Prevalence and Risk Factors of *Toxoplasma Gondii* and *Leishmania* Spp. Infections in Apparently Healthy Dogs in West Shewa Zone, Oromia, Ethiopia

Endrias Zewdu Gebremedhin (✉ endrias.zewdu@gmail.com)

Ambo University

Edilu Jorga Serba

Ambo University

Getachew Kebebew Tola

Ambo University

Solomon Shiferaw Endalew

Ambo University

Lencho Megersa Marami

Ambo University

Asaminew Tesfaye Melkamsew

National Animal Health Diagnostic and Investigation Center

Di Marco Vincenzo

Istituto Zooprofilattico Sperimentale of Sicily, Laboratory of Genetics of Microorganisms, Via Gino Marinuzzi 3, 90129 Palermo, Italy

Maria Vitale

Istituto Zooprofilattico Sperimentale of Sicily, Laboratory of Genetics of Microorganisms, Via Gino Marinuzzi 3, 90129 Palermo, Italy

Research Article

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Abstract

Background

In urban settings, the presence of a high density of human population and contacts with domestic and or stray animals such as dogs and cats can be risk factors for the transmission of zoonotic diseases.

Toxoplasma gondii (*T. gondii*) and *Leishmania* spp. are zoonotic protozoon parasites with great health burdens worldwide.

Methods

A cross-sectional study was used to investigate the antibody prevalence and risk factors of *T. gondii* and *Leishmania* spp. infections in 385 randomly selected dogs of Ambo, Bako, and Gojo towns of West Shewa zone, Oromia regional state, Ethiopia. A questionnaire survey was administered to households to collect data on potential risk factors. Dog sera samples were assayed for *T. gondii* IgG antibodies by using the direct agglutination test while *Leishmania* spp. specific antibodies were tested using an indirect enzyme-linked immunosorbent assay (ELISA) kit. Logistic regression was used for data analysis.

Results

An overall 82.86% (95% CI: 78.71–86.49%) and 92.47% (95% CI: 89.36–94.90%) seroprevalence of *T. gondii* and *Leishmania* spp. was found respectively. Seropositivity for both *T. gondii* and *Leishmania* spp. was found in 82.58% of the dogs. None of the investigated factors were associated with *Leishmania* spp. seropositivity ($p > 0.05$). Furthermore, altitude, sex, breed, housing, feeding, educational level of head of the household, and the living area of dogs were not significantly associated with *T. gondii* seropositivity ($p > 0.05$). The seroprevalence of *T. gondii* was significantly different between the study towns ($p = 0.003$). The risk of *T. gondii* infection was 2.71 times higher in adult dogs than juvenile dogs ($p = 0.043$). Dogs kept simultaneously with other domestic animals had increased odds of *T. gondii* seropositivity as compared to those with no other domestic animals (Adjusted Odds ratio: 1.96, $p = 0.021$).

Conclusions

The high seropositivity and the concomitant presence of antibodies of *T. gondii* and *Leishmania* spp. in dogs suggest the widespread nature of these parasites in the environment and the high potential of transmission to other animals and humans. Further epidemiological studies, isolation and molecular characterization of the parasites, and educational campaigns are suggested.

Introduction

Toxoplasmosis and leishmaniosis are important zoonotic diseases both caused by unicellular parasites. Toxoplasmosis is spread worldwide since *T. gondii* can infect almost all warm-blooded animals and humans and can be transmitted through different routes. Leishmaniasis is a vector-borne infection caused by different *Leishmania* spp transmitted by many sandflies vectors in different geographic areas. The dog population in Ethiopia is unknown and data on dog-related zoonotic diseases is largely scarce. However, rabies, hydatidosis, and toxocariasis are among the broad variety of zoonotic diseases that are transmitted to humans by dogs. Rabies is an endemic priority zoonotic disease to control in Ethiopia where the annual incidence of rabies of 2,33 cases per 100,000 in humans, 412,83 cases per 100,000 in dogs, 19,89 cases per 100,000 in pigs, 67,68 cases per 100,000 in equines, and 14,45 cases per 100,000 in goats has been reported [1]. Hydatid cysts were reported more frequently in women of 40 years of age (4.7%) in Hamar of Ethiopia [2], while an average annual incidence rate of approximately 2.3 cases per 100 000 per year was reported from Bahir Dar, Ethiopia [3].

Dog (*Canis familiaris*) holding in big cities and towns in Ethiopia has increased significantly in recent years along with increased urbanization. Dogs are mainly kept to protect household properties and protection. However, the attitude of keeping dogs as companion animals is also growing with the presently rising trend of urbanization and customizing western culture. In Addis Abeba, some people generate income by breeding and selling exotic dog breeds.

