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Research article

SEROPREVALENCE OF SEROVARS OF PATHOGENIC LEPTOSPIRA IN DOGS AND RED FOXES *(VULPES VULPES)* FROM BOSNIA AND HERZEGOVINA

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The aim of this study was to examine the seroprevalence of *Leptospira* spp. in dogs and red foxes in the entity of the Republic of Srpska, Bosnia and Herzegovina, after heavy rainfall and floods in 2014 and for the two years thereafter. The seroepidemiological study involved testing serum samples from dogs (n = 98) and foxes (n = 112) using MAT (microscopic agglutination test).

Antibodies to at least one *Leptospira* spp. serovar were found in 52.04% of the tested dogs. The dog seroprevalence in 2014 (81.25%) was significantly higher than in 2015 (51.42% $p < 0.0001$) and 2016 (22.5% $p < 0.05$). The highest seroprevalences were for serovars Australis (76.47%), Bratislava (70.58%), Sejroe (66.67%) and Autumnalis (45.09%).

Antibodies to at least one *Leptospira* spp. serovar were detected in 34.82% of the examined red foxes. In 2015, the fox seroprevalence was significantly higher (52.94%) than in 2016 (6.82%) ($p < 0.0001$). The highest seroprevalences were for serovars Sejroe (64.10%), Bratislava (48.72%), Australis (43.59%) and Bataviae (25.64%).

The high seroprevalence of *Leptospira* spp. in dogs and foxes determined during this study indicates the importance of these carnivores in maintaining leptospirosis in the study area, and the potential risk of infection for humans and other animal species that come into contact with these canids. The results obtained indicate that heavy rainfall and intense floods can result in increased *Leptospira* spp. infection in these canids.

Keywords: Leptospirosis; dogs, foxes, MAT, flooding

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INTRODUCTION

Leptospirosis, an infectious disease of many species of animals including humans and one of the most widespread zoonoses in the world, is caused by pathogenic spirochetes of the genus *Leptospira*. Leptospirosis is considered a seasonal disease, with human and animal outbreaks being linked among other things, also to heavy rainfall or flooding [1,2]. Each country, depending on climatic, geological and ecological factors, can host leptospirosis in enzootic areas. *Leptospira* spp. maintain their life cycle by circulating in the environment among domestic and wild animals, which puts them in the group of diseases with natural foci. Although *Leptospira* spp. can survive in humid environments, the main source that promotes their maintenance in the environment is a wide range of domestic and wild animals, in which the spirochaetes inhabit the proximal renal tubules [1]. Small rodents are considered the most important reservoir hosts. However, it is likely that any known species of rodent or mammal, including humans, can act as a reservoir for *Leptospira* spp. [1,3].

Leptospirae are excreted from the kidneys by animal urine, thus contaminating soil, surface waters, rivers and streams, wherein they can survive for weeks and months. *Leptospira* spp. infection occurs through injuries on the skin and mucous membranes during the contact with contaminated urine, aborted fetus tissue, sexual secretions, ingestion of contaminated water or infected tissues, or indirectly, by contact with a contaminated environment. In dogs in endemic areas, the infection usually occurs as a result of contact with water in rivers, lakes or streams that is contaminated with *Leptospirae* or contact with rodents (both in rural and urban areas) [4-8]. However, in rural areas, contact with wildlife that has access to human habitats is also important in the spread of leptospirosis. It is thought that dogs could be important for infection by creating an epidemiological link between reservoir wildlife and humans [8]. At the same time, dogs can serve as indicators of the presence of leptospires in specific environments. The control of leptospirosis, therefore, is important not only from an animal but also from a public health point of view. However, data on the epidemiology of *Leptospira* spp. infections in animals and relevant public health data are lacking in many parts of the world [9].

