



Seroprevalence of canine leishmaniosis in asymptomatic dogs in Kosovo

Betim Xhekaj^{1,2} · Jovana Stefanovska² · Kurtesh Sherif¹ · Agim Rexhepi¹ · Bejo Bizhga³ · Ljubica Rashikj² · Martin Nikolovski² · Edwin Kniha⁴ · Aleksandar Cvetkovikj²

Received: 28 October 2022 / Accepted: 12 December 2022
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2022

Abstract

Canine leishmaniosis (CanL) is a neglected zoonotic disease caused by *Leishmania* spp. *Leishmania infantum* is the species responsible for the zoonotic form of the disease where dogs are reservoir hosts. This study aimed to determine the seroprevalence of CanL in asymptomatic dogs in Kosovo. Blood samples were collected from 285 dogs in all seven regions in Kosovo (35–50 samples per region) from summer 2021 to spring 2022. Sera were tested using enzyme-linked immunosorbent assay (ELISA), and the presence of anti-*Leishmania* IgG was confirmed by an indirect fluorescent antibody test (IFAT). The true overall seroprevalence of CanL of asymptomatic dogs in Kosovo with ELISA was 4.21% (95% CI: 2.42–7.21) while with IFAT was 3.51% (95% CI: 1.92–6.34). The highest rates were found in the Prishtina region to be 8.0% (4/50) by ELISA and 6.0% (3/50) by IFAT, and in the Mitrovica region, the prevalence was 0% (0/40). There were no significant differences among the different regions, gender, age, health status, and breed. These findings highlight the presence of CanL in most regions of Kosovo and underline the veterinary relevance of clinically asymptomatic dogs infected with *Leishmania*.

Keywords *Leishmania infantum* · Asymptomatic dogs · Kosovo · ELISA · IFAT

Introduction

Canine leishmaniosis (CanL) is a neglected zoonotic disease caused by *Leishmania* spp. (Selim et al. 2021). Etiological agents are intracellular protozoa belonging to the genus *Leishmania* (Kinetoplastida, Trypanosomatidae) (Rombolà et al. 2021). The life cycle of *Leishmania* parasites

is heteroxenous and alternates between the female phlebotomine sand fly vectors and numerous mammalian species (including humans) (Bennai et al. 2018). More than 70 vertebrate species are known reservoirs of *Leishmania* spp. including dogs, rodents, and humans (Akhoundi et al. 2016). Leishmaniosis is endemic in five continents and occurs in more than 98 countries, except the southern Pacific and New Zealand. Of 53 described *Leishmania* species, 31 have been reported to infect mammals, and infection with 20 species can cause various clinical manifestations in humans (Derghal et al. 2022).

Leishmania infantum is the species responsible for the zoonotic form of the disease where dogs are the main reservoir hosts and this species is widespread in the Mediterranean region, Central Asia, the Middle East, and some countries of Central and South America (Velez and Gállego 2020). In the Mediterranean, endemic areas include Portugal, Spain, Italy, Greece, Croatia, and other Balkan countries (Šiško-Kraljević et al. 2013; Symeonidou et al. 2021; Iatta et al. 2021; Paz et al. 2022; Priolo et al. 2022). The endemic area has recently expanded to other Northern European countries, which were free from leishmaniosis, and sand

Handling Editor: Julia Walochnik.

✉ Aleksandar Cvetkovikj
acvetkovic@fvm.ukim.edu.mk

- ¹ Faculty of Agriculture and Veterinary, University of Prishtina 'Hasan Prishtina', Bulevardi 'Bill Clinton', P.N., Prishtinë 10000, Kosovo
- ² Faculty of Veterinary Medicine-Skopje, Ss. Cyril and Methodius University in Skopje, Lazar Pop-Trajkov 5-7, Skopje 1000, North Macedonia
- ³ Faculty of Veterinary Medicine, Agricultural University of Tirana, Koder Kamez, 1000 Tirana, Albania
- ⁴ Center for Pathophysiology, Infectiology and Immunology, Institute of Specific Prophylaxis and Tropical Medicine, Medical University Vienna, Kinderspitalgasse 15, 1090 Vienna, Austria

flies have been detected in regions previously mapped as free from sand flies and disease (Kotnik et al. 2021).

Canine leishmaniosis is present in all neighboring countries of the Republic of Kosovo, namely Albania, North Macedonia, Serbia, and Montenegro (Lazri et al. 2008; Bizhga et al. 2013; Pavicevic et al. 2014; Hamel et al. 2016; Ratko et al. 2020; Vaselek 2021; Tasić-Otašević et al. 2022). There are only two studies for CanL in Kosovo. The first data for Kosovo were published in 2008, which showed a seroprevalence in dogs as high as 1.7% (Lazri et al. 2008). In 2016, the observed prevalence rate of anti-*Leishmania* antibodies among stray dogs in the southwestern region of Kosovo was 18.4% (Xhekaj et al. 2020). Dogs are the main reservoir of *Leishmania infantum*, the majority of infected dogs remain asymptomatic with a subclinical presentation. Only a small number manifest typical clinical signs of the disease with numerous clinical signs and immune responses that can be associated with CanL (Mendoza-Roldan et al. 2021). *Leishmania infantum* infections in dogs might present as a chronic subclinical, self-limiting disease, or non-self-limiting condition (Esteve et al. 2015). Asymptomatic dogs appear to be a source of *Leishmania infantum* infection in addition to symptomatic dogs (Barati et al. 2015). In CanL endemic regions, many infected dogs remain asymptomatic and never show symptoms (Bharati 2022). A correct diagnostic process is important to identify both dogs with clinical as well as without clinical signs (asymptomatic) infected with *Leishmania infantum*.