T. gondii is one of the most common parasites on earth, infecting as much as one-third of the world's human population [4]. The health burden of toxoplasmosis has been ranked among the highest of all parasitic diseases [5]. *Toxoplasma gondii* infection in humans occurs by ingestion of tissue cysts from undercooked meat from intermediate hosts (sheep, goat, and pig), by intake of water containing oocysts excreted from the final host (cats), or by the congenital transmission of tachyzoites. However, only a small percentage of infected adults show clinical symptoms of the disease, but pregnant women's infection can be especially harmful to the fetus [4, 6].

Stray dogs and owned dogs with outdoor access, play important role in the epidemiology of *T. gondii* infection. This is due to the practice of feeding dogs on food types from various sources like garbage and food contaminated with soil. The dogs can act as a passive vector for the spread of *Toxoplasma* oocysts in the environment and thus serve as the parasite's environmental sentinel [6]. Like cats, dogs may also serve as a possible source of *T. gondii* infection in humans due to near contact [7]. Although dogs do not develop oocysts, human *T. gondii* oocysts exposures through dogs can occur in connection with the mechanical transport of oocysts from the feces of cats by rolling in foul-smelling substances [8]. Dogs can become infected by the ingestion of *T. gondii* oocysts from cat feces or by the feeding habit of uncooked mutton (carnivorism). Antibodies to *T. gondii* were found worldwide in canine sera, moreover, viable *T. gondii* were also segregated from dogs' muscles and brain tissues [4, 6].

In Ethiopia, a meta-analytical study of IgG seroprevalence for *T. gondii* found a high pooled prevalence in animals (87.72% in cats, 34.59% in small ruminants) and humans (74.73%) with a high risk of sheep and goat reproductive problems and multiple human diseases[9].

Toxoplasmosis in dogs is typically asymptomatic, and the clinical process in the respiratory and hepatic systems is often most noticeable when it occurs. Clinical cases of toxoplasmosis in cats are much more common than in dogs. A high proportion of clinical infections with *T. gondii* are caused by immunosuppressive chemotherapy [10]. However, neurological symptoms have also been identified [11, 12]. The clinical type may be due to the reactivation of latent infection associated with the immunosuppression caused by the canine distemper virus [13].

Leishmaniasis are sandflies (*Phlebotomus* spp.) transmitted diseases of great medical and veterinary importance. There are two major clinical forms of leishmaniasis, cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL, also known as Kala-azar) [14]. Leishmaniasis are neglected tropical and subtropical diseases endemic to 98 countries worldwide [15] including Ethiopia [16]. Visceral leishmaniasis affects about 12 million people worldwide. Around 0.5 million new cases of VL in humans are identified each year and 350 million people are at risk of infection [17]. Ethiopia, India, Bangladesh, Sudan, South Sudan, and Brazil are countries with a high prevalence of visceral leishmaniasis (90% of cases) [15].

In Ethiopia, there are several foci of *Leishmania* spp. infections with frequent outbreaks leading to over 7,000 and 50,000 new cases of visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL) per year, respectively [18]. This has contributed to their identification as a major public health concern. Leishmaniasis, however, remains one of the most-overlooked tropical diseases [19].

A large proportion of infected animals are asymptomatic in endemic areas, and their role is largely unknown in the transmission of leishmaniasis [20]. Generally, the prevalence of leishmaniasis in dogs is high. Dogs are urban domestic reservoirs for *Leishmania* spp. that play an important role in the epidemiology of leishmaniasis by spreading the parasites to humans through the sand flies [21]. In Ethiopia, dogs and hyraxes are the main reservoir hosts for visceral and cutaneous leishmaniasis, respectively [18]. However, poor knowledge of leishmaniasis in the canine population is available. As part of a study to investigate the VL outbreak in Libo Kemkem, Ethiopia, J Alvar, S Bashaye, D Argaw, I Cruz, P Aparicio, A Kassa, G Orfanos, F Parreño, O Babaniyi and N Gudeta [22] reported the genetic material of *Leishmania* in the venous blood of two of the 40 asymptomatic dogs sampled. In north-west Ethiopia, where foci of human VL are common, S Kalayou, H Tadelles, A Bsrat, N Abebe, M Haileselassie, and H Schallig [23] reported an overall seroprevalence of *L. donovani* infection of 27.7% and 14.8% in dogs, using direct agglutination test and Kal-a-azar detect rapid test, respectively.

A study on a meta-analysis of *T. gondii* in animals and humans in Ethiopia revealed that prevalence is generally high being 87.72% in cats, 34.59% in small ruminants, and 74.73% in humans [24]. However, no single published information is available about *T. gondii* infection in dogs in Ethiopia to date. Good knowledge of the prevalence of *T. gondii* and *Leishmania* spp in household dogs may aid in the design and implementation of pertinent disease management strategies and could therefore benefit both animal and human health. Therefore, the present study aims to estimate the seroprevalence and associated risk

factors of *T. gondii* and *Leishmania* spp infections in dogs in Ambo, Bako, and Gojo towns of West Shewa Zone, Oromia, Ethiopia.