Red foxes (*Vulpes vulpes*) are the most common and widespread wild carnivores in Europe, and they are known hosts of many pathogens of wild and domestic animals and humans. Many of these pathogenic agents cause severe diseases in domestic animals and can pose a significant threat to public health [10]. Red foxes are considered an epizootiologically important species in the spread of leptospirosis, bearing in mind the characteristics of the fox life cycle, ie. high reproductive potential, migration of young individuals, easy adaptation to different habitats, and diet (since foxes feed on small rodents that are an important reservoir of *Leptospira* spp.) [11,12].

The incidence of leptospirosis is decreasing in many developed countries, which is most likely related to preventive measures that include rodent population control, application of veterinary and general hygiene regulations and disease control in domestic animals. With the application of these measures, leptospirosis as a disease predominantly occurs in rural areas with the potential to spread after heavy rainfall [13]. The presence of leptospirosis in cattle, pigs, sheep and dogs has been studied to a limited extent in Bosnia and Herzegovina, but for many geographic areas, there are no data on territorial distribution, number of infected animals or the presence of *Leptospira* spp. serotypes that cause infections in animals [14]. In Bosnia and Herzegovina (BiH, which comprises two entities), data on the seroprevalence of canine leptospirosis exist for the entity of the Federation of Bosnia and Herzegovina, while in the entity of the Republic of Srpska, these tests have not been conducted and no data exists. Additionally, this is the first study on the presence of *Leptospira* spp. in the red fox population in BiH. In mid-May 2014, heavy rainfall caused the most serious rain precipitation event in the last 120 years throughout BiH. In 48 h, in some areas of BiH, about 150 l/m^2 of rain fell, which led to large floods in the basins of important rivers: the rivers Sava, Bosnia, Drina and others [15]. The situation posed a high risk of promoting the spread of many infectious diseases in populations of domestic and wild animals.

The aims of this study were to determine, in parts of the Republic of Srpska from 2014 to 2016, the seroprevalence of leptospirosis in shelter dogs and wild foxes and to determine the pathogenic *Leptospira* serovars involved.

MATERIALS AND METHODS

Study area

BiH is located in the western part of the Balkan Peninsula and consists of two entities, the Republic of Srpska and the Federation of BiH. The Republic of Srpska is located in the northeastern part of BiH (42° 33’ and 45° 16’ north latitude and 16° 11’ and 19° 37’ east longitude) and covers an area of 25,053 km^2, about 49% of BiH land mass. It borders Croatia to the north, and Serbia and Montenegro to the east. The Republic of Srpska has numerous river watercourses that are a natural barrier between it and Croatia and Serbia. The climate of Republic of Srpska ranges from temperate continental to Mediterranean. Summers are warm and winters are mostly moderately cold, with an average annual temperature above 10°C. Precipitation is mostly evenly distributed with the highest amount in May to June. The average annual rainfall ranges from 700-1000 mm.

Study design and samples

Blood samples were taken from abandoned dogs of different sexes, breeds and ages (n = 98) that resided in two public dog shelters in the Banja Luka-Lijevče area. The sampled dogs included in the study were different every year and were from Republic of Srpska. The blood of every dog was sampled and analyzed only once. Dog blood was sampled in September 2014 (n = 32), from September to November 2015 (n =

35) and from September to December 2016 (n = 31). There are usually 70-90 dogs in these public shelters, which contain dog kennels. When dogs are admitted to a public shelter, they are isolated in individual pens for ten days, and undergo a general clinical examination to determine their health status. On that occasion, antihelminthic treatment and anti-rabies vaccination are administered, and dogs that are put up for adoption are surgically sterilized. After treatment, dogs enter the adoption procedure or are released onto the streets. During the general clinical examination, 2 ml of blood was sampled aseptically by cephalic vein puncture using vacutainers to determine whether dogs to be adopted had parasitic infections (heartworm, piroplasmosis). Some of the blood samples were submitted to the Veterinary Institute in Banja Luka for *Leptospira* spp. analysis, since in 2014, conditions were favorable for the spread of leptospirosis.

There were no data for any of the dogs on their health status, breed, sex or age, and their vaccination histories were unknown. Given that the blood samples were sampled and submitted for standard diagnostic purposes, no ethical approval was required.

