High seroprevalence of *Leishmania infantum* infection in dogs and its associated risk factors in selected towns of Southwest and West Shewa zones of Oromia, Ethiopia

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Abstract
Background: *Leishmania infantum* infection can result in serious vector-borne zoonotic disease such as visceral leishmaniasis. The objectives of the study were to estimate the seroprevalence and associated risk factors in Weliso, Ambo, and Ejaji towns in the Southwest and West Shewa zones, Oromia, Ethiopia.

Methods: A cross-sectional study was conducted from October 2019 to September 2020. A total of 368 canines were sampled for the study using a basic random sampling procedure. An Indirect ELISA kit was used to test the presence of rK39 antigen eliciting specific antibodies in a serum sample. The association between *L. infantum* seropositivity and possible risk factors was assessed using Pearson's Chi-square and logistic regression tests.

Results: The overall seroprevalence of *L. infantum* in dogs was found to be 84.24% (95% confidence interval [CI]: 80%–88%) with seroprevalences ranging from 76.11% (95% CI: 67%–84%) in Ambo to 79.82% (95% CI: 71%–87%) in Weliso, and higher seroprevalence, in Ejaji 93.84% (95% CI: 89%–97%). In a univariable logistic regression analysis, town (*p* = 0.001), season (*p* = 0.001), mixed living (indoor/outdoor) environment (*p* = 0.003), and kebele (*p* ≤ 0.05) were all found to be significantly linked with *L. infantum* seropositivity. In the multivariable analysis, wet season (*p* = 0.001) and mixed living environments (*p* = 0.025) were found to be independent predictors of *L. infantum* seropositivity.

Conclusions: This is the first comprehensive report of *L. infantum* infection in Ethiopian dogs. The very high seroprevalence suggests that dogs may play an important role in...
1 | INTRODUCTION

Canine visceral leishmaniasis (CVL) is a major vector-borne zoonotic disease mainly caused by Leishmania infantum infection but other species can also be found (Ashford, 2000). Leishmania infantum is an obligate intramacrophage protozoan of the genus Leishmania in the family Trypanosomatidae (Azevedo et al., 2012). The parasite is of great public health and veterinary significance. Leishmaniasis is a neglected tropical disease that is transmitted by the bite of Phlebotomine female sandflies of the genera Phlebotomus and Lutzomyia in the Old and New World, respectively. During a sandfly bite, flagellated promastigotes are delivered to mammalian hosts, where non-flagellated amastigotes proliferate within phagocytic mononuclear cells, completing the Leishmania parasite’s digenetic life cycle. These cells are taken up by the phlebotomine sandfly during feeding for the development into flagellated promastigotes to complete the life cycle (Handman, 2001). Apart from the general species-specific organ tropism, intra-species intrinsic characteristics are also a relevant factor to consider. Dermotropic and viscera-tropic L. infantum strains modulate the sand fly biting time on the host, leading to the delivery of a high or low dose of metacyclic promastigotes into the skin, which will impact the parasite tropism and manifestation of the disease (Maia et al., 2011). Even strains belonging to the same zymodeme were associated with differential infectivity (Baptista Fernandes et al., 2007). 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 (Alemany et al., 2017; Gramiccia & Gradoni, 2005).

In Ethiopia, the following Leishmania species are found in humans and relative vectors have been detected: L. aethiopica (Phlebotomus longipes, Phlebotomus pedifer, Phlebotomus sergenti), L. tropica (Phlebotomus sergenti, Phlebotomus aequalis), L. major (Phlebotomus duboscqi), and L. donovani (Phlebotomus martini, Phlebotomus celiae, Phlebotomus orientalis). Cutaneous leishmaniasis (CL) caused by L. aethiopica is widely distributed throughout the highland areas, whereas visceral leishmaniasis (VL) caused by L. donovani species complex is found in lowland to midland areas (FDRE, 2013). Although the transmission of L. donovani was generally considered anthropogenic, recent shreds of evidence show the detection of the parasite and its vector in domestic dogs in Ethiopia (Bashaye et al., 2009; Bejano et al., 2021; Bsrat et al., 2018).