Results

Seroprevalence

The overall seroprevalence of *T. gondii* infection in dogs of the studied towns was found to be 82.86% (319/385, 95% confidence interval [CI]: 78.71–86.49%), which is significantly different among the studied towns ($\chi^2 = 13.72$, $p = 0.003$).). Of 385 dogs' sera tested for anti-*Leishmania* spp. antibodies, 356 (92.47%, 95% CI: 89.36–94.90%) were seropositive. *Leishmania* spp seropositivity was not significantly different between study towns ($\chi^2 = 0.92$, $p = 0.632$). There was no statistically significant association between *Leishmania* spp seropositivity and independent variables evaluated in the study ($p > 0.05$) (Table 1).

Table 1

The overall seroprevalence of *T. gondii* and *Leishmania* spp. infection in dogs of the study towns

Town	No. tested	<i>T. gondii</i> *		<i>Leishmania</i> spp.	
		No. positive	% prevalence (95% CI)	No. positive	% prevalence (95% CI)
Ambo	169	127	75.15 (67.93–81.46)	157	92.90 (87.93–96.28)
Gojo	68	59	86.76 (76.36–93.77)	61	89.71 (79.93–95.76)
Bako	148	133	89.86 (83.83–94.22)	138	93.24 (87.93–96.71)
Overall	385	319	82.86 (78.71–86.49)	356	92.47 (89.36–94.90)
*Pearson χ^2 (3) = 13.72, $p = 0.003$, CI = Confidence interval					

There was no statistically significant association between *T. gondii* and *Leishmania* spp. seropositivity. The study revealed that 82.58% ($n = 294$) of the studied dogs were seropositive for both *T. gondii* and *Leishmania* spp (Fig. 1).

Age-wise, the highest seroprevalence of *T. gondii* infection was found in adult dogs (84.35%). The presence of cats and other domestic animals in the household were significantly associated with *T. gondii* seroprevalence (Table 2).

Table 2
Results of logistic regression analysis of *T. gondii* prevalence and potential risk factors in selected districts of West Shewa zone, Ethiopia

Variable	Categories	No. tested	No. pos. (% prevalence)	Univariable		Multivariable	
				OR (95% CI)	P	OR (95% CI)	P
Town	Ambo	169	127 (75.15)	1.0		1.0	
	Gojo	68	59 (86.76)	2.17 (0.99–4.75)	0.053		
	Bako	148	133 (89.86)	2.93 (1.55–5.55)	0.001		
Altitude	Highland ($\geq$ 2100 masl)	237	186 (78.48)	1.0		1.0	
	Midland (1600–2100 masl)	148	133(89.86)	2.43 (1.31–4.51)	0.005	2.36 (1.23–4.50)	0.009
Age	Juvenile	27	19 (70.37)	1.0		1.0	
	Adolescent	77	63 (81.82)	1.89 (0.69–5.20)	0.214	2.42 (0.83–7.03)	0.105
	Geriatrics	51	43 (84.31)	2.26 (0.74–6.93)	0.153	2.77 (0.85–8.97)	0.090
	Adult	230	194 (84.35)	2.27 (0.92–5.59)	0.074	2.85 (1.09–7.43)	0.032
Sex	Male	293	239 (81.57)	1.0		1.0	
	Female	92	80 (86.96)	1.51 (0.77–2.96)	0.234	1.62 (0.79–3.32)	0.186
Breed	Exotic	15	11 (73.33)	1.0		1.0	
	Cross	74	61 (82.43)	1.71 (0.47–6.21)	0.417		
	Indigenous	296	247 (83.45)	1.83 (0.56–5.99)	0.316		
Feeding	Cooked	103	83 (80.58)	1.0	-		
	Raw animal	282	236 (83.69)	1.24	0.475		

Table 2
Continued...

Variable	Categories	No. tested	No. pos. (% prevalence)	Univariable		Multivariable	
				OR (95% CI)	P	OR (95% CI)	P
Housing	Indoor	119	93 (78.15)	1.0		1.0	
	Outdoor	106	87 (82.08)	1.28(0.66–2.48)	0.463	1.44 (0.71–2.90)	0.309
	Mixed	160	139 (86.88)	1.85 (0.98–3.48)	0.056	1.55(0.79–3.03)	0.203
PODAH	No	181	142 (78.45)	1.0		1.0	
	Yes	204	177 (86.76)	1.80 (1.05–3.08)	0.032	1.94(1.10–3.42)	0.022
Education of HHH	Secondary	125	100 (80.00)	1.0		1.0	
	Illiterate	47	38 (80.85)	1.06 (0.45–2.47)	0.901	1.02 (0.42–2.48)	0.957
	Tertiary	115	96 (83.48)	1.26 (0.65–2.44)	0.487	1.33 (0.65–2.70)	0.435
	Primary	98	85 (86.73)	1.63 (0.79–3.39)	0.187	1.50 (0.70–3.19)	0.298
Living area/residence	Urban	341	280 (82.11)	1.0			
	Peri-urban	44	39 (88.64)	1.70 (0.64–4.49)	0.285		
Presence of cats in the household	No	214	170 (79.44)	1.0		1.0	
	Yes	171	149 (87.13)	1.75 (1.00–3.06)	0.048	1.65 (0.92–2.95)	0.094