Fox blood samples (n = 112) were collected from January to March 2015 (n = 68) and during 2016 (n = 44). The foxes came from six geographic areas in the Republic of Srpska: Banja Luka-Lijevče (n = 35), Visoka Krajina (n = 8), and western (n = 20), central (n = 28), eastern (n = 12) and southern (n = 9) areas (Figure 1).

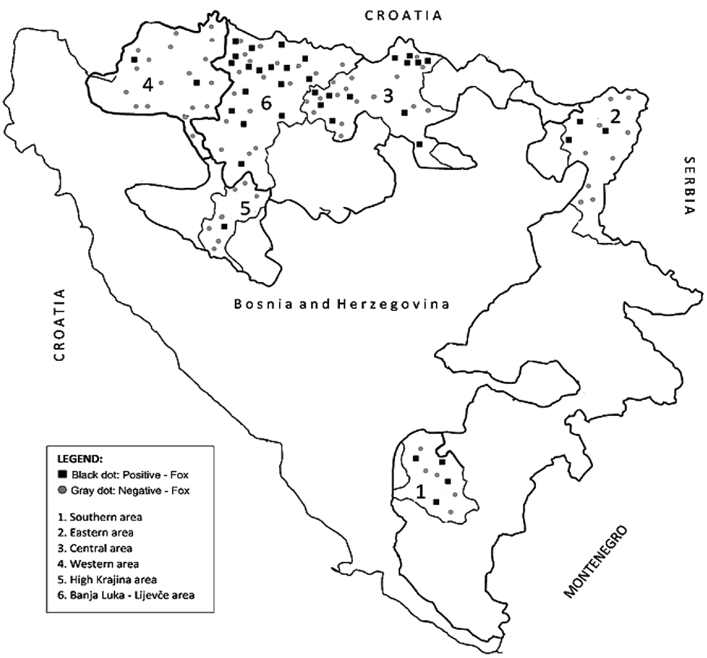

Figure 1. Geographical location of *Leptospira* spp. antibody-negative and -positive red foxes (*Vulpes vulpes*) detected by microscopic agglutination test in the Republic of Srpska, Bosnia and Herzegovina from 2015 to 2016.

Blood was also sampled from the thoracic cavity or heart of animals culled primarily through the rabies monitoring program. The authors declare that no animals were killed for the purpose of this study and that all procedures contributing to this work met the ethical standards of the relevant national and European regulations on the care and use of animals (Directive 2010/63/EC).

Blood samples were centrifuged at 2,000 *g* for 10 minutes, and the sera obtained were stored at -20 °C until analysis.

Serology

Sera were thawed and then examined for the presence of antibodies against pathogenic *Leptospira* by microscopic agglutination test (MAT) according to OIE standards (Office International des Epizooties) [16]. For MAT testing, live cultures of 10 reference strains of *Leptospira* spp. belonging to 8 serogroups were used: Pomona (Pomona strain, Pomona serogroup), Australis (Ballico strain, Australis serogroup), Icterohaemorrhagiae (RGA strain, Icterohaemorrhagiae serogroup), Grippotyphosa (Moskva V strain, Grippotyphosa serogroup), Canicola (Hond Utrecht IV strain, Canicola serogroup), Sejroe (M84 strain, Sejroe serogroup), Bataviae (Swart strain, Bataviae serogroup), Hardjo (Hardjobovis, Sejroe serogroup), Bratislava (Jez strain, Australis serogroup) and Autumnalis (strain Akiyami A, Autumnalis serogroup) (National Collaborating Centre for Reference and Research on Leptospirosis, Royal Tropical Institute (KIT), Amsterdam, the Netherlands).

