

SEROPREVALENCE OF *LEISHMANIA* SPP. IN DOGS IN NORTHERN SERBIA

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Abstract

Leishmaniosis is a zoonotic disease caused by *Leishmania* spp. Serbia was an endemic region for this disease until the second half of the 20th century. Nowadays, canine leishmaniosis appears to be a re-emerging vector-borne disease in Serbia. Its spread is enabled by the presence of *Phlebotomus* spp. sandflies in this region. The objective of this study was to establish the seroprevalence of *Leishmania* spp. in dogs being kept in shelters in Serbia and compare those that apply and those that do not apply prophylactic measures against arthropod vectors. This cross-sectional study involved 336 dogs in two regions in Northern Serbia during 2019-2020. A commercial enzyme-linked immunosorbent assay test was used to establish the prevalence of seropositive dogs. In the shelters that applied prophylactic measures against arthropod vectors, dogs were seronegative (n=52), while in those without these measures, 2.1% (6/284) were seropositive against *Leishmania* spp. In conclusion, the presence of dogs seropositive against *Leishmania* spp. in shelters that did not apply prophylactic measures may indicate that the local population of sandflies is a source of *Leishmania* spp. parasites.

Key words: leishmaniosis, zoonoses, vector-borne disease, diagnostics

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INTRODUCTION

Leishmaniosis is a zoonotic vector-borne disease caused by parasites from the genus *Leishmania*. It is spread by *Phlebotomus* spp. sandflies among dogs, cats, and wild animals such as rabbits or wild canids. Leishmaniosis is endemic in the countries of the Mediterranean basin. Until the mid-decade of the last century, Serbia was endemic for human leishmaniosis (Milovanović and Popović, 1960). The disease was probably introduced to Serbia by troops of soldiers retreating through Greece and Macedonia during and after the Second World War (Milovanović and Popović, 1960), and it became endemic due to the presence of sandflies and poor hygienic and health conditions at that time (Saulic, 1949; Živković, 1980). From 1945 to 1955, the disease spread from Southern to Northern Serbia following the canyons and river basins, being the main paths of human and animal migrations (Milovanović and Popović, 1960). Among infected human patients, mortality was estimated to be higher than 30% (Djorić 1953; Milovanović and Popović, 1960).

This alarming situation called for solutions, and investigation in the field led to the characterisation of autochthonous sandflies *Phlebotomus simici* (Simić, 1954) and later on, another two species, *Phlebotomus neglectus* and *Phlebotomus perfilievi* (Živković, 1982; 1983). During the epidemic period, dichlorodiphenyltrichloroethane (DDT) residual spraying was applied, living conditions for most people were improved, higher hygienic standards were established and medical care became more available, which all lead to a reduction of sandfly density and eradication of the disease (Milovanović and Popović, 1960). In the late 60s, the disease was considered eradicated. After that period, there was a huge gap in research, since leishmaniosis was no longer a disease of interest.

During the last 13 years, *Leishmania* spp. were detected in sandflies again in Serbia, along with cases of canine leishmaniosis (Savić-Jevđenić et al., 2007, Savić et al., 2013). Cases of imported, but later also autochthonous visceral leishmaniosis in humans were reported in Serbia (Dakić et al., 2009, Dokmanović et al., 2012). The detected human cases indicated the risk of leishmaniosis re-emergence in Serbia. Since wild canids are potential reservoirs in the sylvatic cycle, at this point, research on the prevalence of *Leishmania* spp. in jackals in Serbia is particularly relevant (Ćirović et al., 2014).

Leishmaniosis is re-emerging in Serbia through the “back door”. There are no legislative demands regarding *Leishmania* spp. testing for the import or export of dogs throughout Europe. Dogs from Serbia often travel with their owners to endemic countries like Montenegro, Greece, and Croatia, with no controls on their return (Dakić et al., 2011). Several reports of clinical cases of imported canine leishmaniosis in dogs were published in recent years (Savić et al., 2013, Ralić and Jovanović 2011; Aleksić et al., 2009).

