Seroprevalence and associated risk factors of canine *Leishmania infantum* infection in selected towns of Southwest and West Shewa Zones of Oromia, Ethiopia

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Abstract

Background

Canine *Leishmania infantum* infection is a major vector-borne zoonosis. Most cases of canine leishmaniasis are caused by *Leishmania infantum*, but other species can also be found. In the Old and New Worlds, leishmaniasis is spread by the bite of phlebotomine female sandflies of the genera *Phlebotomus* and *Lutzomyia* respectively. The objectives of the study were to estimate the seroprevalence and associated risk factors in Weliso, Ambo, and Ejaji towns of South-West and West Shewa Zones, Oromia, Ethiopia.

Methods

A cross-sectional study was conducted from October 2019 to September 2020. The study population consists of dogs found in the study towns. The study subjects include 368 dogs selected by using a simple random sampling method. A blood sample (5 ml) was collected to test for the presence of rK39 antigen inducing specific antibodies using an Indirect ELISA kit. All the data were analyzed using the STATA version 14 software program. Pearson's Chi-square and logistic regression tests were used to evaluate the association between the seropositivity of canine *Leishmania infantum*s and hypothesized risk factors.

Results

An overall canine *Leishmania infantum* seroprevalence of 84.24% (95% confidence interval [CI]: 80 - 88%) was obtained. In Weliso, Ambo, and Ejaji towns canine *Leishmania infantum* seroprevalence of 79.82% (95% CI: 71- 87%), 76.11% (95% CI: 67- 84%), and 93.84% (95% CI:89 – 97%) were found respectively. Univariable logistic regression analysis showed that town ($P=0.001$), season ($P=0.001$), mixed living environment ($P=0.003$), and Kebele ($P<0.05$) were significantly associated with *Leishmania infantum* seropositivity. However, the multivariable analysis indicated that wet season ($P = 0.001$) and a mixed living environment ($P = 0.025$) were independent predictors of canine *Leishmania infantum* seropositivity.

Conclusion

The seroprevalence of canine *Leishmania infantum* in dogs was very high. Wet season and mixed living environment are predictors of seropositivity. The high percentage of seropositive dogs indicates the potential role of dogs to maintain the infection and the possible spread of *L. infantum* infection to humans. Therefore, improvement of the living environment and health care facilities of domesticated dogs, community awareness and health education, and further epidemiological studies are suggested.

Background

Canine *Leishmania infantum* (CLI) infection is a major vector-borne zoonosis. Most cases of canine leishmaniasis are caused by *L.infantum*, but other species can also be found [1]. *Leishmania infantum* is obligate Intramacrophage protozoa of the genus *Leishmania* in the family *Trypanosomatidae* [2]. The parasite is of great public health and veterinary significance. Leishmaniasis is a neglected tropical disease (NTD) that is transmitted by the bite of *phlebotomine* female sandflies of the genera *Phlebotomus* and *Lutzomyia* in the Old and New World, respectively. About 53 species of the parasite have been described from different regions of the world; Of these, 31 species are known to be parasites of mammals and 20 species are pathogenic to human beings [3, 4].

Canine *Leishmania infantum* causes an important disease that occurs on all continents except Oceania [5]. Leishmaniasis is endemic in 98 countries and 350 million people are at risk of contracting the disease. Leishmaniases are a group of diseases with a wide range of clinical manifestations. There are three forms of leishmaniases, visceral (VL), cutaneous (CL), and mucocutaneous leishmaniasis (MCL). Of the three forms, VL is highly fatal and the most prevalent form in eastern Africa, followed by CL and MCL [6].

There is an estimated incidence of 1 to 1.5 million cases of CL or MCL (mainly occurring in Brazil, Peru, and the Plurinational State of Bolivia) and 500,000 cases of VL primarily in South America, East Africa, and the Indian Subcontinent. More than 90% of the world's VL cases occur in Bangladesh, Brazil, Ethiopia, India, Nepal, and Sudan. Eastern Africa has the second-highest number of VL cases, after the Indian Subcontinent, and the disease is endemic in parts of Eritrea, Ethiopia, Kenya, Somalia, North Sudan, Southern Sudan, and Uganda [6, 7].