Leishmaniasis is a parasitic disease that affects a variety of mammals, including humans. Dogs are the most important species in the epidemiology of the disease among domestic animals. Because dogs are the most common domestic reservoir for zoonotic visceral leishmaniasis (ZVL), they play an important role in the ecology and control of the disease (Lima et al., 2018). CVL is an important disease that occurs in all continents except Oceania (Dantas-Torres et al., 2012). Dogs often show greater than 10% seropositivity before the onset of VL in humans. As a result, dog ownership is a significant risk factor for VL in several endemic countries (Bsrat et al., 2018).

Leishmaniasis is prevalent in 98 countries, and 350 million people are at risk of developing the disease. Leishmaniasis are a group of diseases that appear in a variety of ways. VL, CL, and mucocutaneous leishmaniasis (MCL) are the three types of leishmaniasis. VL in humans is the most lethal and common of the three types in eastern Africa, followed by CL and MCL (Desjeux, 2004).

It is estimated that each year, there will be about 500,000 cases of VL in humans (mostly in South America, East Africa, and the Indian Subcontinent) and 1–1.5 million cases of CL or MCL (primarily in Brazil, Peru, and the Plurinational State of Bolivia). Bangladesh, Brazil, Ethiopia, India, Nepal, and Sudan account for more than 90% of all VL cases worldwide. After the Indian Subcontinent, Eastern Africa has the second-highest number of VL cases. The disease is endemic in Eritrea, Ethiopia, Kenya, Somalia, North Sudan, Southern Sudan, and Uganda (Chappuis et al., 2007; Desjeux, 2004). CL is more widely distributed, with about one third of cases occurring in each of three epidemiological regions, the Americas, the Mediterranean basin, and western Asia from the Middle East to Central Asia. The 10 countries with the highest estimated case counts, Afghanistan, Algeria, Colombia, Brazil, Iran, Syria, Ethiopia, North Sudan, Costa Rica, and Peru, together account for 70%–75% of global estimated CL incidence (Alvar et al., 2012).

Ethiopia is one of the 10 nations with the highest estimated case numbers, which together account for 70%–75% of global estimated VL incidence. Only about 600,000 instances out of an estimated 2 million are recorded (Alvar et al., 2012). In Ethiopia, the epidemiology of VL has changed, with endemic areas spreading continuously (Kebede et al., 2017), possibly due to the current trend of climate change, rapid urbanisation, and massive population movements affecting the range and population density of the insect vectors and reservoir hosts, resulting in a cumulative increase in the rate of human infection (Bashaye et al., 2009).

Other than the previous report of seroprevalence of L. infantum to the genus-specific antigen of the parasite (Gebremedhin et al., 2021), the status of L. infantum-specific seroprevalence in Ethiopian dogs is generally unknown. Therefore, the objectives of the current study were to estimate the seroprevalence and associated risk factors of maintaining and spreading the infection to humans. Improvement of the living environment and health care facilities for dogs and humans as well health education for people awareness is suggested.

KEYWORDS
dogs, Ethiopia, Indirect ELISA, risk factors, seroprevalence, visceral leishmaniasis
Leishmania infection in the towns of Weliso, Ambo, and Ejaji in the Southwest and West Shewa zones, Oromia, Ethiopia.

2 MATERIALS AND METHODS

2.1 Description of the study areas

The research was conducted in Oromia, Ethiopia, in the towns of Weliso in the Southwest Shewa zone, and Ambo and Ejaji in the West Shewa zone (Figure 1). Weliso is one of the 14 districts that make up the Southwest Shewa zone. Weliso town is the administrative centre of the Weliso district. It is located 114 kilometres southwest of Addis Ababa, with a longitude of 37°57'59.99" E and a latitude of 8°31'59.99" N, with an elevation of 2063 m above sea level (m.a.s.l.). The annual rainfall and temperature are 950–2718.3 mm and 13.6–25°C, respectively.