The first objective of this study is to establish the current seroprevalence of *Leishmania* spp. in dogs being kept in animal shelters in Northern Serbia, and compare those that apply and those that do not apply prophylactic measures against arthropod vectors.

The second objective is to discuss the epizootic situation of canine leishmaniosis in Serbia, from those first studies that recognised the disease in dogs, and to point out the potential risk this disease presents for public health.

MATERIALS AND METHODS

A total of 336 dog serum samples were analysed for the presence of specific antibodies against *Leishmania* spp. The sera were from dog shelter dogs, located in Novi Sad, Budisava, Srbobran, Zrenjanin, and Djurdjevo, all in Northern Serbia. The study was conducted during 2019-2020. Dogs were fed on commercial diets and were chosen randomly, regardless of their sex and age. The animals' health status was recorded, and special attention was given to clinical signs such as skin and eye lesions, and cachexia. Blood samples were collected into plain tubes and after clotting, serum was transferred to new tubes and kept at -20°C until further analysis. Blood samples were collected during a regular health check-up, so no additional invasive procedures were performed for this study. Sera were tested for antibodies against *Leishmania* spp. using a commercial enzyme-linked immunosorbent assay according to the manufacturer's instructions (INgezim Leishmania; Ingenasa, Spain). The sensitivity and specificity of this commercial ELISA test is 90-95%.

RESULTS

The housing of dogs in different shelters differed slightly, but all dogs had the access to the outdoors (Table 1).

Table 1. Epidemiological data on dogs from shelters 1-5, and number (%) of dogs with antibodies to *Leishmania* spp.

Shelter	N° of animals	Housing	Preventive Treatment against VBD	Location	No of positive samples (%)
1	94	In separate pens; 1-3 dogs per pen	No	Novi Sad	1 (1.06%)
2	52	Indoor and outdoor free	Yes	Djurdjevo	0
3	65	Outdoor in pens for 7-10 dogs	No	Budisava	0
4	55	Outdoor in fenced compounds for 20-30 dogs	No	Srbobran	3 (5.45%)
5	70	Outdoor in fenced compounds for 20-30 dogs	No	Zrenjanin	2 (2.86%)

From a total of 336 dogs in shelters, six (1.8%) were positive, with antibodies against *Leishmania* spp. found in those dogs' sera. These seropositive dogs were housed in

shelters that do not apply prophylaxis against arthropod vectors. In shelters where this treatment is applied, there were no *Leishmania*-seropositive dogs. Four of the positive dogs were females and two were males. The positive dogs were of different ages, from 1 to 6 years old. None of the seropositive dogs had any visible skin or eye lesions, although two of them had poor body condition.

DISCUSSION

During the previous 13 years, several similar studies were performed on dogs in Serbia (Table 2). In a study from 2007, a group of 16 dogs that lived in the same household was examined. Three of them had arrived from Greece several years earlier, and they had cachexia, skin lesions and lameness (Savić et al., 2013). They were seropositive against *Leishmania* spp. Another four dogs without a history of travel were also seropositive. In total, 7 of 16 dogs (43%) were found seropositive for *Leishmania* (Savić et al., 2013).

The same year (2007), another group of seven dogs living together was examined, two of which were seropositive against *Leishmania* spp. One of the positive dogs (male) had constantly travelled all over Europe for dog shows, but the other (female) had never left Serbia. They both had epistaxis, skin, and eye lesions. These two dogs lived very closely in the same household. The other five dogs were seronegative, but they were not in constant contact with two positive dogs and they lived in an outside kennel (Savić-Jevđenić et al., 2007).

During the period 2008-2010, 23 dogs were examined for the presence of specific antibodies against *Leishmania* spp., and most of them (21) had spent a short time (3-10 days) in Mediterranean countries (Italy, Montenegro, Greece), while two dogs had never left Serbia. Dogs that had spent time abroad were checked upon their return to Serbia and were seronegative for *Leishmania*. Interestingly, the two dogs that never travelled had clinical symptoms of canine leishmaniosis and positive serology findings. Clinical symptoms were epistaxis, cachexia, pale mucosa, skin lesions and blindness (Savić et al., 2010).