Symptomatic dogs are easy to diagnose based on clinical signs. In addition, a standard diagnostic approach should be applied to confirm clinical leishmaniosis, typically starting with quantitative serology such as IFAT (indirect fluorescent antibody test) and ELISA (enzyme-linked immunosorbent assay). Quantitative serological tests have been widely used in clinical and epidemiological studies on *Leishmania infantum*, due to their high diagnostic performance with close to 100% sensitivity and specificity (Cavalera et al. 2022). While high antibody levels usually confirm leishmaniosis, medium to low or even unclear test results urge further testing by cytology, histopathology, or PCR of aspirates from cutaneous lesions, lymph nodes, bone marrow, and spleen (Miró et al. 2008; Solano-Gallego et al. 2009). For testing asymptomatic dogs, which represent the largest infected population in endemic areas up to 85%, serological methods should be primarily used. However, seropositive dogs should be managed according to antibody levels. Specimens with high titers should be treated equally to sick dogs and specimens with low titers should be monitored and retested. Noteworthy, sensitive PCR-based approaches can be combined with serology, but results should be interpreted carefully as PCR positivity is highly dependent on the stage of infection and tissues used (Solano-Gallego et al. 2009).

In Kosovo, there are no recent data regarding the seroprevalence of leishmaniosis in dogs, particularly on the distribution of *Leishmania infantum* in all regions. This study aimed to determine the seroprevalence of leishmaniosis in dogs in Kosovo and to determine the association of seroprevalence with geographical location (region), sex, age, health status, and breed.

Materials and methods

Study area

The present study was conducted in the Republic of Kosovo, a landlocked country located in the center of the Balkan Peninsula in Southeastern Europe. It lies between latitudes 41 and 43° N, and longitudes 20 and 22° E. Kosovo is divided into seven regions: Prishtina (01), Mitrovica (02), Peja (03), Prizreni (04), Ferizaj (05), Gjilani (06), and Gjakova (07) (Fig. 1). The study area has a continental climate with Mediterranean and Alpine influences. There are no data regarding the number of dogs in Kosovo. The dogs are either stray, kept in private households, or on farms (shepherd dogs).

Sampling and data collection

This study was based on a randomized sampling of dogs from private households, stray dogs (kept in shelters), and shepherd dogs. Blood samples (5–7 ml of blood collected from the cephalic vein) were collected from 285 dogs from all seven Kosovo regions for a period of 1 year starting from summer 2021 to spring 2022. All samples were collected following the basic ethical principles and were marked by the name of the dog or chip number, location, age, breed, sex, and health status.

Thirty-five to fifty samples were taken from each region (Prishtina $n = 50$, Mitrovica $n = 40$, Peja $n = 35$, Prizreni $n = 40$, Ferizaj $n = 40$, Gjilani $n = 40$, and Gjakova $n = 40$) (Fig. 1). Dogs were grouped by gender as 141 males and 144 females. According to age, dogs were categorized as follows: 1–2 years ($n = 41$), 2–3 years ($n = 64$), 3–4 years ($n = 60$), 4–6 years ($n = 67$), 6–8 years ($n = 23$), 8–10 years ($n = 14$), and > 10 years ($n = 16$). According to the health status, the dogs were categorized as normal ($n = 245$) or disrupted ($n = 40$), which included dogs with random pathology unrelated to the CanL (dermatitis, arthritis, tumor, vasculitis, etc.). According to the breed status, 176 dogs were classified as mix breed, and 109 were classified as pure-breed dogs.

The samples were transported from the field to the laboratory under cool conditions and were allowed to clot at room temperature. The blood was centrifuged for 5 min at 800 rotations (rpm), and the sera were separated, placed into a