Ethiopia is one of the ten countries with the highest estimated case counts, which together account for 70 to 75% of global estimated VL incidence. Of the estimated 2 million cases, only around 600,000 are reported [8]. The epidemiology of VL has changed in Ethiopia, with endemic areas spreading continuously [9]. This is in line with the existing trend of climate change, rapid urbanization, and massive population movements affecting the range and population density of the insect vectors and reservoir hosts of the disease, resulting in a cumulative elevation in the rate of human exposure to the infection [10].

Leishmaniasis infects numerous species of mammals, including humans. Among domestic animals, dogs are the most important species in the epidemiology of the disease. Dogs play an important role in the ecology and control of zoonotic visceral leishmaniasis (ZVL) as they are the predominant domestic reservoir for the disease, with greater than 10% seropositivity often evident in dogs before the emergent of VL that has been observed in humans [11]. Hence, dog ownership is a major risk factor of VL in multiple endemic countries [12]. The digenetic life-cycle of the *Leishmania* parasite involves the delivery of flagellated promastigotes to mammalian hosts during a sandfly bite, where non-flagellated amastigotes develop within phagocytic mononuclear cells. These cells are taken up by the phlebotomine sandfly during feeding and then develop into flagellated promastigotes, completing the lifecycle [13].

Apart from a general species-specific organotropism of *Leishmania infantum*, intra-specie intrinsic characteristics are also a relevant factor to consider. Dermo-tropic and viscera-tropic *L. infantum* strains modulate the sandflies biting time on the host leading to the delivery respectively, of a high or low dose of meta-cyclic promastigotes into the skin which will impact the parasite tropism and manifestation of the disease [14]. Even strains belonging to the same zymodeme have been associated with differential infectivity [15].

The objectives of the current study were to estimate the seroprevalence and associated risk factors of CLI in Weliso, Ambo, and Ejaji towns of South-West and West Shewa Zones, Oromia, Ethiopia.

Methods

The study was carried out in the towns of Weliso in the South-West Shewa Zone, and Ambo and Ejaji in the West Shewa Zone, Oromia, Ethiopia (Figure 1). Weliso district is one of the 14 districts found in the South-West Shewa Zone. Weliso town is an administrative town of Weliso district, and the South-West Shewa Zone, which is located 114 km southwest of Addis Ababa and the district has a longitude of 37° 57' 59.99" E and latitude of 8°31' 59.99" N with an elevation of 2063 meters above sea level (m.a.s.l). The annual rainfall and a temperature range from 950-2,718.3mm and 13.6°C-25°C respectively.

Ambo town is an administrative town of Ambo district, and the West Shewa Zone, which is located 115 km West of Addis Ababa and the town has a latitude of 8°59'N and longitude of 37°51'E and an elevation of 2101m.a.s.l. The annual rainfall and a temperature range from 800-1000mm and 15°C-29°C respectively. The mean temperature is 18.6°C. Whereas, Ejaji town is the administrative town of Elu Gelan district, which is located 90 km West of the zonal capital, Ambo, and 215 km West of Addis Ababa. Ejaji town has a longitude of 37° 9.8'E and a latitude of 8° 59.9'N and has an altitude ranging from 1565-1790 m.a.s.l. The annual rainfall and a temperature range from 2000-2300mm and 27°C and 30°C respectively.

The climatic conditions of the study areas vary from very hot (lowland) to mid-temperate (midland) and cold temperatures (highland).

The study population and study animals

The study population consists of dogs found in the study towns. Local, cross, and exotic breeds of dogs are found in the study towns. Nevertheless, local breeds are predominantly found in the study areas, followed by a cross and exotic breeds of dogs. Dogs are kept for house guard in most parts of urban areas in the country; however, in rural areas they are also used for protecting livestock from thieves in the evening and hunting. In this study, healthy dogs above six months of age, both sexes, and different kinds of breeds were included. A total population of 7,098 dogs is found in the current study towns. Of these, the highest population of dogs is found in Ejaji (2,816), followed by Ambo (2,180), and Weliso (2,102) towns. Dogs aged 6 months; 6 months to 1.5 years, 1.6 to 7 years, and above 7 years were considered as juvenile, adolescent, adult, and geriatrics respectively [16].