Another study in Serbia was performed in 2014. In that study, 10.6% of 170 sera samples from randomly chosen dogs with no clinical symptoms were seropositive for specific antibodies against *Leishmania* spp. (Savić et al., 2015).

Table 2. *Leishmania*-seropositive dogs according to ELISA test in Serbia, 2007-2020.

Year	No. of positive dogs Total no. of dogs examined (%)	Location	Reference
2007	2/7 (28.6)	Sremski Karlovci	Savić-Jevđenić et al., 2007
2007	7/16 (43.7)	Čenej	Savić et al., 2013
2008-2010	2/23 (8.7)	Novi Sad	Savić et al., 2010
2014	18/170 (10.6)	Novi Sad and surrounding area	Savić et al., 2015

During the period from 2007 to 2020, including the current study, 552 dog serum samples were analysed for the presence of antibodies against *Leishmania* spp. Dogs were housed differently (pets, military, hunting, shelters) and lived in various conditions (in houses, yards, pens, shelters). To the best of our knowledge, at the time of sampling, sandflies were not identified in the locations that were studied, even though they were present in the wider region. Seropositive samples for *Leishmania* came from dogs that travelled abroad, but also from dogs that lived together with *Leishmania*-seropositive dogs or from dogs that had never travelled anywhere. The present study is important and specific, as dogs from the shelters we investigated had no record of traveling out of Serbia, at least to the best of our knowledge. The fact that seropositive cases were found in shelters that did not use prophylactic measures against arthropods indicates the source of infection could be the local population of sandflies infected with *Leishmania* spp. As the number of seropositive dogs was low, it is possible to hypothesise that the population of sandflies is not dense or that those infected with *Leishmania* spp. are relatively rare.

Although the seroprevalence of *Leishmania* spp. in this study was low, a comprehensive analysis of the history of our research on the seroprevalence of this parasite highlights the need for active surveillance of this disease among the dogs as potential reservoirs.

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Authors' contributions

DM was in charge of organising the sampling of material and communication with shelters; MŽS and SM were involved in sampling and laboratory analysis; IP was involved in sampling; AP planned the study design; SO was involved in writing the manuscript; SS was involved in sampling, laboratory analysis and writing of the manuscript.

Competing interests

The authors declare that they have no competing interests.

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SEROPREVALENCIJA *LEISHMANIA* SPP. KOD PASA U SEVERNOJ SRBIJI

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Kratak sadržaj

Lajšmanioza je zoonozno oboljenje koje izazivaju paraziti tipa Lajšmanija (*Leishmania* spp.), a Srbija je bila endemsko područje do druge polovine 20. veka. Danas je lajšmanioza pasa emergentna vektorska bolest u Srbiji koja se ponovo ljavlja. Širenje oboljenja je moguće zbog prisustva vektora *Phlebotominae* spp. u regionu. Cilj ovog istraživanja je bio da se utvrdi seroprevalenca *Leishmania* spp. kod pasa koji žive u prihvatilištima u Srbiji i da se uporede nalazi kod onih koji koriste i koji ne koriste profilaktičke mere protiv artropodnih vektora. U istraživanje je bilo uključeno 336 pasa iz dva regiona u Srbiji tokom perioda 2019–2020. Za određivanje prevalencije seropozitivnih pasa korišćen je komercijalni ELISA test. U prihvatilištima gde su se primenjivale profilaktičke mere zaštite pasa od vektora psi su bili seronegativni (n=52), dok je u onima gde se nisu primenjivale mere 2,1% pasa (6/284) bilo seropozitivno na *Leishmania* spp. Zaključujemo da prisustvo seropozitivnih pasa na *Leishmania* spp. u prihvatilištima koja ne primenjuju profilaktičke mere zaštite mogu biti indikator da je lokalna populacija mušica izvor *Leishmania* spp. parazita.

Ključne reči: lajšmanioza, zoonoze, vektorske bolesti, dijagnostika