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Full Research Article

A SEROLOGICAL SURVEY OF CANINE LEPTOSPIROSIS IN THE CITY OF BELGRADE, SERBIA

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Abstract

Canine leptospirosis is a zoonosis caused by bacteria belonging to the genus *Leptospira*. Dogs are one of the animal species involved in the cycle of preservation and transmission of leptospirosis in urban areas. Serological testing for the presence of specific antibodies against *Leptospira* spp. in dogs was continuously performed between 2010 and 2020 in the city of Belgrade. At the request of the owners themselves, other veterinary laboratories or laboratory clinics, 179 blood sera from 179 dogs were examined in the Laboratory for Immunology, Scientific Institute of Veterinary Medicine of Serbia. Blood sera samples from dogs were examined using the standard microscopic agglutination test (MAT) for the presence of specific antibodies against seven different serovars of *Leptospira*: *Pomona*, *Icterohaemorrhagiae*, *Grippotyphosa*, *Sejroe*, *Canicola*, *Bataviae*, and *Australis*. The number of seropositive dogs was 17/179 (9.5%). Among all examined sera, the highest titre of seropositive samples was to serovar *Icterohaemorrhagiae* (10/17, 58.8%), followed by *Pomona* (4/17, 23.5%), and serovar *Canicola* (3/17, 17.6%). Specific antibodies for serovars *Grippotyphosa*, *Sejroe*, *Bataviae* and *Australis* were not detected in any of the dog sera. Cross-reaction (the presence of two or three titres with different values where one of them was higher than others) between different serovars was diagnosed in a low number of sera (n=4), with the following serovars: *Icterohaemorrhagiae* and *Pomona* (n=3) and *Pomona* and *Canicola* (n=1). The confirmed specific antibody titres for *Leptospira* spp. were between 1:100 to 1:3000 (5 sera had titres of 1:100, 7 had titres of 1:300, 4 had titres of 1:1000, and 1 serum had a titre 1:3000). Monitoring canine leptospirosis is a useful tool in preventing leptospirosis in Belgrade.

Key words: Belgrade, dogs, *Leptospira* spp., MAT

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INTRODUCTION

On a global scale, leptospirosis is one of the most widespread zoonotic diseases (Adler and de la Peña Moctezuma, 2010; Langston and Heuter, 2003). The causative agent of leptospirosis is a pathogenic, Gram negative, spirochete bacterium from the *Leptospiraceae* family, genus *Leptospira.* Pathogenic *Leptospira* are divided into 25 serogroups, with more than 300 serovars (Picardeau, 2013). *Leptospira* survives in moist soil and standing waters, and the cycle of transmission involves susceptible species, natural reservoirs, and the environment. Risk factors for the occurrence of leptospirosis are deficient hygiene, water spills due to increased rainfalls, floods, high temperatures, increase in animal population density including reservoir species, inadequate waste disposal, poor drainage, and sewers (Ebi and Schmier, 2005; Levett, 2001). The presence of *Leptospira* reservoirs, i.e. the field mouse (*Apodemus agrarius*) and the common vole (*Microtus arvalis*) in high densities in fields, as well as the grey rat (*Rattus norvegicus*), the black rat (*Rattus rattus*), and the house mouse (*Mus musculus*) from the immediate farm and human environment present a constant threat for leptospiral infection of domestic animals and humans (Stanojević et al., 2003).

From an epizootiological aspect, dogs in urban areas are significant animal species included in the natural leptospirosis cycle and transmission of the pathogen to humans. Certain *Leptospira* serovars are host specific. In dogs, serovars *Canicola* and *Icterohaemorrhagiae* are commonly present, although recently, there was a report of increasing detections of uncharacteristic *Leptospira* spp., for which dogs are not recognized as the primary hosts (Samokovlija et al., 2010).

The course of canine leptospirosis can be peracute, acute (most common), subacute, or chronic. The clinical symptoms include fever, dehydration, and inflammatory processes in the muscles, meninges, or kidneys. Moreover, a loss of appetite, abdominal pain, icterus, nosebleeds, fatigue, and/or hind leg paralysis is commonly seen in the diseased dogs (Valčić et al., 2014). Dogs that recover occasionally excrete *Leptospira* in their urine for a very long time, several months to two years after the infection, and they can also be lifelong carriers (Dmitrović, 2002; Faine et al., 1999).