Study design and sampling methods

This cross-sectional study was carried out from October 2019 to September 2020. Dogs were brought to the selected places in the study areas arranged for sample collection. Dog owners were invited to the area through microphone announcement with the help of the *kebele* (the smallest formal administrative unit in a town) administration members. The study animals were selected by using a simple random sampling method. This consists of randomly drawing “n” individuals from “N” people in a sampling frame list. Dog owners were informed to handle their dogs properly up to the end of the sample collection process. Dogs that cannot get to the selected locations were sampled in their homes.

Inclusion and exclusion criteria

Agreement by the dog owner to participate in the study was an inclusion factor. Accessible dogs whose owners are willing for sampling and dogs above six months of age were included in the study while highly debilitated, sick dogs and those under treatment were excluded. Dogs known for their extreme aggressiveness were also excluded from the study.

Sample size determination

The desired sample size for the study was calculated by the formula given by Thrusfield[17] assuming 50% expected seroprevalence of CLI in the study areas (since there was no previous study in the current study areas) and a 5% level of precision. The sample size (n) is determined by the formula:

$$n = \frac{1.96^2 \times P_{exp}(1-P_{exp})}{d^2}$$

Where, n sample size required, 1.96 = the value of Z at 95% confidence interval, P_{exp} = expected seroprevalence, d = desired absolute precision

$$n = \frac{1.96^2 \times 0.5(1-0.5)}{0.05^2} = 384 \text{ dogs}$$

Although, the calculated sample size was 384, due to kit shortage 368 dogs were tested (i.e., 146, 113, and 109 dogs from Ejaji, Ambo, and Weliso towns respectively).

Sampling procedure and techniques

Before blood collection, personal protective pieces of equipment like disposable gloves, gowns, and waste management materials were made ready and used properly. The dogs were restrained in portable dog crush, by rope, and dog catcher accordingly. Then, the site of blood collection (cephalic and lateral saphenous vein) was washed with soap and water, shaved with a sterile surgical blade, disinfected with

70% isopropyl alcohol, and tied by the tourniquet. A blood sample (5ml) was collected from each dog by intravenous cannulation with plain vacutainer tubes and needles.

Each tube was individually identified and the blood was allowed to clot and then centrifuged at 3000 revolutions per minute (rpm) for 5-10 min to obtain serum. Centrifugation took place in the field, i.e., during the evening in the town of each study area. The serum samples were then immediately transported using an icebox containing ice packs to the Ambo University Zoonosis and Food Safety Laboratory and stored at -20°C until serologically tested. Finally, the serum samples were transported using an icebox to Sebeta National Animal Health Diagnostic and Investigation Center (NAHDIC) for Indirect ELISA Serological examination.

I-ELISA

All the collected serum samples were tested for the presence of rK39 antibodies against CLI following the protocol of the manufacturer of the indirect ELISA kit (IDvet Innovative Diagnostics, ID Screen®, *Leishmania* Indirect, France).

Data management and analysis

The raw field data obtained from sampled dogs and findings of laboratory examination were entered into Microsoft Excel 2019 Spreadsheet to create a database followed by refining and coding. The collected data were analyzed using the STATA version 14 software program (Stata Corp. College Station, USA). Descriptive statistics were used to summarize the data. Pearson's Chi-square () and logistic regression (univariable and multivariable) tests were used to evaluate the association between hypothesized potential risk factors like town, season, *kebele*, sex, age, breed, living environment, and presence of other domesticated animals in the households with CLI seropositivity. Additionally, the collinearity (confounding) of the risk factors was also analyzed and from collinear risk factors, only one independent variable with a strong biological association with the disease outcome was selected and used for the final logistic regression model. Those variables with $P \leq 0.25$ in the univariable logistic regression analysis were further analyzed using multivariable logistic regression after checking for multicollinearity. In all the cases $P < 0.05$ was set for significance.