Table 2. Continued

Variable	Categories	No. tested	No. pos. (% prevalence)	Univariable		Multivariable	
				OR (95% CI)	P	OR (95% CI)	P
Family size of a dog-owning household	≤ 4	114	93 (81.58)	1.0			
	≥ 5	271	226 (83.39)	1.13 (0.64-2.01)	0.666		
HHH	Protestant	248	199 (80.24)	1.0			
	Orthodox	118	102 (86.44)	1.57 (0.85-2.90)	0.149		
	<i>Waqefeta</i>	8	7 (87.50)	1.72 (0.21-14.34)	0.614		
	Muslim	11	11 (100.00)	-	-		
Marital status of dog-owning HHH	Divorce	25	20 (80.00)	1.0			
	Married	343	283 (82.51)	1.18 (0.43-3.27)	0.751		
	Single	17	16 (94.12)	4.00 (0.42-37.78)	0.226		

PODAH=presence of other domestic animals in the household, HHH= head of the household, RHHH=religion of the head of the household

Full model= HLX2 =7.70, P-Value=0.4632, Se= 99.37, Sp=1.52, PPV=82.98, NPV, 33.33, ROC= 0.6993

Best fitting model= HLX2 =5.89, P-Value=0.6594, Se= 99.37, Sp=3.03, PPV=83.20, NPV, 50.0, ROC= 0.6741

Risk factors

Toxoplasma gondii infection

As indicated in Table 2 below, univariable logistic regression analysis showed that the likelihood of *T. gondii* seropositivity was 2.93 times higher in dogs of Bako town as compared to Ambo (p = 0.001). Similarly, the risk of *T. gondii* seropositivity in dogs was 1.8 times higher in households where other domestic animals are found (p = 0.032). The *T. gondii* seropositivity of dogs is significantly associated with the presence of cats in dog-owning households (OR = 175, 95% CI: 1.00–3.06, p = 0.048).

In the multivariable logistic regression analysis, the risk of *T. gondii* infection in adult dogs was 2.71 times higher as compared to Juvenile dogs ($p = 0.043$). The likelihood of getting seropositive dogs was 1.96 times high in households where other domestic animals are present than when they were absent ($p = 0.021$). Thus, the age of dogs and the presence of other domestic animals in the household were independent predictors of *T. gondii* seropositivity. On the other hand, altitude, sex, housing, and presence of cats in the household showed no significant association with *T. gondii* seropositivity in the final model ($p > 0.05$) (Table 2).

***Leishmania* spp. infection**

All independent variables investigated were non-collinear with each other except district vs altitude ($r = -0.87$). Based on the univariable logistic regression analysis, variables such as the way of life of dogs, community type, and presence of cats in the household had $p < 0.25$ and hence entered into the multivariable model. As a result, none of the risk factors investigated were independent predictors of *Leishmania* spp infection ($p > 0.05$) (Table 3).

Table 3

Results of logistic regression analysis of seroprevalence of *Leishmania* spp infection and potential risk factors in selected districts of West Shewa zone, Ethiopia

Variable	Categories	No. tested	No. pos. (% prevalence)	Univariable		Multivariable	
				OR (95% CI)	P	OR (95% CI)	P
Town/location	Gojo	68	61 (89.74)	1.0			
	Ambo	169	157 (92.90)	1.50 (0.56–3.99)	0.415		
	Bako	148	138 (93.24)	1.58 (0.58–4.36)	0.373		
Altitude	Highland	237	218 (92.0)	1.0			
	Midland	148	138 (93.2)	1.20 (0.54–2.66)	0.649		
Age	Adolescent	77	70 (90.91)	1.0			
	Geriatrics	51	47 (92.16)	1.18 (0.33–4.24)	0.805		
	Adult	230	213 (92.61)	1.25 (0.50–3.15)	0.631		
	Juvenile	27	26 (96.30)	2.6 (0.30–22.17)	0.382		
Sex	Female	92	85 (92.39)	1.0			
	Male	293	271 (92.49)	1.01 (0.42–2.46)	0.975		
Breed	Cross	74	68 (91.89)	1.0			
	Indigenous	296	274 (92.57)	1.10 (0.43–2.82)	0.844		
	Exotic	15	14 (93.33)	1.24 (0.14–11.08)	0.850		

Table 3
continued.....