Ambo town is the administrative centre of the Ambo district of the West Shewa zone, located 115 kilometres West of Addis Ababa at the latitude of 8°59'N, the longitude of 37°51'E, and elevation of 2101 m.a.s.l. The yearly rainfall and temperature range from 800 to 1000 mm and 15 to 29°C, respectively. The average temperature is 18.6°C. Ejaji town, on the other hand, is the administrative centre of the Elu Gelan district, which is 90 kilometres West of the zonal headquarters, Ambo, and 215 kilometres West of Addis Ababa. Ejaji town is located at a latitude of 8°59.9’N and a longitude of 37°9.8’E, with an altitude of 1565–1790 m.a.s.l. The average annual rainfall and temperature are 2000–2300 mm and 27–30°C, respectively. The research locations’ climatic characteristics range from extremely hot (lowland) through temperate (middle) to cold temperatures (highland).

2.2 The study population and study animals

Dogs from the research towns make up the study population consisted of the local, cross, and exotic breeds, but native dog breeds predominated in the studied locations. In most regions of the country, dogs are kept as house guards; but, in rural areas, they are also utilised for hunting and for defending livestock from robbers in the dark. Healthy dogs over the age of 6 months, both sexes, and various breeds were involved in this study. The current study towns have a combined population of 7098 owned dogs. The town of Ejaji has the highest number of dogs (2816), followed by Ambo (2180) and Weliso (2102). Juvenile, adolescent, adult, and geriatric dogs were classified as 6 months, 7 months to 1.5 years, 1.6–7 years, and over 7 years, respectively (Kiflu et al., 2017).

2.3 Study design and sampling methods

From October 2019 to September 2020, a cross-sectional study was undertaken. Dogs were brought to certain locations in the research sites to gather samples. A simple random sampling procedure was used to pick the research animals. This entails selecting "n" members at random from a sample frame list of "N" individuals. At the end of the sample collecting process, dog owners were advised to properly handle their canines.

2.4 Inclusion and exclusion criteria

The dog owners’ willingness to participate in the study was a criterion for inclusion. The study included dogs over 6 months of age who were accessible and whose owners were willing to be sampled, whereas dogs who are much debilitated, sick, and under treatment were omitted (Figure 2). Dogs with a history of being overly aggressive were also excluded from the study.

2.5 Sample size determination

The required sample size for the study was estimated using Thrusfield (2005) formula, which assumed a 50% expected seroprevalence (Pexp) of CLI in the study areas (as no previous study had been conducted in the present study areas) and a 5% level of precision (d). The sample size (n) is calculated using the following formula: 

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

where n is the sample size required, 1.96 is the value of Z at a 95% confidence interval (CI), Pexp is the expected seroprevalence, and d is the desired absolute precision.

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

Even though the sample size was projected to be 384 dogs, only 368 dogs were tested due to a kit shortage (i.e., 146, 113, and 109 dogs from Ejaji, Ambo, and Weliso towns, respectively). Almost 50% of sera (188) were collected during the wet seasons (March 2020 to September 2020).

2.6 Sampling procedure and techniques

During sampling, personal protective equipment, 70% isopropyl alcohol, and a tourniquet were used. In order to collect 5 ml of blood from each dog, cephalic or lateral saphenous veins were cannulated using vacutainer tubes and needles, and the dogs were adequately restrained or fastened. After coagulation of blood samples, serum was obtained by centrifugation for 5–10 min at 3000 revolutions per minute (rpm). The serum samples were kept at ~20°C until serology testing.

2.7 Laboratory testing using I-ELISA

All sera samples were tested for the presence of rK39 antibodies against L. infantum following the manufacturer’s instructions for the Indirect ELISA kit (IDvet Innovative Diagnostics, ID Screen®, Leishmania Indirect, France). However, since L. infantum is within the L.
donovani complex and recombinant k39 protein which is conserved in the *L. donovani* complex, cross-reaction between *L. infantum* and *L. donovani* is likely. The S/P% was computed as follows: $S/P\% = \frac{\text{OD}_{450\text{ value of the sample} - \text{OD}_{450\text{ value of the negative control}}}}{\text{mean OD}_{450\text{ value of the positive control} - \text{OD}_{450\text{ value of the negative control}}}} \times 100$. This was done in order to interpret the results. The kit was validated for testing canine sera (Pourquier et al., 2007). Any sample with $S/P\% \leq 40\%$, $\geq 50\%$, and $40\%–50\%$ was regarded as negative, positive, and doubtful, respectively. The suspicious sera were tested again. The kit has a 99.1% specificity and 98.5%
sensitivity with this cut-off (Mahshid et al., 2014; Solano-Gallego et al., 2014).