Ethical statement

The study protocols were approved by the ethical review committee of the Ambo University, Mamo Mezemir Campus, School of Veterinary Medicine. Each study subject involved in the study gave oral consent to participate and only those individuals who showed their willingness were selected to participate in the study. All dogs were enrolled in this study with informed permission from their caretakers, and all dogs sampled were examined with the assistance and acceptance of their owners. Blood samples were collected by the researcher according to the good practices of veterinary medicine

i.e., all animals were handled strictly by following the good animal handling practice to minimize animal sufferings during sampling.

Results

Frequency distribution analysis of potential risk factors

From the overall 368 dogs studied, the majority are from Ejaji (146/368, 39.67%), followed by Ambo (113/368, 30.71%), and Weliso (109/368, 29.62%) towns (Table 1).

Seroprevalence

The current study estimated an overall Canine *L. infantum* seroprevalence of 84.24% (95% CI: 80 – 88%). In Weliso, Ambo, and Ejaji towns Canine *L. infantum* seroprevalence of 79.82% (95% CI: 71 - 87%), 76.11% (95% CI: 67 - 84%), and 93.84% (95% CI: 89 - 97%) was found respectively. The seroprevalence was significantly high ($P < 0.05$) in dogs of Ejaji town (93.84%) compared to Ambo (76.11%) [Table 2].

Risk factors

All independent variables considered in this study were non-collinear with each other except town and season ($r = 0.6312$), *kebele* and season ($r = 0.5338$), and *kebele* and town ($r = 0.5782$). Among collinear independent variables season was selected for the final multivariable model. Furthermore, those independent variables with univariable $P \leq 0.25$ such as season, breed, and living environment were selected for the multivariable logistic regression analysis. CLI seropositivity was significantly associated with a wet season ($P = 0.001$) and a mixed living environment ($P = 0.025$). The likelihood of CLI seropositivity of dogs was 2.91 times higher in the wet than the dry season. The likelihood of CLI seropositivity of dogs was also 2.19 times higher in dogs living in a mixed environment (indoor and outdoor) as compared to indoor living dogs (Table 2).

Discussion

Seroprevalence

The current study revealed an overall high canine *L. infantum* seroprevalence (310/368, 84.24%). Of this, the highest seroprevalence was observed in Ejaji town (137/146, 93.84%) followed by Weliso (87/109, 79.82%), and Ambo (86/109, 76.11%). The current study findings were significantly higher than the canine leishmaniasis seroprevalences of 13.9% reported in Benishangul Gumuz Regional State, Ethiopia [18], 40% in dogs in North-West Ethiopia [19], 6.9% in domesticated dogs in Eastern Sudan [20], 11.7% in stray dogs, 9.7% in National Guard dogs, and 5.9% in farm dogs in Algeria [21].

Much lower Seroprevalences CLI than the present study have also been reported previously such as 6.5% in an endemic focus of Satluj river valley of Himachal Pradesh (India) [22], 35% VL among dogs in VL endemic areas of Mymensingh district, Bangladesh [23], 26% *L. infantum* infection in dogs in Spain [24], 6.31% in Portugal [25], 4.9 to 12.6% in a public kennel in Bologna, Italy [26], 5.2% (L'Aquila, Abruzzo region)

to 21.8% (Campobasso, Molise region) in Italy [27], 22% in Brazil [28], 22% in Colombia [29], and 12% in the central region of Colombia [30].

The variation in seroprevalence between the current study and the aforementioned reports might be possibly due to variations in geographical location, i.e., geographical regions with various weathers have shown different seroprevalence of *L.infantum* in dogs. Since the seroprevalence of CLI and HVL is associated with weather conditions and humidity in endemic areas [31]. It could also possibly be due to the variation in the suitability of the environment, activities of sandflies, and the country's prevention and control strategies against CLI in predominant reservoir host, and vectors of leishmaniasis. Noticeably, the prevalence of canine leishmaniasis also varies between different regions depending on the serological tests used in the diagnosis [30].

Risk factors

The current study revealed that wet season ($P = 0.001$) and mixed living environment ($P = 0.025$) were significantly associated with CLI seropositivity possibly owing to the different activities of sandflies in these regions favored by suitable environmental conditions for its breeding and more exposure of dogs to sandfly bites. The lower infection in household dogs is possibly due to the lower chance of exposure to sandfly bites [32].