Variable	Categories	No. tested	No. pos. (% prevalence)	Univariable		Multivariable	
				OR (95% CI)	P	OR (95% CI)	P
Housing	Outdoor	106	95 (89.62)	1.0		1.0	
	Indoor	119	110 (92.44)	1.42 (0.56–3.56)	0.461	1.19 (0.46–3.07)	0.719
	Mixed	160	151 (94.38)	1.94 (0.78–4.86)	0.156	1.74 (0.69–4.41)	0.243
PODAH	Yes	204	187 (91.67)	1.0		1.0	
	No	181	169 (93.37)	1.28 (0.59–2.76)	0.528		
Education of HHH	Secondary	125	113 (90.40)	1.0		1.0	
	Primary	98	90 (91.84)	1.19 (0.47–3.05)	0.710		
	Tertiary	115	108 (93.91)	1.64 (0.62–4.32)	0.318		
	Illiterate	47	45 (95.74)	2.39 (0.51–11.10)	0.266		
Living area/residence	Peri-urban	44	38 (96.36)	1.0		1.0	
	Urban	341	318 (93.26)	2.18 (0.84–5.70)	0.111	2.05 (0.77–5.47)	0.152
Presence of cats	No	214	194 (90.65)	1.0		1.0	
	Yes	171	162 (94.74)	1.86 (0.82–4.19)	0.137	1.72 (0.75–3.93)	0.197

Discussion

In this study, the seroprevalence and risk factors for *T. gondii* and *Leishmania* spp infections were carried out on 385 dogs to understand the epidemiology and control measures against the diseases in dogs as well as for public health interventions. The current finding revealed that the seroprevalence in apparently healthy dogs in the studied areas for these two important zoonotic protozoon parasites was very high.

The *T. gondii* seroprevalence (82.86%) in dogs of the current study corroborates well with the previous meta-analysis prevalence reports from Ethiopia in cats (87.72%) but higher than the reports in small ruminants (34.59%) [9]. The high seroprevalence of *T. gondii* infection in this study is an indication of the widespread contamination of the urban environment of the towns with the parasite. Previous studies in seropositive sheep and goats [28], backyard chicken [29], and pig [24] in central Ethiopia demonstrated the isolation of viable tissue cysts indicating that these animals might serve as a source of infection for dogs. Dogs are likely to acquire *T. gondii* infection through oral uptake of bradyzoite tissue cysts from infected preys such as birds, rodents, and the carcass of other dead animals or through ingestion of water contaminated with oocysts of free-roaming cats or tissue cysts from human leftover food available in the garbage [4, 30]. Moreover, the warm moist temperature and the high percentage of relative humidity in the towns might be favorable for the survival of the *T. gondii* oocysts [4].

Very high seroprevalence of 98% (50/51) from stray dogs in Giza, Egypt A El Behairy, S Choudhary, L Ferreira, O Kwok, M Hilali, C Su and J Dubey [6], and 67.3 % (68/101) from dogs of Veracruz, Mexico [31] have been reported using MAT [31], which is comparable with the current study (82.86%).

The seroprevalence of *T. gondii* infection in the present study was very high as compared to the 35.8% (42/118) seroprevalence reported from Brazil [32] and 50% (21/42) in rural Vietnam [33] using modified agglutination test (MAT). A lower level of *T. gondii* infection has been reported from the People's Republic of China (8.24%) [34] and Angola (15.5%) [35] using MAT. Similarly, a relatively lower seroprevalence of 25% in Nigeria [36], 26.9% in Brazil, [37], 32.0% in Trinidad and Tobago [38], and 7.9% in pet dogs in Taiwan [39].

The difference in seroprevalence among different studies might be related to the difference in climate, lifestyle, the behavior of dogs [35], sensitivity and specificity and a cut-off value of serological tests [40], type of antigen used (whole parasite vs purified/recombinant), geographical location, sample size, the diagnostic test used, and cat density [4]. Hence, a comparison of prevalence figures across different studies might be difficult.

Univariable logistic regression analysis showed that there was a significant difference in the seroprevalence of *T. gondii* infection concerning the three towns in that it was high in Bako ($p = 0.001$) as compared to Ambo town. This might indicate that climate considerably influences the risk of *T. gondii* exposure. The warm and moist environment coupled with the more abundance of cats and the source of infection for dogs (cats, the meat of infected domestic or wild animals containing tissue cysts) in Bako town might explain the high seroprevalence. It has been well documented that seroprevalence varies according to the density of cats, density of intermediate hosts [7], geographical location, and even within the same region from place to place [4].

The finding that adult dogs had a significantly higher *T. gondii* seroprevalence (84.35%) compared with dogs from the juvenile age group (70.37%) agrees with the previous report [41]. In this study, there was a considerable increase in seroprevalence as the age of dogs increase from juvenile (70.37%) to geriatrics (84.31%) stage and the odds of acquiring *T. gondii* infection in adult dogs is nearly 2.71 times higher as

compared to juvenile dogs ($p = 0.043$). As the age of dogs increases the likelihood of acquiring *T. gondii* infection from the environment increases i.e., postnatal/horizontal infection is the main route of infection [4, 35–37, 39, 42, 43]. Moreover, the lifelong persistence of IgG antibodies once infected might also add to the high prevalence in older dogs [4].