Differing *Leptospira* serovars can be present depending on the geographical region. In Europe, the most common serovars in dogs are *Icterohaemorrhagiae*, *Grippotyphosa, Bratislava*, *Pomona*, and *Canicola* (Ellis, 2010). The first research on leptospirosis in Serbia began after World War II. In Serbia, dog leptospirosis was first described as caused by serovar *Sejröe* (Trbić and Paunović 1955), and positive serological tests for antibodies against leptospirosis were found in 43.2% of dogs in Belgrade and Pančevo (Trbić and Vučković, 1959). Between 1975 and 1980, serological tests were performed on 844 blood sera from dogs in the territory of Belgrade in five urban districts, revealing 67 (7.94) sera from dogs were positive for *Leptospira* antibodies, and *Icterohaemorrhagiae* was the most common serovar (Trifunović et al., 1977). A more recent study of 317 blood sera from street dogs confirmed the following serovars: *Icterohaemorrhagiae* (17,

5.36%), *Pomona* (6, 1.89%), *Canicola* (2, 0.63%), *Grippotyphosa* (1, 0.31%) and *Bataviae* (1, 0.31%) (Elezović et al., 2011).

In other European countries, many studies showed the frequent occurrence of dog leptospirosis. In Berlin, Germany, serological research was conducted on 329 blood sera from dogs from small animal clinics (Mayer-Scholl et al., 2013). Microscopic agglutination tests (MAT) showed serogroup *Australis* (24%) was predominant, followed by *Grippotyphosa* (20%) and *Pomona* (9%) (Mayer Scholl et al., 2013). Serological examination of 3028 blood sera from dogs in Italy with MAT showed a seroprevalence of 29.9% (904 positive agglutinations in tested dogs). The leading serovar was *Bratislava* (serogroup Australis), found in 39.3% of dogs (Tagliabue, et. al 2016). In Switzerland, the examination of 377 blood sera from dogs resulted in positive MAT for serovars *Australis* (14.9%) and *Bratislava* (8.8%) (Delaude et al., 2017).

Considering the recorded occurrence of leptospirosis in dogs in Serbia, the aim of this paper was to present data on seropositivity and the presence of examined serovars of *Leptospira* spp. in dogs living in the city of Belgrade in the period from 2010 to 2020.

MATERIALS AND METHODS

Whole blood samples from dogs living in Belgrade were submitted to the Laboratory of Immunology of the Scientific Institute of Veterinary Medicine of Serbia, Belgrade in the period from 2010 to 2020. Samples were examined at the request of owners or were submitted by other veterinary laboratories or small animal veterinary clinics. Only one sample per dog was submitted for examination upon the reasonable suspicion of canine leptospirosis (dogs presenting sudden fever, stiffness in muscles and legs, weakness, depression, lack of appetite, vomiting, diarrhoea, petechiae, yellow skin, mild swelling of the lymph nodes, difficulties in breathing). Therefore, there was no possibility for follow-up or repetitive diagnostics. Furthermore, there are no available data on vaccination against canine leptospirosis. Blood sera were extracted from the whole blood samples and analysed.

To detect specific antibodies against *Leptospira*, the standard serological diagnostic MAT (OIE, 2019) was used. Serological examination was performed using a panel of seven *Leptospira* serovars: *Pomona*, *Icterohaemorrhagiae*, *Grippotyphosa*, *Sejroe*, *Canicola*, *Bataviae*, and *Australis*.

The cut-off titre for positive samples was 1:100, and this was also used as a preliminary blood serum test. After the preliminary test, those sera with specific *Leptospira* spp. antibodies were examined, and the titre values were determined for sera dilutions 1:300, 1:1000, 1:3000. The reaction was visualized on an Axiostar plus dark field microscope, Carl Zeiss, Jena, Germany.

RESULTS

We examined 179 dog serum samples (each from an individual dog), of which 17/179 (9.5%) were seropositive for antibodies against *Leptospira* spp. Table 1 shows the number of examined and the number of positive sera from 2010 to 2020.

Table 1. Total number of examined sera from individual dogs, seropositive sera and the percentage of seropositive dog sera between 2010 and 2020.

Year	Dog sera (No.)	Seropositive dog sera (No.)	Seropositive dog sera (%)
2010	10	0	0
2011	14	1	7.1
2012	13	3	23
2013	15	1	6.6
2014	10	1	10
2015	12	0	0
2016	10	0	0
2017	24	4	16.6
2018	17	2	11.8
2019	41	4	9.7
2020	13	1	7.7
Total	**179**	**17**	**9.5**

Among all examined sera, the highest titre of seropositive samples was to *Icterohaemorrhagiae* (10/17, 58.8%), followed by *Pomona* (4/17, 23.5%), and serovar *Canicola* (3/17, 17.6%) (Table 2). Specific antibodies for serovars *Grippotyphosa*, *Sejroe*, *Bataviae* and *Australis* were not diagnosed in any of the dog sera.