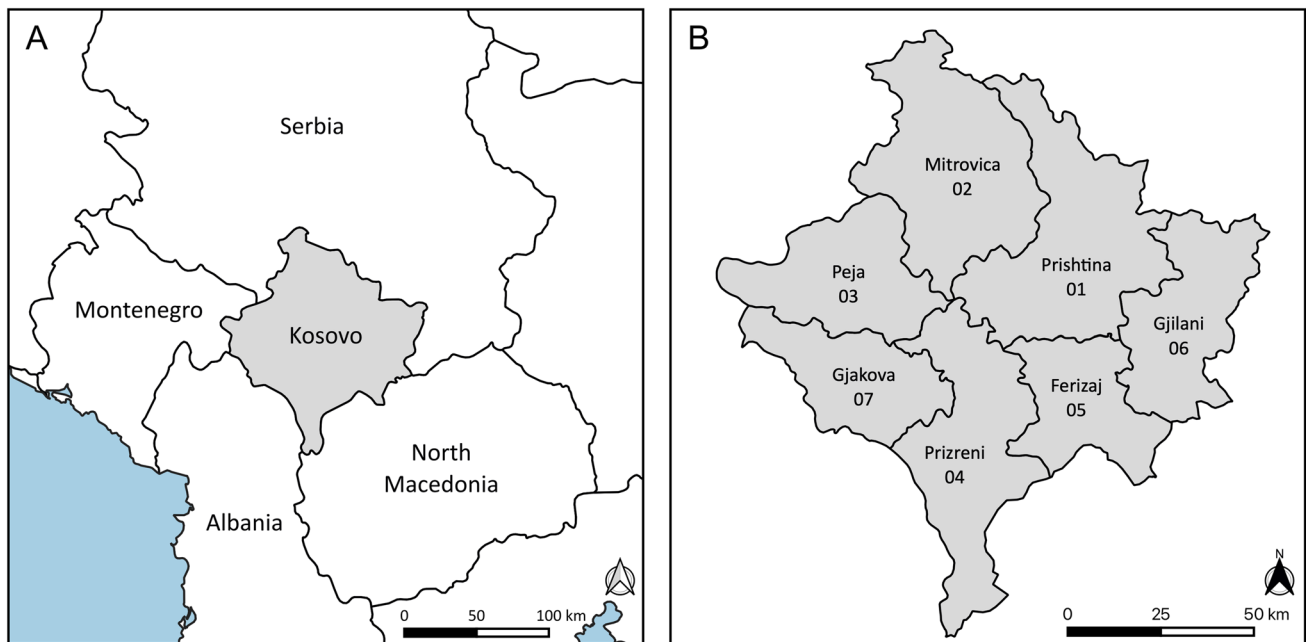


Fig. 1 Map of Kosovo: **A** position in the Balkan Peninsula; **B** map of Kosovo's regions. Country border and sea data made with Natural Earth. First-level administrative divisions, Kosovo, 2015 taken from <https://earthworks.stanford.edu/catalog/stanford-zh532mm5047>

microtube (1.5 ml), and stored at -20°C until further serological examination.

Serological testing

All collected sera were tested using a competitive enzyme-linked immunosorbent assay (ELISA), and confirmation was obtained by an indirect fluorescent antibody test (IFAT). The ELISA used was ID Screen® Leishmaniasis Indirect Test (VET-Innovate ID Diagnostics, France). The assay was carried out according to the manufacturer's instructions. The diagnostic sensitivity of this kit, as stated by the manufacturer, is 98.54% (95% CI: 94.29–99.75%) and the diagnostic specificity is 99.18% (95% CI: 98.22–99.62%). The optical density (OD) for each sample was read at a wavelength of 450 nm. The results of the leishmaniosis antibody detection were calculated as a percentage of the positive or negative control, respectively, as indicated by the manufacturer. All sera were also tested by IFAT MegaFLUO® LEISH (MEG-ACOR Diagnostic GmbH, Austria), a test kit for the semi-quantitative immunofluorescence detection of IgG antibodies against *Leishmania infantum* in the plasma or serum of the dog, which was performed at the Faculty of Veterinary Medicine—Skopje in North Macedonia. The diagnostic sensitivity of this kit, as stated by the manufacturer, is 98.2% and the diagnostic specificity is 92.3%. Anti-*Leishmania* antibodies were detected using FITC anti-dog IgG conjugate as described in the manufacturer's instructions. The sera were classified as positive if promastigote cytoplasmatic or

membrane fluorescence was observed at a serum dilution of 1:160 or higher.

Statistical analysis

To test whether there were significant differences among the different regions, gender, age, health status, and breed, a power analysis (GPower v.3.9.1.2, Franz Faul, Kiel University, Germany) was performed a priori to assess the power of the chi-square analysis (Pearson or Fisher's exact test) (SPSS, IBM®). The preset effect size of 0.3, α probability of 0.05, appropriate sample size ($n = 273\text{--}285$), and degrees of freedom ($df = 1\text{--}14$) were used according to the analysis. Chi-square results were considered relevant if the obtained power was ≥ 0.95 and $p < 0.05$.

Results

The overall prevalence rate of CanL antibodies among asymptomatic dogs in all seven regions of Kosovo was 4.21% (95% CI: 2.42–7.21%), representing 12 out of 285 samples in the ELISA test and 3.51% (10/285, 95% CI: 1.92–6.34%) tested on IFAT.

The distribution of positive ELISA and IFAT samples by region was as follows: Prishtina (33% and 30%), Gjakova (17% and 30%), Prizreni (17% and 10%), Ferizaj (17% and 10%), Peja (8% and 10%), and Gjilani (8% and 10%). The

Mitrovica region samples were negative (Fig. 2). Detailed descriptive statistics are shown in Table 1.

The seropositive samples by gender were distributed as follows: 67% and 33% for ELISA and 70% and 30% for IFAT