The significant association between *T. gondii* infection in dogs and the presence of cats in the dog-owning households confirms the previous observation that toxoplasmosis prevalence is high in areas where cats are present abundantly. If cat-owning households there will be an ample chance to contaminate animals' farmlands, feed, and water leading to infection of domestic animals including dogs [4].

Very high seroprevalence of *Leishmania* spp. infection was observed in the present study (92.47%) in contrast to I Rohousova, D Talmi-Frank, T Kostalova, N Polanska, T Lestinova, A Kassahun, D Yasur-Landau, C Maia, R King, and J Votypka [44] that reported relatively lower seropositivity of 55.9% (19/34) and PCR positivity of 5.9 % (2/34) in dogs of Northwestern Ethiopia. However in the Ethiopian region considered in the study (Oromia), no data are available on the competent vector populations present, so we cannot exclude that dogs might be the preferential hosts for the sand-flies of this area. A complex relationship between hosts, parasites, and sand fly vectors, makes quite intricate the transmission of *Leishmania* spp. as suggested also by the so-called paradox of Cyprus where a high seroprevalence for *L. infantum* in the dog population does not correspond to leishmaniasis cases and seroprevalence in humans; two transmission cycles seem to run in parallel in Cyprus: in dogs with *L. infantum* and humans with *L. donovani* [45]. The expansion of agricultural activities, increased urbanization, the abundance of reservoir hosts (e.g. hyraxes) and the biological vectors (sandflies) adaptation of the parasites and vectors might also contribute to the high seroprevalence [18, 23, 43]. Moreover, the weak health infrastructure and poor or absence of disease and vector control programs in dogs as well as humans of the current study areas, are additional contributing factors.

Although *Leishmania* infection of dogs ranging from 60 to 80% has been reported in endemic areas [46], the current seroprevalence was much higher and less related to the factors considered in this study compared to *Toxoplasma* since no statistically significant variations were detected among the three cities. This might suggest that infection transmission through a vector such as sand-flies for *Leishmania* might be related to environmental, structural, and human factors that are similar in the three cities considered in this study. Moreover, vector-borne diseases are influenced by environmental changes and socioeconomic factors such as sanitary conditions, malnutrition, population movement, or poor housing. A recent study in Nepal for human leishmaniasis in endemic districts found that houses with natural floors increased the risk of infection by eightfold, walls made from straw, leaves, and/or bamboos increased by threefold, walls with cracks, especially in the bedroom, increased by threefold and proximity to a livestock shed increased the risk by fourfold [47]. Anthropogenic factors tend to reorient the composition and behavior of sand fly vectors. To date, there are at least 50 different sand fly species transmitting leishmaniases [48].

In this study, contrary to our expectation, there was no significant difference in the seroprevalence of *T. gondii* and *Leishmania* spp. infections between indoor and outdoor kept dogs. In Ethiopia, exotic and crossbred dogs are mostly kept indoors while indigenous dogs live outdoors. However, the infection rate of both parasites was considerably high in both canine populations. For *T. gondii* infection this might be explained by the fact that both populations are fed with food waste and raw meat instead of that commercial or adequately cooked food. For *Leishmania* infection, the shelters for dogs are not built to avoid sandflies access and indoor conditions cannot assure the absence of the vectors. Due to the complex relationship between human, animal hosts, parasites, and sand fly vectors, the transmission of *Leishmania* spp. is intricate. Nevertheless, the absence of a statistically significant association between seroprevalence of *Leishmania* spp and potential risk factors considered in this study should prompt further studies in the future to identify the risk factors.

The high percentage of concurrent infection of dogs with *T. gondii* and *Leishmania* spp. (82.58%) as well as the absence of significant difference in the seroprevalence of the two parasites across altitudes, sex, breeds, housing and living areas/residence (urban vs peri-urban), might suggest the ubiquitous nature of the parasites and that these factors have a similar risk of infection. as reported by other researchers elsewhere [37, 39, 41, 46]. Besides, the lack of association of *T. gondii* seropositivity with breed and sex of dogs might have probably be overshadowed by the high exposure to the parasite at a very young age [31, 36]. In agreement with the present study, S Kalayou, H Tadelles, A Bsrat, N Abebe, M Haileselassie, and H Schallig [23] also reported the absence of a significant association between sex, housing, and place of residence and *L. donovani* seroprevalence in dogs of northwest Ethiopia.

The study identified widespread *T. gondii* and *Leishmania* spp. infections in the canine population along with the contributing risk factors for the transmission. Such information may serve in the efforts to minimize the risk of zoonosis in humans. The asymptotically infected dogs living together or very close to humans identified in the current study might serve in the maintenance of *Leishmania* spp. and *T. gondii* parasites to other animals and humans. Thus, because of the high seroprevalence and the poor or non-existent veterinary medical care for dogs, high HIV/AIDS prevalence, the overall inadequate personal hygiene, and environmental sanitation in the studied towns, these zoonotic parasites might be of great public health concern since asymptotically infected dogs might be the source of infection for humans [42].