Each live *Leptospira* serovar was cultivated in 10 ml of Ellinghausen–McCullough–Johnson–Harris (EMJH) medium at 28 ± 1°C for at least 5 but no more than 8 days depending on the serovar. The resulting bacterial concentration was approximately $3x10^8$ *Leptospira*/ml and was determined according to the McFarland scale. Sera were diluted 1:50. The same amount of antigen and diluted serum was placed in the wells of a microtiter plate to give a final dilution of 1:100 in the screening test. The plates were incubated at 28 ± 1°C for 2-4 h and subsequently examined by dark-field microscopy. Titers were defined as the highest serum dilution in which ≥50% of the added antigen suspension was agglutinated. Anti-*Leptospira* positive dog serum (Veterinary Institute, Croatia) and reference positive rabbit serum (Royal Tropical Institute, Amsterdam, Netherland) were used as positive controls.

Sera that were positive in a 1:100 titer for a particular *Leptospira* serotype were further tested in double serial dilutions from 1:100 to 1:12800. These tests were conducted in the Serological Laboratory of the Dr Vaso Butozan Veterinary Institute of the Republic of Srpska in Banja Luka.

Statistical analysis

In each group, the seroprevalence was calculated as the number of animals with *Leptospira*-positive sera divided by the total number of animals studied. Chi-square (χ2) test and Fisher's exact test were used to determine the statistical significance of seroprevalence differences between animal species and years. In all the analyses, the confidence level was maintained at 95% and statistical analyses were considered significant at $p<0.05$. Statistical Package for Social Sciences (SPSS) v23 (IBM SPSS Statistics, Armonk, NY) and Microsoft Office Excel 2010 (Microsoft Corp., Redmond, WA, USA) were used for statistics processing.

RESULTS

Seroprevalence in Dogs

Antibodies to the tested *Leptospira* spp. serovars were found in 52.4% (51/98) of the dogs examined. The highest seroprevalence in dogs, 81.25% (26/32), occurred in 2014, and this was significantly higher than in both 2015 ($p <0.05$; 51.42%; 18/35) and 2016 ($p <0.0001$; 22.58%). The seroprevalence in 2015 was also significantly higher than in 2016 ($p <0.05$). The overall prevalences of the serovars in dogs were: Australis 76.47% (39/51), Bratislava 70.59% (36/51), Sejroe 66.67% (34/51) Autumnalis 45.09% (23/51), Pomona 31.37% (16/51), Grippotyphosa 17.65% (9/51), Icterohaemorrhagiae 5.88% (3/51), Canicola 5.88% (3/51) and Bataviae 3.92% 2/51). None of the dogs were positive for serovar Hardjo (Table 1).

In 2014, the most prevalent serovars in dogs were Australis (88.5%), Bratislava (69.23%), Sejroe (69.23%), Autumnalis (57.69%) and Pomona (42.30%) (Table 1). Antibodies to three or more serovars were found in 61.5% (16/26) of the dog sera samples. Antibody titers from 1/100 to 1/400 were registered in 46.15% (12/26) of the dog sera samples and titres from 1/800 to 1/6400 in 53.84% (14/26) of the dog sera samples.

In 2015, the most prevalent serovars in dogs were: Sejroe 83.33% (15/18), Australis 77.8%, (14/18), Bratislava 72.2% (13/18) and Autumnalis 33.33% (6/18). Serovar Pomona was significantly less prevalent, detected in 16.66% (3/18) of the dogs. Serovar Bataviae was registered for the first time in one animal (5.55%; 1/18) (Table 1). The presence of antibodies to two serovars was determined in 16.67% (3/18) of the dog sera samples. Antibody titers from 1/100 to 1/400 were registered in 77.77% (14/18) of the dog sera samples and antibody titers from 1/800 to 1/1600 in 22.22% (4/18) of the dog sera samples.

In 2016, serovar Bratislava was the most prevalent serovar in dogs (71.4%; 5/7). Serovar Canicola was detected for the first time in three dogs (42.86%, 3/7), while antibodies to Australis, Pomona, Grippotyphos and Autumunalis were detected in only two dogs (28.6%), and serovars Sejroe and Bataviae were each detected in one animal only (Table 1). Antibody titers <1/800 were found in 71.42% (5/7) of dog sera samples and titers from 1/800 to 1/3200 in 28.57% (2/7) of dog sera samples.