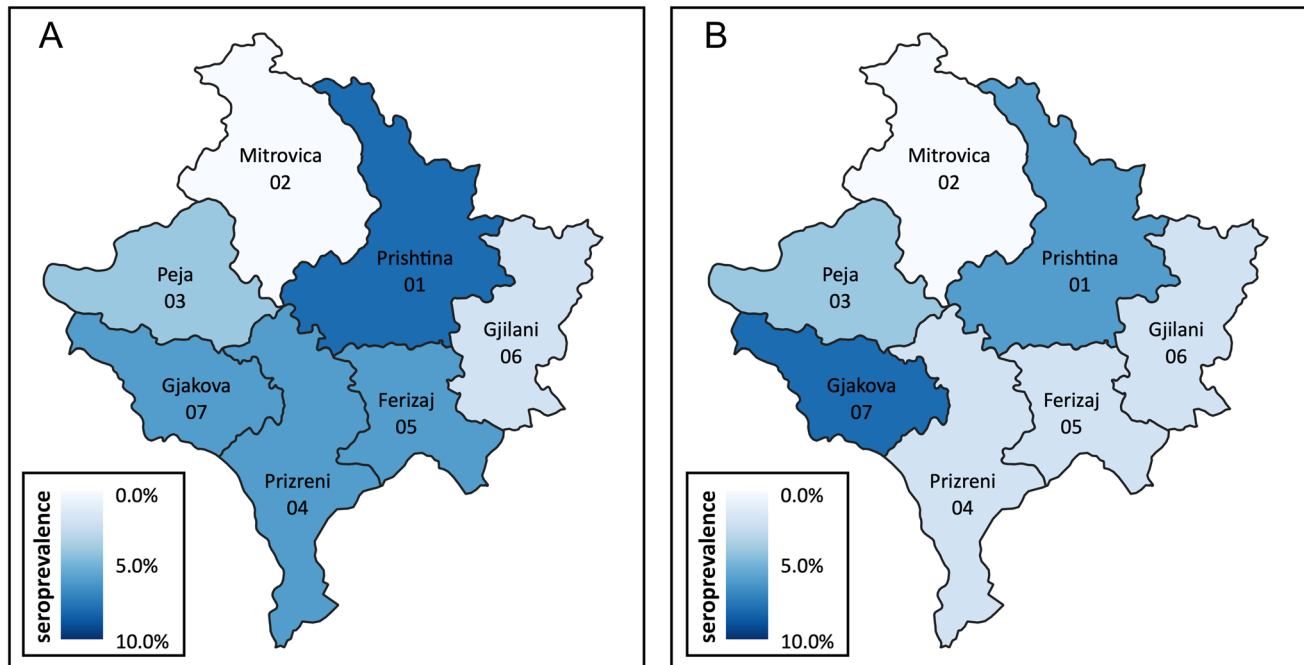


Fig. 2 *Leishmania infantum* seroprevalence by region in Kosovo: **A** seroprevalence by ELISA; **B** seroprevalence by IFAT. First-level administrative divisions, Kosovo, 2015 taken from <https://earthworks.stanford.edu/catalog/stanford-zh532mm5047>

Table 1 Seroprevalence of CanL in Kosovo related to different variables

Parameter	Factor	No. of samples	No. of positive ELISA	No. of positive IFAT	% ELISA	95% CI ELISA	% IFAT	95% CI IFAT
Location	Prishtina 01	50	4	3	8.0	3.15–18.84	6.0	2.06–16.22
	Mitrovica 02	40	0	0	0	0–8.76	0	0–8.76
	Peja 03	35	1	1	2.86	0.51–14.53	2.86	0.51–14.53
	Prizreni 04	40	2	1	5.0	1.38–16.5	2.5	0.44–12.88
	Ferizaj 05	40	2	1	5.0	1.38–16.5	2.5	0.44–12.88
	Gjilani 06	40	1	1	2.5	0.44–12.88	2.5	0.44–12.88
	Gjakova 07	40	2	3	5.0	1.38–16.5	7.5	2.58–19.86
Sex	Male	141	8	7	5.67	2.9–10.8	4.96	2.43–9.89
	Female	144	4	3	2.78	1.1–6.92	2.08	0.71–5.95
Age	1–2 years	41	0	0	0	0–8.57	0	0–8.57
	2–3 years	64	0	0	0	0–5.66	0	0–5.66
	3–4 years	60	2	1	3.33	0.92–11.36	1.67	0.29–8.86
	4–6 years	67	6	6	8.96	4.17–18.19	8.96	4.17–18.19
	6–8 years	23	1	1	4.35	0.77–20.99	4.35	0.77–20.99
	8–10 years	14	2	2	14.29	4.01–39.94	14.29	4.01–39.94
	> 10 years	16	1	0	6.25	1.11–28.33	0	0–19.36
Health status	Normal	245	11	9	4.49	2.53–7.86	3.67	1.94–6.83
	Disrupted	40	1	1	2.5	0.44–12.88	2.5	0.44–12.88
Breed status	Mix breed	176	8	7	4.55	2.32–8.71	3.98	1.94–7.98
	Specified breed	109	4	3	3.67	1.44–9.06	2.75	0.94–7.78

in males and females, respectively. The seropositive samples by age on ELISA and IFAT were distributed as follows: 50% and 60% for 4–6 years, 17% and 20% for 8–10 years, 17% and 10% for 3–4 years, 8% and 10% for 6–8 years, 8% and 0% for the > 10 years group, and 0% for 1–2 and 2–3 years, respectively. The seropositive samples by health status on ELISA and IFAT were distributed as follows: 92% and 8% for normal, and 90% and 10% for disrupted health status, respectively. The seropositive samples by breed status on ELISA and IFAT were distributed as follows: 67% and 33% for the specified breed, and 70% and 30% for the mix-breed dogs. There were no any significant differences among the different regions, gender, age, health status, and breed.

Discussion

This study provides a recent report of *Leishmania infantum* seropositivity in asymptomatic dogs in Kosovo. The wide distribution of anti-*Leishmania* antibodies in local dogs from six of seven regions of Kosovo indicates established leishmaniosis transmission cycles among asymptomatic dogs within the country. Overall, the seroprevalence rate of leishmaniosis in asymptomatic dogs was 4.21% in ELISA and 3.51% in IFAT, which complies with reports from previous studies in the neighboring countries. Reported prevalence rates ranged from 5.1 to 10.6% in Albania, 2.5 to 20.3% in North Macedonia, 2.1 to 43.7% in Serbia, and 5.9 to 72.7% in Montenegro (asymptomatic and symptomatic dogs, respectively) (Lazri et al. 2008; Bizhga et al. 2013; Pavicevic et al. 2014; Hamel et al. 2016; Ratko et al. 2020; Vaselek 2021; Tasić-Otašević et al. 2022).