A relatively high percentage of infected dogs having outdoor access as compared to owned dogs without outdoor access might suggest dogs that have outdoor access might be the potential source for human infection. Free-roaming is an important factor for achieving infection [33]. The likelihood of CLI seropositivity of dogs in the wet season (AOR=2.91) and mixed living environment (AOR=2.19) was significantly higher compared to the dry season and indoor living environment respectively.

The current study findings were in close agreement with the studies conducted in Himachal Pradesh, India; where domestic, as well as stray dogs, suggesting that they are perhaps harboring the *L. donovani-infantum* and providing a constant reservoir for the infection [22], in Mymensingdistrict, Bangladesh; where the living status of the dogs (street or owned) was a potential risk factor [23], and in Italy, where being a dog hosted in a kennel, seems not to be a factor increasing the probability that dogs show seropositivity, even in long-term sheltering conditions [27].

However, the current findings disagree with the studies conducted in Algeria; where the infection was significantly more prevalent in older dogs than younger ones, in stray dogs than owned dogs, and rural dogs than urban ones [21], in Brazil, where the re-emergence of the disease appears to be a consequence of the discontinuation of control programs [28], and in the central region of Colombia, where owned dogs were strongly associated with canine leishmaniasis [30].

Limitations of the research

This study is believed to have certain constraints. Mainly, it is a financial and diagnostic kit shortage. From the calculated sample size (n=384), only 368 dogs were tested due to diagnostic kit shortage. The findings of this study should be cautiously interpreted for all dog populations in the study areas. In the absence of stray dog ownership, the current study was limited to only those dogs that have an owner, and also rural dog populations were not included in the study. This can often create challenges to generalize the findings of the current research to the whole dog population in the area. Canine *L. infantum* is largely uninvestigated and neglected in Ethiopia. Inadequate information is available about CLI in Ethiopia in general and absent in the selected study areas in particular. Hence, it was difficult to gain enough information and to discuss the results obtained with other studies conducted in similar study areas. Therefore, the current study results were discussed with many reports from abroad.

Conclusions

In the current study, a very high percentage of an overall CLI seroprevalence (84.21%) in dogs was reported. Wet season and mixed living environment of dogs were predictors of CLI seropositivity. The high percentage of seropositive dogs indicates the potential role to maintain the infection and possible spread of *L. infantum* infection to humans. Therefore, improvement of living environment and health care facilities of domesticated dogs, community awareness and health education, and further epidemiological studies are suggested.

Declarations

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Ethics approval and consent to participate

This research project was approved by the ethical committee for animal experimentation of Ambo University, Ethiopia (Ref. No. RD/AREC/004/2016). All animals were handled strictly following the good animal handling practice to minimize animal sufferings during sampling. Informed verbal consent was obtained from the dog owners.

Consent for publication

Not applicable.

Availability of data and materials

The data supporting the conclusions of this article are included within the article and anonymized data could be shared upon request to the corresponding author.

Competing interest

The authors declare that they have no competing interests.

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Authors' contributions

WW Participated in proposal preparation, collection of data, analysis of data, and manuscript drafting. EZ conceived the research idea, participated in proposal preparation, supervising, data analysis and interpretation, manuscript reviewing, and commenting. CD and DZ did an Indirect ELISA serological test and reviewed the manuscript. VMLP and MV participated in the design, drafting, and critical review of the manuscript. All Authors read and approved the final manuscript.

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Abbreviations

AOR: adjusted odds ratio; CI: confidence interval; CL: cutaneous leishmaniasis; CLI: canine *Leishmania infantum*; COR: crude odds ratio; ELISA: enzyme-linked immunosorbent assay; HVL: human visceral leishmaniasis; M.A.S.L.: meter above sea level; MCL: mucocutaneous leishmaniasis; NTD: neglected tropical disease; NAHDIC: national animal health diagnostic and investigation center; POS: positive; RPM: revolution per minute; VL: visceral leishmaniasis; ZVL: zoonotic visceral leishmaniasis.

