. infantum MON-24 demonstrated as the causal agent of the cutaneous form of CL in the Pre-Rif region of Morocco (Haralambous et al. 2007). Estimation of the pooled prevalence in this period was increased to 24% (95% CI [0.10–0.25]; p = 0.0001).
addition, the Moroccan Ministry of Health announced that it revised the NLCP and published its updated version in 2010 (Ministry of Health 2010). In this edition, the program has reserved a strategic axis for the control of dogs suspected as the reservoirs of *L. infantum* in Morocco, while humans remain the only reservoir of *L. tropica* (MMH 2010).

In addition, several epidemiological studies have reported the frequent coexistence of human cases of cutaneous leishmaniasis due to *L. tropica* and *L. infantum* in temperate zones, especially in the center and north part of the country, whereas this coexistence has not been noticed with cases due to *L. major*, whose reservoir is the rodent “*Meriones Shawi*”. Referring to the results of this study, the simultaneous occurrence of skin lesions due to *L. infantum* and *L. tropica* in the Moroccan population in humid area may be justified by the presence of the dog which has been shown to be the reservoir of both species. In addition, the presence of the sand fly vector is very common in the Moroccan territory (El-Mouhdi et al. 2020a, b, c; 2021).

Certainly, human leishmaniasis is a public health problem in Morocco, the visceral form is fatal in the absence of treatment and the cutaneous form due to *L. tropica* is endemic in several areas of the country, it presented alone 43% of all cases reported (MMH 2010; El-Mouhdi et al. 2020a, b, c). It is suggested that the control strategy against the canine reservoir of leishmaniasis in Morocco should be renewed taking into consideration these new data. In this way, the effective control of the canine reservoir will reduce the human cases of leishmaniasis due to *L. infantum* and *L. tropica*. The culling of stray and symptomatic dogs as the only strategy to prevent human risk is outdated (MMH 2010). A holistic approach must be adapted in the control of leishmaniasis and particular emphasis must be placed on dog population management adapted to the country context if Morocco is to eliminate mortality from visceral leishmaniasis and achieve Sustainable Development Goals by 2030 (El-Mouhdi et al. 2019).

Therefore, it emerges from this spatiotemporal meta-analysis that, on the one hand, the high prevalence rates that were reported during the first period in the coastal provinces persisted during all periods, on the other hand, high prevalence rates in the central regions appeared from the third period and continued in the fourth period where high prevalences were recorded in the southern regions. This means that, over time, CL spread from the north to the center and then to the south of the country by gaining new foci. This can be explained by the wandering lifestyle of stray dogs carrying the infection through hamlets and villages that forces them to move in search of food and shelter (Kholoud et al. 2020). However, it can also be explained by exposure to other factors that may be directly related to the dog (intrinsic factors) or related to the external environment (extrinsic factors).

For the intrinsic factors, our results show that seropositivity increased with the age of the dog, it was higher in adult dogs older than 4 years compared to the youngest ones of less than 2 years. This may be explained by the long latency period of the disease in the dog after infection (Oliva et al. 2006), or by the increased contact with the sand fly vector.

Furthermore, the risk of *Leishmania* was twice as high in healthy dogs with asymptomatic clinical status. This can be explained; on the one hand, by the culling of clinically suspected stray dogs under the NLCP (MMH 2010), on the other hand, by the attractiveness of asymptomatic dogs to sand flies (Belo et al. 2013; Shokri et al. 2017). We assume that the very difficult lifestyle of Moroccan dogs reflects the clinically suspect aspect which in reality lived in lack and abuse. In addition, recent studies conducted among Moroccan citizens and health professionals have shown that dogs are poorly known as reservoirs of leishmaniasis (El-Mouhdi et al. 2020a, b, c). However, the sex of the dog was not found to be associated with leishmaniasis infection. These results confirm those found in the primary results in Morocco (Rami et al. 2003; Fellah et al. 2014) and those found in Brazil (Belo et al. 2013).

Regarding extrinsic factors, the results of our analysis showed that CL in Morocco was present in all altitude ranges but with a high prevalence in altitudes above 1000 m. This can be explained by the ecological preferences of the proven vectors of *L. infantum* in Morocco, namely *Phlebotomus perniciosus*, *P. ariasi* and *P. longicuspis*, which are very active at high altitudes (Boussaia et al. 2008). Indeed, Boussaia et al (2008) showed that *Larroussius* species, *P. perniciosus* and *P. ariasi* are very frequent in the altitudes 1000–1400 m and *P. longicuspis* was very active in the altitudes 800–1000 m.

Comparison of the combined prevalence according to the environment revealed that it was higher in rural area compared to urban area. This difference can be explained by the abundance of the canine population in rural area because of their usefulness to farmers. In fact, using to guard livestock, rural Moroccan families own an average of 2 to 4 dogs per family, (Nejjjar et al. 1998). In urban area, public and garbage dumps environment favors the installation of canine populations (Kholoud et al. 2020), and consequently, the local transmission cycle of CL.

In sum, dogs have no predators in these two environments, they have a short and rapid reproductive cycle, with an average of five to six pups, the number of dogs increases rapidly as its food supply is assured (Pal 2003). Therefore, the likelihood of the risk of spreading *Leishmania* will be more increased. This reinforces the relevance of designing a dog population management strategy in Morocco based
on the promotion of the dog lifestyle in collaboration with government authorities, community and veterinary leaders through education and awareness actions, especially to dog owners. Thus, the control of CL requires courageous actions that allow dog owners to proceed to (a) registration of their dogs, (b) implementation of regulations on breeding, veterinary monitoring and sale of dogs, (c) sterilization procedures, (d) application of protective collars against insect bites, and (e) establishment of direct legislation to dog owners (World Organisation for Animal Health 2021).

Conclusion

This systematic review and meta-analysis have provided an update on the prevalence of CL in Morocco and its risk factors but we highlight a scarcity of publication about CL compared to human leishmaniasis in Morocco. Our results contribute to a better understanding of the status of the disease, the main pathogenic strains circulating in the country and its regional and provincial distribution. This allows policymakers to predict area at risk for human visceral leishmaniasis where direct their control efforts. Nevertheless, it is necessary to conduct awareness campaigns among dog owners to improve the quality of life of these animals by insisting on veterinary follow-up, the application of anti-Leishmania collars and stalls that respect their dignity. Referring to “one health” standards, future efforts must be continued by integrating holistic approaches in the management of zoonotic diseases. The dog is not only a reservoir of leishmaniasis; it is also a victim of sand fly bites.

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Declarations

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