Our study shows a non-significantly higher prevalence in male compared to female dogs. Similar findings were reported among *L. infantum*-infected dogs in Iran (Fakhar et al. 2022). While we did not observe an age-dependent seroprevalence, dogs aged 8–10 years showed the highest seroprevalence, and dogs younger than 3 years did not have anti-*L. infantum* antibodies. In endemic areas, seroprevalence is often reported to increase with each passing year of life; thus, age is considered an important factor for the infection with *Leishmania* spp. (Rombolà et al. 2021; Tamponi et al. 2021). On the contrary, other authors reported a bimodal age distribution of seroprevalence peaking at very young (1–2 years) and old age (7–8 years). This was explained by the breed composition of screened dogs, whereby some breeds (e.g. Boxer, German Shepherd) are more sensitive to the disease than others and have a higher chance to develop a disease at an early age. Latent infections will be active at a progressive age with immunosuppression or comorbidity (Gálvez et al. 2010).

The current study included only dogs without symptoms of CanL. There was no significant difference between the

seropositive samples from dogs with normal and disrupted health status (cachexia, alopecia, lethargy, epistaxis, or skin lesions). Only one seropositive dog with disrupted health status showed moderate dermatitis with no other symptoms of the disease. There was also no significant difference in seropositivity between the dogs with a mix or specified breed status.

The first report of CanL in Kosovo was published in 2008 reporting a seroprevalence of 1.7% (Lazri et al. 2008). Considering that samples analyzed by Lazri et al. (2008) mainly originated from only three regions, namely Prishtina, Gjakova, and Ferizaj, our observed seroprevalence rates were more than four times higher in Prishtina region and two times higher in the two other respective regions, clearly indicating an increased transmission in the last decade. In another report from 2016, the prevalence rate of anti-*Leishmania* antibodies among stray dogs in the southwestern region of Kosovo was 18.4% (Xhekaj et al. 2020). The high discrepancies between these observed prevalence rates might be a result of a focally performed sample collection. While our seroprevalence results are presented by the seven different regions of Kosovo, Xhekaj et al. (2020) analyzed samples of a much lower geographic scale, namely in four municipalities of Southwestern Kosovo, where subsequently high rates of neutralizing antibodies against *Toscana phlebovirus* (TOSV) and *Sicilian phlebovirus* (SFSV) have been detected in cattle (Ayhan et al. 2017). Furthermore, *Ph. neglectus*, a proven vector for *L. infantum*, was observed to be the predominant sand fly species in these regions (Vaselek et al. 2020), which might contribute to locally established *L. infantum* transmission cycles.

Nine sand fly species of two genera are reported in Kosovo. Eight species belong to the genus *Phlebotomus*: *Ph. papatasi* (Scopoli, 1786), *Ph. perfiliewi* Parrot, 1930, *Ph. tobbi* Theodor & Lourie, 1930, *Ph. neglectus* Tonnoir, 1921, *Ph. simici* Nitzulescu, 1931, *Ph. balcanicus* (Theodor, 1958), *Ph. alexandri* (Sinton, 1928), and *Ph. mascittii* Grassi, 1908, and only one species belongs to the genus *Sergentomyia*, namely *Se. minuta* (Rondani, 1843) (Vaselek et al. 2020). Of these, *Ph. papatasi*, *Ph. balcanicus*, *Ph. neglectus*, *Ph. perfiliewi*, and *Ph. tobbi* are proven vectors of *L. infantum* (Sáez et al. 2018). Additionally, CanL caused by *L. infantum* is zoonotic and, thus, poses a significant medical risk for human infection.

Although not significant, the highest seroprevalence was detected in the most populated Prishtina region, which also constitutes the homonymous capital city of the country (more than 200,000 inhabitants). Considering the high number of dog shelters and stray dogs in this region, particularly in the capital city, as well as the potential zoonotic spillover of *L. infantum* to humans, this area might be of high public health relevance. However, there are few data on the sand fly distribution and human leishmaniosis in the Prishtina region.

A study for the detection of anti-*Leishmania* antibodies in Austrian Armed Forces returning from Kosovo showed that 55 out of 261 soldiers (21.1%) had anti-*Leishmania* antibodies, of these 13.8% showing positive and 7.3% showing borderline results. These results demonstrate a considerable risk of exposure to *Leishmania* spp. in Kosovo and an urge for a one-health approach (Kniha et al. 2020).

According to the World Health Organization (WHO), leishmaniosis is one of the most significant and neglected tropical diseases. The main etiologic agent of canine visceral leishmaniosis is *Leishmania infantum*, which is a major global zoonotic parasite potentially fatal to dogs and humans (Peris et al. 2021).