The limitations of this cross-sectional survey include failure to collect data on clinical manifestations of dogs to relate it with seropositivity. Nevertheless, the findings for these zoonotic parasites indicate the magnitude of infections and that dogs might be an important reservoir posing likely health risks for animals and humans.

To the best of the knowledge of the authors, this is the first report of seroprevalence of *T. gondii* infection as well as co-infection of *T. gondii* and *Leishmania* spp from household dogs in Ethiopia.

Conclusions

The results showed very high infection rates and the concomitant presence of *T. gondii* and *Leishmania* spp. in dogs of the studied areas suggesting the widespread nature of the parasites in the urban environments and the big potential risk of transmission to humans and other animals. The age of dogs and the presence of other domestic animals in households, owning dogs are predictors of *T. gondii* seropositivity. None of the investigated variables were independent predictors of *Leishmania* spp. seropositivity. Further studies to isolate, identify the genotype and virulence of the parasites, preferably from clinical cases, as well as the contribution of dogs in the transmission of the infections to humans along with hygienic measures and educational campaigns, is imperative.

Materials And Methods

Study areas

The study was carried out in three district towns of West Shewa Zone of Oromia regional state, Ethiopia, namely Ambo, Bako, and Gojo towns.

Ambo town is the administrative center of Ambo district and West Shewa Zone located 114 Km West of Addis Ababa, the capital city of Ethiopia. The town is located at 8°59'N latitude, 37,051'E longitude, and at an altitude of 2100 meters above sea level (masl). The average annual temperature and rainfall are 22 °C and 900 mm respectively. The town has a total human population of 74, 843 out of which 39,192 are males and 35,651 are females.

Bako town, the administrative center of Bako Tibe district, is located 260 Km West of Addis Ababa. The town has a longitude and latitude of 9°08'N 37°03'E with an elevation of 1743 masl. The average annual temperature is 19.7°C while the rainfall is 1281 mm.

According to the Central Statistical Agency, in 2005 Bako town has an estimated total human population of 18,641 of whom 9,370 are men and 9,271 women.

Gojo town is the administrative center of Jeldu district located 120 Km West of Addis Ababa. The town is found at an altitude of 2550 masl, latitude and longitude of 65° 27' to 38°49' and 31° 38' to 22° 19'E, respectively. The mean annual temperature for the town is 20°C; it receives an annual rainfall of 2500 mm. The human population of Jeldu district is 202,655 of which 102,796 are females and the remaining 99,859 males [25].

The three towns have bimodal rainfall characterized by a small rainy season from February to May and a big rainy season from July to September. The dry season extends from October to January [26].

Study population

A total of 385 dogs were analyzed for the two parasitic infections: 169 from Ambo 68 from Gojo and 148 from Bako.

Animals and samples

Domestic /owned/ dogs (*Canis familiaris*) from each randomly selected "Gotes" (Gote is a subdivision of *Kebele* containing 20–30 households) were sampled house to house. "Kebele" refers to the smallest administrative unit of a town). The dogs in the study area are mainly used for guarding and companionship. Dogs are fed with whatever food is available (household leftover, animal products). The veterinary service provided to the dogs is quite inadequate and consequently, the vast majority of the studied dogs received no rabies vaccination and /or other treatments. Dogs above three months of age were sampled to avoid transcolostral antibodies [4]. The age of dogs ranged from 3 to 168 months, with an average value of 33 months.

Study design

A cross-sectional household survey was undertaken in Ambo, Bako, and Gojo towns of West Shewa Zone, Oromia Regional State, from January 2015 to June 2017.

Sample size and sampling technique

Since there is no previous *T. gondii* seroprevalence study in Ethiopia, 50% expected prevalence, 5% desired absolute precision, and 95% confidence interval were used to calculate the required sample size using the formula: $N = 1.96^2 p_{\text{exp}} (1-p_{\text{exp}}) / d^2$ [27], where n = required sample size, p = expected prevalence d = desired absolute precision. Therefore, the calculated sample size was 384. There was no accessible data on the dog population in the three towns. Thus, it was assumed that the population of dogs in the towns is evenly distributed multi-stage sampling procedure was employed to select households for this study. There are three, two, and one *Kebele* in Ambo, Bako, and Gojo towns respectively. From each "Kebeles," four "Gotes" were randomly selected using the list of *Gotes* in each *Kebeles* (sampling frame) provided by local administrators. The index household in a *Gote* was randomly selected and subsequent households were surveyed door to door. From the sample size considered for the study ($n = 385$), 169 samples were collected from Ambo town while 148 and 68 samples were collected from Bako and Gojo towns respectively.

Blood sample collection

Five milliliters of whole blood was aseptically collected from the cephalic vein of each dog using a plain vacutainer tube. The blood samples were kept at room temperature and allowed to clot in a slanted position in a cool place and serum was separated by centrifugation at 3000 RPM for 10 minutes, transferred into cryovials, labeled, and stored at -20°C until laboratory assay was carried out.