The test was deemed to be valid if both the mean value of the Positive Control O.D. (ODPC) is greater than 0.350 (ODPC > 0.350) and the ratio of the mean values of the Positive and Negative Controls (ODPC and ODNC) is greater than 3 (ODPC/ODNC > 3).

2.8 Data management and analysis

The raw field data from the sampled canines, as well as the results of the laboratory evaluation, were recorded into a database using Microsoft Excel 2019 Spreadsheet, which was then refined and coded. The STATA 14 software tool was used to analyse the obtained data (Stata Corp. College Station, USA). To summarise the data, descriptive statistics were employed. The association between hypothesised potential risk factors like town, season, kebele, sex, age, breed, living environment, and the presence of other domesticated animals in the households with seropositivity was evaluated first using Pearson’s chi-square ($\chi^2$) followed by logistic regression (univariable and multivariable) tests.

Furthermore, the risk factors collinearity (confounding) was investigated, and only one independent variable with a substantial biological connection with the disease outcome was chosen and employed in the final logistic regression model. After verifying for multicollinearity, variables having $p \leq 0.25$ in the univariable logistic regression analysis were further investigated using multivariable logistic regression. The significance level was set at $p \leq 0.05$ in all situations.

3 RESULTS

3.1 Frequency distribution analysis of potential risk factors

Of 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. In this study, the frequencies of categories of independent variables (town, season, kebele, sex, age, breed, living environment, and presence of other animals) considered as risk factors to acquire L. infection are summarised below (Table 1).

3.2 Seroprevalence

The current study estimated an overall seroprevalence of 84.24% (95% CI: 80%–88%). In Weliso, Ambo, and Ejaji towns, 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 Weliso (79.82%) and Ambo (76.11%) (Table 2).
### TABLE 1
Results of the frequency distribution of the potential risk factors of CVL in Ambo, Ejaji, and Weliso towns

| Variables          | Category     | Frequency | %   |
|--------------------|--------------|-----------|-----|
| Town               | Weliso       | 109       | 29.62 |
|                    | Ambo         | 113       | 30.71 |
|                    | Ejaji         | 146       | 39.67 |
| Season             | 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  |
| Sex                | 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 |
| 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 |
| Leishmania status  | Negative     | 58        | 15.76 |
|                    | Positive     | 310       | 84.24 |

### 3.3 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 the collinear independent variables, the season was selected for the final multivariable model. Furthermore, those independent variables with a univariable $p \leq 0.25$, such as season, breed, and living environment, were selected for the multivariable logistic regression analysis. Seropositivity was significantly associated with a wet season ($p = 0.001$) and a mixed living (indoor/outdoor) environment ($p = 0.025$). The likelihood of *L. infantum* seropositivity in dogs was $2.91$ times higher in the wet than in the dry season. The likelihood of 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).

### 4 DISCUSSION

#### 4.1 Seroprevalence

This is the first evidence on the seroprevalence and risk factors of *L. infantum* infection in dogs of West and Southwest Sheewa zones of Oromia in particular and Ethiopia in general. The current investigation found a high seroprevalence of *L. infantum* infection (310/368, 84.24%). The study provided baseline data on seroprevalence and risk factors of CVL infection and suggests the need for similar studies in other regions of the country to have a comprehensive status of the infection in animals and humans as well. This is essential since VL (Bashaye et al., 2009; Bejano et al., 2021; Bsrat et al., 2018; Kebede, 2017; Kenubih et al., 2015) and CL (WHO, 2014a; Yohannes et al., 2019) are wide spread public health problems predominantly in the lowland and highland parts of Ethiopia, respectively.