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Table 1. Number (percentage) of positive reactions and titres in dogs to *Leptospira* spp. serovars, tested by microscopic agglutination test during 2014-2016 in the Republic of Srpska, Bosnia and Herzegovina

***Leptospira* spp. serovars**	**Positive reactions**	**Titre**						
	***N* (%)**	**100**	**200**	**400**	**800**	**1600**	**3200**	**6400**
Australis*	23 (88.46)	5	3	2	2	6	1	4
**	14 (77.77)	3	4	5	1	1	-	-
***	2 (28.57)	0	2	-	-	-	-	-
Total	39 (76.47)	8	9	7	3	7	1	4
Sejroe*	18 (69,23)	5	4	3	4	2	-	-
**	15 (83.33)	2	6	5	1	1	-	-
***	1 (14.28)	-	-	1	-	-	-	-
Total	34 (66.67)	7	10	9	5	3	-	-
Icterrohaemorhagiae*	3 (11.54)	-	1	1	1	-	-	-
**	-	-	-	-	-	-	-	-
I***	-	-	-	-	-	-	-	-
Total	3 (5.88)	-	1	1	1	-	-	-
Grippotyphosa*	6(23.07)	2	2	1	1	-	-	-
**	1 (5.55)	1	-	-	-	-	-	-
***	2 (28.57)	1	1	-	-	-	-	-
Total	9 (17.65)	4	3	1	1	-	-	-
Bratislava*	18 (69.23)	1	2	5	3	4	2	1
**	13 (72.22)	4	5	1	3	-	-	-
***	5 (71.43)	0	1	3	0	0	1	-
Total	36 (70.59)	5	8	9	6	4	3	1
Pomona*	11 (42.30)	3	6	1	1	-	-	-
**	3 (16.67)	1	1	-	-	1	-	-
***	2 (28.57)	-	1	-	-	1	-	-
Total	16 (31.37)	4	8	1	1	2	-	-
Bataviae*	-	-	-	-	-	-	-	-
**	1 (5.55)	-	1	-	-	-	-	-
***	1(14.28)	-	1	-	-	-	-	-
Total	2 (3.92)	-	2	-	-	-	-	-
Canicola*	-	-	-	-	-	-	-	-
**	-	-	-	-	-	-	-	-
***	3 (42.86)	2	1	-	-	-	-	-
Total	3 (5.88)	2	1	-	-	-	-	-
Autumnalis*	15 (57.69)	4	6	4	1	-	-	-
**	6 (33.33)	3	-	1	1	1	-	-
***	2 (28.57)	-	-	-	2	-	-	-
Total	23 (45.09)	7	6	5	4	1	-	-
Hardjo* ** ***	-	-	-	-	-	-	-	-

*2014; **2015; ***2016

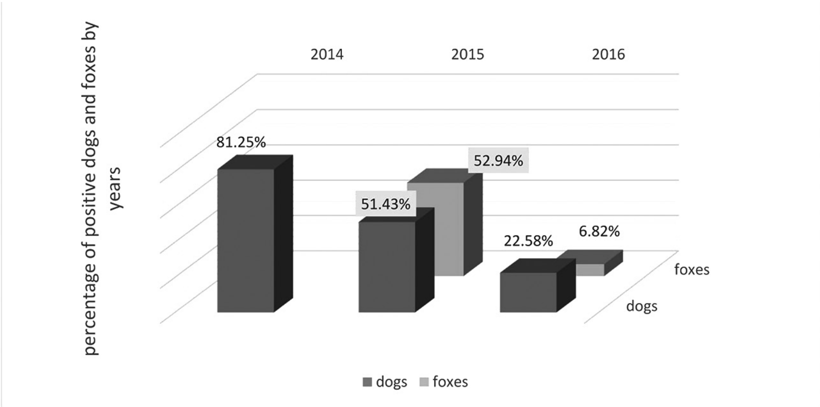

Figure 2. Overall annual percentages of examined dogs and foxes that harboured antibodies to *Leptospira* spp.