Diagnosis of leishmaniosis is complex and is an example of an infection with overt clinical signs of disease due to the high prevalence of subclinical infection (Strube and Mehlhorn 2021). To diagnose CanL, serological tests are a useful tool to detect specific antibodies against *Leishmania* spp. and to determine the spread of the disease, particularly among the high number of asymptomatic dogs. In our study, we applied two tests, ELISA and IFAT, considering that these are the most frequently used serological methods. ELISA is a highly sensitive method for screening large numbers of samples in epidemiological studies and is well-suited to be applied in field conditions (Selim et al. 2021). In our study, seroprevalence rates detected by ELISA were generally higher compared to IFAT results, with one exception of the Gjakova region. These results are in line with other studies, in which ELISA tests showed higher sensitivities compared to IFAT, particularly lacking sensitivity in samples of asymptomatic dogs (Mettler et al. 2005; Rodríguez-Cortés et al. 2013). Thus, as we screened dogs without apparent signs of CanL, better diagnostic performance by ELISA is not surprising. Furthermore, the results of IFAT vary by the subjective interpretation of the operator's skills and different cut-off titers are used by laboratories (Ferroglia and Vitale 2006). Based on the exceptionally high diagnostic performance of our applied ELISA kit (ID Screen®) observed by Solano-Gallego et al. (2014), ELISA-based methods should be favored over IFAT when screening asymptomatic dog populations. However, we need to highlight that sensitivity of antibody detection is generally lower in early or in asymptomatic canine infections and the possibility of false negative samples should not be neglected (Leontides et al. 2002).

The current study highlights the urgent need to enhance the knowledge about the disease among health authorities in Kosovo. Dog owners should receive more information on how to protect their pets from being infected and stop the transmission of the disease. Currently, four vaccines against leishmaniosis in dogs have been commercialized: Leishmune®, Leish-Tec®, CaniLeish®, and Letifend®. However, none of these is licensed in Kosovo. Baxarias et al. (2022) have reported that 39.8% of dog owners in Europe vaccinate

their dogs against leishmaniosis, whereas 86.2% apply repellents as the first mode of action. Insecticide-impregnated dog collars (e.g. deltamethrin, imidacloprid, or permethrin) are highly effective in protecting dogs from sand fly bites. Thereby, uncollared dogs living with or close to collared dogs were shown to have 3.5 times higher risk of being infected (Matsumoto et al. 2022). In Kosovo, pet dogs are mainly kept outside. Considering the high number of stray dogs as well as their frequent contact with pet dogs, the broad application of insecticide-impregnated dog collars is highly recommended, particularly in regions with high *Leishmania* seroprevalences.

Although *Leishmania infantum* is pathogenic for humans, no data on human infections exist in Kosovo to date. The present study underlines the necessity to increase the capacity of local authorities for better control of CanL in Kosovo. In addition, extensive entomological fieldwork is required to further elucidate the epidemiology of this zoonotic disease. The future control and prevention of leishmaniosis in Kosovo should be based on an integrated one-health approach of surveillance of animals, sand flies, and humans.

Conclusions

Canine leishmaniosis was qualitatively confirmed in Kosovo. It is indicative that *L. infantum* transmission cycles are active in different regions of Kosovo, particularly in the Prishtina region. Quantitatively, there was neither an association between seropositivity and asymptomatic dogs nor with the regional distribution, sex, age, breed, and health status.

Acknowledgements The authors would like to thank all colleagues who helped to collect the blood samples and everyone who contributed to this study.

Author contribution Conceptualization, B.XH., J.S., A.C., B.B., A.R., and K.SH.; validation, Lj.R. and E.K.; formal analysis, M.N.; investigation, B.XH. and J.S.; resources, B.XH., E.K., J.S., A.R., and K.SH.; writing—original draft preparation, B.XH. and A.C.; writing—review and editing, A.C., E.K., and M.N.; supervision, J.S. and A.C.; project administration, J.S.; funding acquisition, B.XH. and K.SH. All authors have read and agreed to the published version of the manuscript.

Funding This research was partially funded by the Ministry of Education, Science and Technology of the Republic of Kosovo, Project number: 2–1775.

Data availability The data presented in the study are available on request from the corresponding author.

Declarations

Competing interests The authors declare no competing interests.

Ethical approval No Ethics Commission is established in the Republic of Kosovo. This study was conducted in compliance with the regula-

tions of the Department of Hygiene, Welfare and Ethology of Animals, Faculty of Agriculture and Veterinary, University of Prishtina 'Hasan Prishtina'. Sampling was performed following the approval of the Faculty from 19 March 2021. Scientific research works that include investigation of vector-borne emerging diseases in dogs are performed to diagnose animal diseases and improve animal welfare. No suffering was caused during the sample collection.

Consent to participate Informed consent was obtained from all owners of dogs involved in the study.

Conflict of interest The authors declare no competing interests.