Table 2. The number and percentage of dog sera seropositive for the examined *Leptospira* spp.

***Leptospira* spp. serovar**	**Seropositive dog sera (No.)**	**Seropositive dog sera (%)**
Pomona	4	23.5
Icterohaemorrhagiae	10	58.8
Grippotyphosa	0	0
Sejroe	0	0
Canicola	3	17.6
Bataviae	0	0
Australis	0	0
Total	**17**	**9.5**

The confirmed specific antibody titres for *Leptospira* spp. were between 1:100 and 1:3000 (5 sera had titres of 1:100, 7 had titres of 1:300, 4 had titres of 1:1000, and 1 serum had a titre of 1:3000).

Cross-reaction (the presence of two or three serovar reacting titres with different values, where one of them was higher than others) between different serovars was diagnosed in a low number of sera (n=4), with the following serovars: *Icterohaemorrhagiae* and *Pomona* (n=3) and *Pomona* and *Canicola* (n=1).

DISCUSSION

In our study, we found 17/179 (9.5%) of dog sera from Belgrade, Serbia, were seropositive to at least one of the *Leptospira* serovars examined. This rate of seropositivity was lower than those in Bulgaria (57.9%) (Sabev et al. 2015), Spain (25.8%) (López et al., 2019), Croatia (23.9%) (Slavica et al., 2016) and Bosnia and Herzegovina (22.3%) (Lindtner Knific et al., 2019), even though the examined population types of dogs differed. This might be explained by the number of sera/dogs that were analysed as well as by the fact that most of the dogs included in this study were living in flats and so were presumably without constant exposure to *Leptospira*, even though they demonstrated clinical symptoms possibly attributed to canine leptospirosis. Furthermore, we lack data about previous vaccination against canine leptospirosis in the examined dogs. Therefore, some of the positive results might also be due to the presence of vaccinal antibodies.

Examination of dog sera for specific antibodies against *Leptospira* spp. in this study proved the long-lasting presence of serovar *Icterohaemorrhagiae* in Belgrade dogs, followed by *Pomona* and *Canicola*. The serovar *Icterohaemorrhagiae* was previously recorded as the most abundant (Vojinović et al., 2015; Elezović, et al. 2011; Trifunović, et al., 1977). Dogs in urban areas, and presumably in Belgrade too, can have frequent contact with rodents and their excreta. Since rodents are reservoirs for serovar *Icterohaemorrhagiae* (Vukićević et al., 1999), this might offer an explanation as to why this serovar predominated in our study. However, vaccination against canine leptospirosis, for which we have no data, might also be a possible explanation. The results shown in this research regarding the prevalence of serovar *Icterohaemorrhagiae* are in compliance with results by López et al. (2019), Vojinović et al. (2015) and Sabev et al. (2015). Historically, the dominant serovar in Belgrade's dog population was *Icterohaemorrhagiae*. The same is true for the swine population in the territory of Belgrade, in which this serovar was noted as one of the most frequent serovars (Vojinović et al., 2014). This speaks in favour of the spread and importance of *Icterohaemorrhagiae* as a causative agent of leptospirosis in the territory of Belgrade.

Similar results of serovar composition and prevalence were described in other European cities. For example, in Sofia (Bulgaria), authors found serovars *Icterohaemorrhagiae* (52.3%) and *Canicola* (43.1%) in blood sera from stray dogs to be the most common

(Sabev et al. 2015). In Spain between 2015 and 2017, the most common serovar in owned dogs was *Icterohaemorrhagiae* at 19.4% among 1310 blood sera from dogs examined by MAT with a panel of eight *Leptospira* serovars (López et al., 2019).

Regarding our neighbouring countries, in Bosnia and Herzegovina and Croatia, serovar *Pomona* was the most frequently found. A greater number of dogs that tested positive for antibodies against *Pomona* serovar was observed also in our current study compared with previous studies in Belgrade. In Bosnia and Herzegovina, authors analysed 300 blood sera from dogs in three categories from 12 cities. MAT with a panel of 12 *Leptospira* serovars was used, and for eight of them, seropositive antibodies were confirmed. Altogether 22.3% (67/300) dogs were seropositive, and peak seropositivity was confirmed for serovar *Pomona* (38, 42.7%) (Lindtner Knific et al., 2019). In Croatia between 2006 to 2015, 535 sera from domestic carnivores (dogs and cats) were examined for 12 different *Leptospira* serovars by MAT (Slavica et al., 2016). They established a seroprevalence of 23.9%, and the most common serovars in dog populations were *Pomona* (30.8%), *Icterohaemorrhagiae* (20.1%), and *Grippotyphosa* (20.1%) (Slavica et al., 2016).