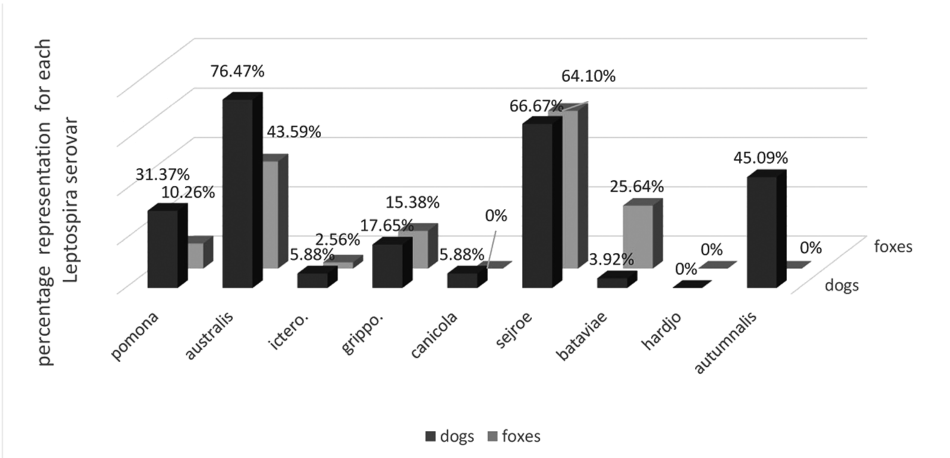

Figure 3. Overall percentages of antibodies to 10 pathogenic *Leptospira* determined in dogs and foxes in the Republic of Srpska

In 2015, the most prevalent serovars in dogs were: Sejroe 83.33% (15/18), Australis 77.8%, (14/18), Bratislava 72.2% (13/18) and Autumnalis 33.33% (6/18). Serovar Pomona was significantly less prevalent, detected in 16.66% (3/18) of the dogs. Serovar Bataviae was registered for the first time in one animal (5.55%; 1/18) (Table 1). The presence of antibodies to two serovars was determined in 16.67% (3/18) of the dog sera samples. Antibody titers from 1/100 to 1/400 were registered in 77.77% (14/18) of the dog sera samples and antibody titers from 1/800 to 1/1600 in 22.22% (4/18) of the dog sera samples.

In 2016, serovar Bratislava was the most prevalent serovar in dogs (71.4%; 5/7). Serovar Canicola was detected for the first time in three dogs (42.86%, 3/7), while antibodies to Australis, Pomona, Grippotyphos and Autumunalis were detected in only two dogs (28.6%), and serovars Sejroe and Bataviae were each detected in one animal only (Table 1). Antibody titers <1/800 were found in 71.42% (5/7) of dog sera samples and titers from 1/800 to 1/3200 in 28.57% (2/7) of dog sera samples.

Seroprevalence in Red Foxes

Antibodies to *Leptospira* serovars were found in 34.82% (39/112) of the foxes examined. In 2015, a significantly higher (p <0.0001) seroprevalence (52.94%) was found than in 2016, when just 6.82% of foxes harboured *Leptospira* antibodies. The highest overall seroprevalences were for serovars Sejroe 64.10% (25/39), Bratislava 48.72%, (19/39) and Australis 43.59% (17/39). In foxes, other serovars were less prevalent: Bataviae 25.64% (10/39), Grippotyphosa 15.38% (6/39). Pomona 10.26% (4/39), while Icterohaemorrhagiae was detected in only one fox (2.56%). None of the foxes were positive for serovars Canicola, Hardjo or Autumnalis (Table 2). Antibodies to just one serovar were found in 17 foxes (43.59%), antibodies to two serovars were found in four foxes (10.26%), antibodies to three serovars were found in 13 foxes (33.33%), and antibodies to four serovars were found in four foxes (10.26%).