References

- Akhoundi M, Kuhls K, Cannet A et al (2016) A historical overview of the classification, evolution, and dispersion of *Leishmania* parasites and sandflies. *PLoS Negl Trop Dis* 10:e0004349. <https://doi.org/10.1371/journal.pntd.0004349>
- Ayhan N, Sherifi K, Taraku A et al (2017) High rates of neutralizing antibodies to toscana and sandfly fever sicilian viruses in livestock, Kosovo. *Emerg Inf Dis* 23:989–992. <https://doi.org/10.3201/eid2306.161929>
- Barati M, Mohebbi M, Alimohammadian MH et al (2015) Canine visceral leishmaniasis: seroprevalence survey of asymptomatic dogs in an endemic area of northwestern Iran. *J Parasit Dis* 39:221–224. <https://doi.org/10.1007/s12639-013-0325-2>
- Baxarias M, Homedes J, Mateu C et al (2022) Use of preventive measures and serological screening tools for *Leishmania infantum* infection in dogs from Europe. *Parasit Vectors* 15:134. <https://doi.org/10.1186/s13071-022-05251-5>
- Bennai K, Tahir D, Lafri I et al (2018) Molecular detection of *Leishmania infantum* DNA and host blood meal identification in *Phlebotomus* in a hypoendemic focus of human leishmaniasis in northern Algeria. *PLoS Negl Trop Dis* 12:e0006513. <https://doi.org/10.1371/journal.pntd.0006513>
- Bharati K (2022) Human genetic polymorphism and Leishmaniasis. *Infect Genet Evol* 98:105203. <https://doi.org/10.1016/j.meegid.2021.105203>
- Bizhga B, Laci D, Dharmo G et al (2013) Survey for canine leishmaniosis. *J Anim Vet Adv* 12:442–446
- Cavaleria M, Zatelli A, Donghia R et al (2022) Conjunctival swab real time-PCR in *Leishmania infantum* seropositive dogs: diagnostic and prognostic values. *Biology (basel)* 11:184. <https://doi.org/10.3390/biology11020184>
- Derghal M, Tebai A, Balti G et al (2022) High-resolution melting analysis identifies reservoir hosts of zoonotic *Leishmania* parasites in Tunisia. *Parasit Vectors* 15:12. <https://doi.org/10.1186/s13071-021-05138-x>
- Esteve LO, Saz SV, Hosein S, Solano-Gallego L (2015) Histopathological findings and detection of toll-like receptor 2 in cutaneous lesions of canine leishmaniosis. *Vet Parasitol* 209:157–163. <https://doi.org/10.1016/j.vetpar.2015.03.004>
- Fakhar M, Derakhshani-nia M, Goharhehi S, et al. (2022) Domestic dogs carriers of *Leishmania infantum*, *Leishmania tropica* and *Crithidia fasciculata* as potential reservoirs for human visceral leishmaniasis in northeastern Iran. *Vet Med Sci* 1–8. <https://doi.org/10.1002/vms3.929>
- Ferroglio E, Vitale F (2006) Diagnosis of leishmaniosis: between old doubts and new uncertainties. *Vet Res Commun* 30:35–38. <https://doi.org/10.1007/s11259-006-0008-x>
- Gálvez R, Miró G, Descalzo MA et al (2010) Emerging trends in the seroprevalence of canine leishmaniasis in the Madrid region (central Spain). *Vet Parasitol* 169:327–334. <https://doi.org/10.1016/j.vetpar.2009.11.025>
- Hamel D, Shukullari E, Rapti D et al (2016) Parasites and vector-borne pathogens in client-owned dogs in Albania. Blood pathogens and seroprevalences of parasitic and other infectious agents. *Parasitol Res* 115:489–499. <https://doi.org/10.1007/s00436-015-4765-8>
- Iatta R, Mendoza-Roldan JA, Latrofa MS et al (2021) *Leishmania tarentolae* and *Leishmania infantum* in humans, dogs and cats in the Pelagie archipelago, southern Italy. *PLoS Negl Trop Dis* 15:e0009817. <https://doi.org/10.1371/journal.pntd.0009817>
- Kniha E, Walochnik J, Poepl W et al (2020) *Leishmania* spp. seropositivity in Austrian soldiers returning from the Kosovo. *Wien Klin Wochenschr* 132:47–49. <https://doi.org/10.1007/s00508-019-01598-5>
- Kotnik T, Moreno J, Šoba B et al (2021) Canine leishmaniasis prevalence in the Slovenian dog population. *J Vet Res* 65:161–167. <https://doi.org/10.2478/jvetres-2021-0028>
- Lazri T, Duscher G, Edelhofer R et al (2008) Infektionen mit arthropodenübertragenen Parasiten bei Hunden im Kosovo und in Albanien unter besonderer Berücksichtigung der Leishmanieninfektionen. *Wien Klin Wochenschr* 120:54–58. <https://doi.org/10.1007/s00508-008-1076-4>
- Leontides LS, Saridomichelakis MN, Billinis C et al (2002) A cross-sectional study of *Leishmania* spp. infection in clinically healthy dogs with polymerase chain reaction and serology in Greece. *Vet Parasitol* 109:19–27. [https://doi.org/10.1016/S0304-4017\(02\)00201-7](https://doi.org/10.1016/S0304-4017(02)00201-7)
- Matsumoto PSS, Taniguchi HH, Pereira VBR et al (2022) Efficacies of insecticide dog collars against visceral leishmaniasis in low and high-income areas and the effects for non-collared neighbor dogs. *Acta Trop* 235:106626. <https://doi.org/10.1016/j.actatropica.2022.106626>
- Mendoza-Roldan JA, Latrofa MS, Iatta R et al (2021) Detection of *Leishmania tarentolae* in lizards, sand flies and dogs in southern Italy, where *Leishmania infantum* is endemic: hindrances and opportunities. *Parasit Vectors* 14:461. <https://doi.org/10.1186/s13071-021-04973-2>
- Mettler M, Grimm F, Capelli G et al (2005) Evaluation of enzyme-linked immunosorbent assays, an immunofluorescent-antibody test, and two rapid tests (immunochromatographic-dipstick and gel tests) for serological diagnosis of symptomatic and asymptomatic *Leishmania* infections in dogs. *J Clin Microbiol* 43:5515–5519. <https://doi.org/10.1128/JCM.43.11.5515-5519.2005>
- Miró G, Cardoso L, Pennisi MG et al (2008) Canine leishmaniosis — new concepts and insights on an expanding zoonosis: part two. *Trends Parasitol* 24:371–377. <https://doi.org/10.1016/j.pt.2008.05.003>
- Pavicevic Z, Lausevic D, Pejovic N (2014) Seroprevalence of dogs to *Leishmania infantum* antigens in the territory of Crna Gora. *Vet Glas* 68:301–309. <https://doi.org/10.2298/VETGL1406301P>
- Paz MLQM, Casimiro T, Correia JHD, Leal RO (2022) The relevance of screening for vector-borne diseases in dogs with proteinuria living in an endemic region: a retrospective study. *Vet Sci* 9:266. <https://doi.org/10.3390/vetsci9060266>
- Peris MP, Esteban-Gil A, Ortega-Hernández P et al (2021) Comparative study of real-time PCR (TaqMan Probe and Sybr Green), serological techniques (ELISA, IFA and DAT) and clinical signs evaluation, for the diagnosis of canine leishmaniasis in experimentally infected dogs. *Microorganisms* 9:2627. <https://doi.org/10.3390/microorganisms9122627>
- Priolo V, Martínez-Orellana P, Pennisi MG et al (2022) *Leishmania infantum* specific humoral and cellular immune responses in cats and dogs: a comparative cross-sectional study. *Vet Sci* 9:482. <https://doi.org/10.3390/vetsci9090482>
- Ratko S, Darko M, Gorana V et al (2020) Seroprevalence of *Leishmania* spp. in dogs in Northern Serbia. *Vet Glas* 74:144–153