Questionnaire survey

A pre-tested structured questionnaire was prepared and administered to dog owners during blood sample collection. The close-ended questions asked include sex (male, female), breed (exotic, cross, indigenous), housing system (indoor, outdoor, mixed), feeding (cooked animal products, household leftover, raw animal products), presence of other domestic animals in the household (cattle, sheep, goats, horse, mule,

donkey, cats, chicken), educational level of dog owner (illiterate, primary, secondary, tertiary), presence of cat/s in the household (yes, no), living area/residence (urban, peri-urban), marital status (single, married, divorced), the religion of the head of the household (Protestant, Orthodox, *Waqefeta*, Muslim), and family size of the dog-owning household (≤ 4 , ≤ 5), The age of dogs was categorized as a juvenile (6 weeks to 6 months), young (6 months to 18 months), adult (18 months to 7 years), and geriatric (greater than 7 years) based on owners information.

Laboratory test

Sera samples were transported to the National Animal Health and Diagnostic Center (NAHDIC) in ice packs and stored at -20 °C until assayed. *T. gondii* IgG antibody was determined from each sample using a commercially available, Direct Agglutination Test kit (Toxo screen DA, biomerieux®, France) following the instructions of the manufacturer. Sera were assayed at a screening dilution of 1/40 and 1/4000 to avoid the false-negative results that might occur at low dilutions when using sera with high antibody titers. *T. gondii* infection was diagnosed when a serum sample gave a positive reaction indicated by a clear agglutination above half of the well at a dilution of 1: 40 or 1: 4000 or both. Sedimentation of antigen at the bottom of the well was considered as a negative result. Positive and negative controls were included in each test. All the collected serum samples were tested for the presence of antibodies against *Leishmania* spp. following the protocol of the manufacturer of the indirect enzyme-linked immunosorbent assay (ELISA) kit (VetLine, NovaTec Immundiagnostica GmbH, Germany). According to the manufacturer, the sensitivity and specificity of the kit is > 98%.

Data analysis

Questionnaire and laboratory data were entered into Microsoft Excel Spreadsheet. Coded data was transferred into STATA version 14.0 for Windows (Stata Corp. College Station, TX, USA). The association of the seroprevalence with putative risk factors was first statistically analyzed using Pearson's Chi-square test. Seroprevalence figure by DAT (for *T. gondii* infection) and ELISA (for *Leishmania* spp. infection) were considered as dependent variables. Age, sex, breed, feeding, housing, town, altitude, residence place, presence of cats, presence of other domestic animals, family size, marital status, and religion were the independent/explanatory variables investigated. Univariable and multivariable logistic regressions were used to identify the predictors of *T. gondii* seropositivity. Non-collinear variables with p-value < 0.25 in univariable analysis were further analyzed using multivariable logistic regression to identify risk factors of seropositivity and obtain adjusted Odds ratios with 95% confidence interval (CI). The 95% confidence level for the subgroup and overall prevalence values were calculated using the exact binomial test. Differences were considered statistically significant at $p < 0.05$.

Declarations

Ethics approval and consent to participate

The Research and Ethics Review Committee of Ambo University approved the experimental protocols. The study protocols complied with the guidelines of the Research and Ethical Review Committee of Ambo

University and that of the guideline of the Animal Research Reporting for In Vivo Experiments. Blood samples were collected by the researchers (veterinarians) after getting informed consent from the owners of the dogs. All efforts were made to minimize animal suffering during sample collection. As an incentive, all dogs were vaccinated for rabies, and antiparasitic drugs were administered for control of internal and external parasites. Written informed consent was obtained from all people who participated in the study.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing Interest

The authors declare that they have no competing interests

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Authors' contributions

EZG designed the study, analyzed the data, and drafted the manuscript. EJS, GKT, SSE, and LMM participated in the field questionnaire survey, blood sample collection, and enrichment of the drafted manuscript. AT contributed in the laboratory testing of sera samples, interpretation of results, and enriched the manuscript. MV and V di M participated in the study design and edition of the article. All authors have read and approved the manuscript.

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Author Details

¹Ambo University, School of Veterinary Medicine, Department of Veterinary Sciences, P.O.Box 19, Ambo, Ethiopia. ²Ambo University, School of Veterinary Medicine, Department of Veterinary Laboratory Technology, P.O.Box 19, Ambo, Ethiopia. ³National Animal Health Diagnostic and Investigation Center, P.O.Box, 04, Sebeta, Ethiopia. ⁴Istituto Zooprofilattico Sperimentale of Sicily, Laboratory of Genetics of Microorganisms, Via Gino Marinuzzi 3, 90129 Palermo, Italy

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Figures

Figure 1

The rate of co-infection of T. gondii and Leishmania spp. in dogs of the study towns