Cross-reaction between serovars, otherwise commonly present in leptospirosis diagnostics by MAT test (Adler and Faine 1978), was established in a small number of our dog sera (n=4): serovar *Icterohaemorrhagiae* and serovar *Pomona* (n=3) and serovars *Pomona* and *Canicola* (n=1). Interpretation of the results can be difficult due to cross-reactivity between serovars, especially in the acute phase of the disease (Levett, 2001). In order to be sure of the diagnosis of acute infection in positive sera, it is necessary to examine paired sera and determine a fourfold and higher increase in antibody titre. This was, unfortunately, not possible in this study. Nonetheless, in the case of serum from a dog with the clinical symptoms testing positive for the presence of antibodies against *Leptospira* spp., it is necessary to examine paired sera and monitor the level of antibody titre to determine if the obtained results actually indicate acute canine leptospirosis or not.

CONCLUSION

In the Belgrade area in the period from 2010 to 2020, 17/179 (9.5%) of submitted dog sera (each from an individual dog) were seropositive for *Leptospira* spp. by MAT. As the number of dogs in Belgrade increases, we recommend this population (especially stray and hunting dogs) be examined in a larger scale epizootiological monitoring programme, considering the potential threat to human health. Furthermore, suitable collaboration with veterinarians in the field needs to be established to ensure adequate sample collection when leptospirosis is suspected.

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Authors' contributions

VD, ŽJ, performed the analyses, VD and VA designed the study and drafted the manuscript, SS, ZSZ and SLJ critically reviewed the manuscript.

Competing interests

The author(s) declare that they have no competing interests.

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SEROLOŠKO ISPITIVANJE LEPTOSPIROZE PASA NA TERITORIJI GRADA BEOGRADA, SRBIJA

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Kratak sadržaj

Leptospiroza pasa je zoonoza izazvana bakterijom iz roda *Leptospira*. Psi u gradskim sredinama su važni u očuvanju i prenošenju leptopsiroze. Serološka ispitivanja na prisustvo specifičnih antitela na *Leptospira* spp. kod pasa na teritoriji grada Beograda su kontinuirano vršena u periodu između 2010. i 2020. godine. Na zahtev samih vlasnika, drugih veterinarskih laboratorija i veterinarskih klinika, ispitano je 179 krvnih seruma pasa u Laboratoriji za imunologiju Naučnog instituta za veterinarstvo Srbije. Krvni uzorci pasa pregledani su testom mikroskopske aglutinacije (MAT) na prisustvo specifičnih antitela na sedam različitih serovarijeteta (sv.) *Leptopsira* spp.: *Pomona*, *Icterohaemorrhagiae*, *Grippotyphosa*, *Sejroe*, *Canicola*, *Bataviae* i *Australis*. Broj seropozitivnih pasa je iznosio 17/179 (9,5%). Među svim ispitanim serumima, najveći broj seropozitivnih uzoraka na serovarijetet *Icterohaemorrhagiae* – 10/17 (58,8%), praćen serovarijetetom *Pomona* – 4/17 (23,5%) i serovarijetetom *Canicola* – 3/17 (17,6%). Specifična antitela na serovarijete *Grippothyphosa*, *Sejroe*, *Bataviae* i *Australis* nisu otkrivena u ispitanim krvnim serumima pasa. Unakrsna reakcija između različitih serovarijeteta u jednom serumu je bila dijagnostikovana u malom broju uzoraka (n=4) sa sledećim serovarijetetima: *Icterohaemorrhagiae* i *Pomona* (n=3) i *Pomona* i *Canicola* (n=1). Ustanovljeni titar specifičnih antitela na *Leptospira* spp. kretao se od 1:100 do 1:3000 (5 uzoraka titra 1:100, 7 titra 1:300, 4 titra 1:1000 i 1 uzorak titra 1:3000). Praćenje leptospiroze pasa je koristan postupak u prevenciji pojave leptospiroze u Beogradu.

Ključne reči: Beograd, psi, *Leptospira* spp., MAT