The highest seroprevalence was found in Ejaji town (137/146, 93.84%), followed by Weliso (87/109, 79.82%) and Ambo (86/109, 76.11%). Canine leishmaniasis seroprevalence of 13.9% in Benishangul Gumuz Regional State, Ethiopia (Bejano et al., 2021), 40% in dogs in North West Ethiopia (Kenubih et al., 2015), 6.9% in domesticated dogs in Eastern Sudan (Hassan et al., 2009), 11.7% in stray dogs, 9.7% in National Guard dogs, and 5.9% in farm dogs in Algeria (Adel et al., 2016) were significantly lower than the current study findings.

Much lower seroprevalence has also been reported previously, including 6.5% in an endemic focus of the Satluj river valley in Himachal Pradesh (India) (Sharma et al., 2009), 35% VL among dogs in endemic areas of Mymensingh district, Bangladesh (Islam et al., 2017), 26% *L. infantum* infection in dogs in Spain (Solano-Gallego et al., 2001), 6.31% in Portugal (Cortes et al., 2012), 4.9%–6.6% in a public kennel in Bologna, Italy (Balldari et al., 2011), 5.2% (L’Aquila, Abruzzo region) to 21.8% (Campobasso, Molise region) in Italy (De-Massis et al., 2020), 22% in Brazil (Leal et al., 2018), 22% in Colombia (Zambrano-Hernandez et al., 2015), and 12% in the central region of Colombia (Picón et al., 2020).

The difference in seroprevalence between the current study and the aforementioned publications could be potentially related to differences in geographical location, that is the differing seroprevalence of *L. infantum* in dogs in different geographical regions with varying climates. In endemic locations, the seroprevalence of CVL and human visceral leishmaniasis is linked to climatic conditions and humidity (Mohebali et al., 2011). It could also be attributed to differences in environmental appropriateness, sandfly activities, and preventive and control efforts for the most common reservoir host and vectors of leishmaniasis in different countries.

#### 4.2 Risk factors

Wet season ($p = 0.001$) and indoor/outdoor mixed living environment ($p = 0.025$) were found to be significantly associated with seropositivity in the current study, possibly due to the different activities of...
TABLE 2
Logistic regression analysis results of the association between canine *Leishmania infantum* seropositivity and potential risk factors

| Variable   | Category     | No. tested | No. Pos. (%) | Univariable COR (95% CI) | p-value | Multivariable AOR (95% CI) | p-value |
|------------|--------------|------------|--------------|--------------------------|---------|---------------------------|---------|
|            |              |            |              | Univariable              |         | Multivariable             |         |
|            |              |            |              | p-value                  |         | p-value                   |         |
|            |              |            |              | AOR (95% CI)             |         |                           |         |
| 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   | –                         | –       |
|            | Yahí 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   | –                         | –       |
| 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   | –                         | –       |

Abbreviations: AOR, adjusted odd ratio; CI, confidence interval; COR, crude odd ratio; Juvenile and ado., juvenile and adolescent; No., Number; Pos., Positive.

*Statistically significant (p ≤ 0.05).

sandflies in these regions, which are favoured by suitable environmental conditions for breeding and more exposure of dogs to sand fly bites. The reduced infection rate in home dogs may be attributable to a lower risk of sand fly bites (Moshfe et al., 2009).

A high percentage of infected dogs have outdoor access, suggesting that dogs with outdoor access could be a potential source of human infection, since free roaming is a key feature in establishing infection (Kumthekar et al., 2014).

When compared to the dry season (adjusted odd ratio [AOR] = 2.91) and indoor living environment (AOR = 2.19), the likelihood of seropositivity in dogs was considerably greater in the wet season (AOR = 2.91) and mixed living environment (indoor/outdoor) (AOR = 2.19).

The current findings were in close agreement with studies conducted in Himachal Pradesh, India, where domestic and stray dogs were found to harbour the *L. infantum* and serve as a constant reservoir for the infection (Sharma et al., 2009). Our findings also agree with the report from Mymensing district, Bangladesh, where the dogs’ living status (street or owned) was a potential risk factor (Islam et al., 2017).

In other studies conducted in Algeria, North Africa, the infection was significantly more prevalent in older dogs than younger ones, in stray dogs than owned dogs, and rural dogs than urban ones (Adel et al., 2016).