- Rodríguez-Cortés A, Ojeda A, Todolí F et al (2013) Performance of commercially available serological diagnostic tests to detect *Leishmania infantum* infection on experimentally infected dogs. *Vet Parasitol* 191:363–366. <https://doi.org/10.1016/j.vetpar.2012.09.009>
- Rombolà P, Barlozzari G, Carvelli A et al (2021) Seroprevalence and risk factors associated with exposure to *Leishmania infantum* in dogs, in an endemic Mediterranean region. *PLoS ONE* 16:e0244923. <https://doi.org/10.1371/journal.pone.0244923>
- Sáez VD, Morillas-Márquez F, Merino-Espinosa G et al (2018) *Phlebotomus langeroni* Nitzulescu (Diptera, Psychodidae) a new vector for *Leishmania infantum* in Europe. *Parasitol Res* 117:1105–1113. <https://doi.org/10.1007/s00436-018-5788-8>
- Selim A, Shoulah S, Abdelhady A et al (2021) Seroprevalence and risk factors associated with canine leishmaniasis in Egypt. *Vet Sci* 8:236. <https://doi.org/10.3390/vetsci8100236>
- Šiško-Kraljević K, Jerončić A, Mohar B, Punda-Polić V (2013) Asymptomatic *Leishmania infantum* infections in humans living in endemic and non-endemic areas of Croatia, 2007 to 2009. *Euro-surveillance* 18:1–8. <https://doi.org/10.2807/1560-7917.ES2013.18.28.20533>
- Solano-Gallego L, Koutinas A, Miró G et al (2009) Directions for the diagnosis, clinical staging, treatment and prevention of canine leishmaniasis. *Vet Parasitol* 165:1–18. <https://doi.org/10.1016/j.vetpar.2009.05.022>
- Strube C, Mehlhorn H (2021) Dog parasites endangering human health. Springer International Publishing, Cham. <https://doi.org/10.1007/978-3-030-53230-7>
- Symeonidou I, Angelou A, Theodoridis A et al (2021) Canine leishmaniasis in Greece: an updated countrywide serological study and associated risk factors. *Pathogens* 10:1129. <https://doi.org/10.3390/pathogens10091129>
- Tamponi C, Scarpa F, Carta S et al (2021) Seroprevalence and risk factors associated with *Leishmania infantum* in dogs in Sardinia (Italy), an endemic island for leishmaniasis. *Parasitol Res* 120:289–300. <https://doi.org/10.1007/s00436-020-06973-0>
- Tasić-Otašević S, Savić S, Jurhar-Pavlova M et al (2022) Molecular survey of *dirofilaria* and *leishmania* species in dogs from Central Balkan. *Animals* 12:911. <https://doi.org/10.3390/ani12070911>
- Vaselek S (2021) Canine leishmaniasis in Balkan – a review of occurrence and epidemiology. *Acta Trop* 224:106110. <https://doi.org/10.1016/j.actatropica.2021.106110>
- Vaselek S, Oguz G, Ayhan N et al (2020) Sandfly surveillance and investigation of *Leishmania* spp. DNA in sandflies in Kosovo. *Med Vet Entomol* 34:394–401. <https://doi.org/10.1111/mve.12451>
- Velez R, Gállego M (2020) Commercially approved vaccines for canine leishmaniasis: a review of available data on their safety and efficacy. *Trop Med Int Heal* 25:540–557. <https://doi.org/10.1111/tmi.13382>
- Xhekaj B, Alishani M, Rexhepi A et al (2020) Serological survey of canine leishmaniasis in southwestern region of Kosovo. *Vet Ital* 56:47–50. <https://doi.org/10.12834/VetIt.1345.7407.5>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.