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Tables

Table 1: Results of the frequency distribution of the potential risk factors of CLI in the study towns

Variables	Category	Frequency	%
Towns	Weliso	109	29.62
	Ambo	113	30.71
	Ejaji	146	39.67
Seasons	Wet	180	51.09
	Dry	188	48.91
Kebele	Burka Gudina	15	21.47
	Ayetu	23	19.57
	Hora	28	18.48
	Yahi Geda	41	11.41
	Ejersa	42	11.14
	Goba	68	7.61
	Hora Ayetu	72	6.25
	Gora	79	4.08
	Female	59	83.97
	Male	309	16.03
Age	Geriatrics	40	73.37
	Juvenile and Adolescent	58	15.76
	Adult	270	10.87
Breed	Exotic and cross	64	82.61
	Local	304	17.39

Table 1: Results of the frequency distribution of the potential risk factors of CLI in the study towns (continued).

Variables	Category	Frequency	%
Living environment	Outdoor	60	63.86
	Indoor	73	19.84
	Mixed	235	16.3
Presence of other animals	No	132	64.13
	Yes	263	35.87
<i>Leishmania</i> Status	Negative	58	15.76
	Positive	310	84.24

Table 2: Logistic regression analysis results of the association between CLI seropositivity and potential risk factors.

Variable	Category	No. tested	No. Pos. (%)	Univariable		Multivariable	
				COR (95% CI)	P-value	AOR (95% CI)	P-value
Town	Ambo	113	86(76.11)	1	1	-	-
	Weliso	109	87(79.82)	1.24(0.66-2.35)	0.506	-	-
	Ejaji	146	137(93.84)	4.78(2.14-10.65)	0.001*	-	-
Season	Dry	188	145(77.13)	1	1	1	1
	Wet	180	165(91.67)	3.26(1.74-6.12)	0.001*	2.91(1.51-5.62)	0.001*
Kebele	Ejersa	42	29(69.05)	1	1	-	-
	Hora	28	21(75.00)	1.34(0.46-3.95)	0.590	-	-
	Yahi Geda	41	31(75.61)	1.39(0.53-3.66)	0.505	-	-
	Hora Ayetu	72	55(76.39)	1.45(0.62-3.39)	0.392	-	-
	Burka Gudina	15	14(93.33)	6.27(0.74-52.90)	0.091	-	-
	Gora	79	74(93.67)	6.63(2.17-20.27)	0.001*	-	-
	Goba	68	64(94.12)	7.17(2.15-23.90)	0.001*	-	-
	Ayetu	23	22(95.65)	9.86(1.2-81.19)	0.033*	-	-
Sex	Female	58	46(79.31)	1	1	-	-
	Male	310	264(85.16)	1.46(0.72-2.96)	0.294	-	-
Age	Juvenile and ado.	58	46(79.31)	1	1	-	-
	Adult	270	227(84.07)	1.38(0.67-2.81)	0.380	-	-
	Geriatrics	40	37(92.50)	3.22(0.84-12.25)	0.087	-	-

Table 2: Logistic regression analysis results of the association between CLI seropositivity and potential risk factors (cont.).

Variable	Category	No. tested	No. Pos. (%)	Univariable		Multivariable	
				COR (95% CI)	P-value	AOR (95% CI)	P-value
Breed	Exotic and cross	64	49(76.56)	1	1	1	1
	Local	304	261(85.86)	1.86(0.96-3.6)	0.067	1.02(0.5-2.11)	0.950
Living environment	Indoor	73	53(72.60)	1	1	1	1
	Outdoor	60	51(85)	2.14(0.89-5.13)	0.089	1.85(0.75-4.57)	0.182
	Mixed	235	257(87.12)	2.68(1.41-5.11)	0.003*	2.19(1.11-4.33)	0.025*
Presence of other animals	No	132	109(82.58)	1	1	-	-
	Yes	236	201(85.17)	1.21(0.68-2.15)	0.513	-	-

No. = Number, Pos. = Positive, COR = Crude Odd Ratio, AOR = Adjusted Odd Ratio, Juvenile and ado. = Juvenile and adolescent

Figures

Study Areas

Study Towns

Figure 1

Maps showing the study areas (West Shewa Zone and Elu Gelan District Land Administration and Use Office, 2003).