In Brazil, the re-emergence of the disease appears to be a consequence of the discontinuation of control programs (Leal et al., 2018), and in the central region of Colombia, owned dogs were strongly associated with canine leishmaniasis (Picón et al., 2020). However, the prevalence of canine leishmaniasis varies by geographic area and depends also on the serological assays employed to diagnose the disease (Adel et al., 2016; Picón et al., 2020).

Dogs that have outdoor access can act as the primary reservoir host for the *Leishmania* infection and potentially serve as a sentinel of human VL which can be lethal. The high seroprevalence of *L. infantum* infection in the current study might potentially have significant effects for infection transmission to humans through insect vectors, with adverse consequences. Even though most seropositive dogs in our study were healthy, the infectivity of dogs presenting *Leishmania*...
infection is not exclusively linked to the symptomatic stage of the disease since asymptomatic but seropositive dogs can transmit the parasite to the competent sand fly vectors (Molina et al. 1994). It takes a long time to treat cases of VL, and total parasite eradication is not always attainable. In some conditions, relapses are also frequent (Aiello & Moses, 2016).

Poor sanitation and degraded areas around houses, the lack of local awareness of the vector, and greater exposure to sand flies, especially in the early evening, favour the spread of *L. infantum* infection in urban settings (Moreno et al., 2005). Leishmaniasis is frequently associated with poverty, along with illiteracy, a weakened immune system, malnutrition, gender inequality, a lack of resources, and inadequate housing (WHO, 2014b). The diagnosis and treatment of the disease puts a huge financial strain. Even though there is a huge socioeconomic impact of the disease (Okwor & Uzonna, 2016), data on the epidemiology and economic impact of leishmaniasis in Ethiopia are inadequate.

### 4.3 Limitations of the research

Because diagnostic kits were limited, the test was only performed on 368 dogs, and interpretation of the results for all canine populations in the research areas should be done with caution. The current study only included dogs with owners in the cities (stray and rural dogs were not included). This makes extrapolating the findings of the current study to the total dog population in the area challenging. Sand fly data were not collected to further support the findings. Canine *Leishmania* infection is mostly unstudied and disregarded in Ethiopia, and there is a scarcity of information in general. As a result, it was difficult to compare the findings of this study to official data produced locally; hence, the findings were compared to reports from around the world.

### 5 CONCLUSIONS

The study showed a high seroprevalence of *L. infantum* infection in dogs in some regions of Ethiopia for the first time. The high seropositivity in the studied urban towns represents a specific cause for concern. *Leishmania* seropositivity was predicted by the wet season and dogs’ mixed living environment. The large proportion of asymptomatic seropositive dogs suggests that they may play a role in maintaining and spreading of *Leishmania* infection to people and animals. As a result, it is recommended that the living environment and health care facilities for dogs be improved, as well as community awareness, health education, and further research on the epidemiology, economic impact, and vaccine development are suggested.

### AUTHOR CONTRIBUTIONS

Weraka Weya participated in proposal preparation, collection of data, analysis of data, and manuscript drafting. Endrias Zewdu Gebremedhin conceived the research idea and participated in proposal preparation, supervising, data analysis and interpretation, manuscript reviewing, and commenting. Chala Dima and Demeke Zewde did an Indirect ELISA serological test and reviewed the manuscript. Vincenzo Di Marco Lo Presti and Maria Vitale participated in the design, drafting, and critical review of the manuscript. All authors read and approved the final manuscript.

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### CONFLICT OF INTEREST

The authors declare no conflict of interest.

### FUNDING INFORMATION

The authors received no specific funding for this work.

### DATA AVAILABILITY STATEMENT

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

### ETHICS STATEMENT

All study participants were informed about the study objective as per the recommendation of Ambo University’s research ethical guideline. The questionnaire was administered to the head or members of households after getting verbal consent to participate because some of the participants do not read and write. The research project was reviewed and 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 minimise animal sufferings during sampling. All participants’ full cooperation and voluntary participation (verbal consent) were acquired by assuring them of the anonymity of their involvement.

### PEER REVIEW

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