Table 2. Number (percentage) of positive reactions and titres in foxes to *Leptospira* spp. serovars, tested by microscopic agglutination test during 2014-2016 in the Republic of Srpska, Bosnia and Herzegovina

***Leptospira* spp. serovars**	**Positive reactions**	**Titre**						
	***N* (%)**	**100**	**200**	**400**	**800**	**1600**	**3200**	**6400**
Australis*	15 (41.66)	6	6	2	1	-	-	-
**	2 (66.66)	2	-	-	-	-	-	-
Total	17 (43.59)	8	6	2	1	-	-	-
Sejroe*	24 (66.66)	13	7	3	1	-	-	-
**	1 (33.33)	1	-	-	-	-	-	-
Total	25 (64.10)	14	7	3	1	-	-	-
Icterrohaemorhagiae*	1 (2.77)	1	-	-	-	-	-	-
**	-	-	-	-	-	-	-	-
Total	1(2.56)	1	-	-		-	-	-
Grippotyphosa*	6 (16.66)	6	-	-	-	-	-	-
**	-	-	-	-	-	-	-	-
Total	6 (15.38)	6	-	-	-	-	-	-
Bratislava*	18 (50.0)	8	4	2	1	-	1	2
**	1 (33.33)	1	-	-	-	-	-	-
Total	19 (48.72)	9	4	2	1	-	1	2
Pomona*	3 (8.33)	3	-	-	-	-	-	-
**	1 (33.33)	1	-	-	-	-	-	-
Total	4 (10.26)	4	-	-	-	-	-	-
Bataviae*	10 (27.77)	7	1	2	-	-	-	-
**	-	-	-	-	-	-	-	-
Total	10 (25.64)	7	1	2	-	-	-	-
Canicola* **	-	-	-	-	-	-	-	-
Autumnalis* **	-	-	-	-	-	-	-	-
Hardjo* **	-	-	-	-	-	-	-	-

*2015; **2016

Antibody titers from 1/100 to 1/400 were registered in 89.74% (35/39) of fox sera, while titers from 1/800 to 1/6400 were registered in 10.25% (4/39) of fox sera. The highest titer (1/6400 to serovar Bratislava) was determined in two sera (5.13%) (Table 2). Serovar Bratislava was found in foxes from five of the six geographic locations examined, while serovar Icterrohaemorhagiae was detected only in animals from Visoka Krajina (Table 3). The highest seroprevalence occurred in foxes from the central area, and it was statistically significantly higher (Fisher's exact test, p <0.05) than in animals from the western area, while no statistically significant difference was found in the seroprevalences in foxes from the other geographic locations.

Table 3. Number (percentage) of tested and positive fox serum samples that harboured antibodies to individual *Leptospira* spp. serovars in the geographic locations studied

Study area	No. Exam.	Positive No. (%)	Aus.	Sej.	Ictero	Gripp.	Brat.	Pom.	Bata.
Banja Luka- Lijevče	35	17 (48.57)	10 (25.64)	9 (23.08)	-	5 (12.82)	9 (23.08)	1 (2.56)	5 (12.82)
Visoka Krajina	8	1 (12.50)	-	-	1 (50.0)	1 (50.0)	-	-	-
Western	20	2 (10.00)	-	-	-	-	1 (50.0)	1 (50.0)	-
Central	28	12 (42.86)	4 (15.38)	10 (38.46)	-	-	5 (19.23)	2 (7.69)	5 (19.23)
Eastern	12	3 (25.00)	1 (20.0)	2 (40.0)	-	-	2 (40.0)	-	-
Southern	9	4 (44.44)	2 (25.0)	4 (50.0)	-	-	2 (25.0)	-	-
Total:	112	39 (34.82)	17 (43.59)	25 (64.10)	1 (2.56)	6 (15.38)	19 (48.72)	4 (10.26)	10 (25.64)

Abbreviations: Icterohaemorrhagiae (Ictero), Bratislava (Brat), Sejroe (Sej), Pomona (Pom), Australis (Aus), Grippotyphosa (Gripp), Bataviae (Bata); Autumnalis, Canicola and Hardjo were not detected

DISCUSSION

Due to its high degree of forest cover with numerous wild animals, plentitude of watercourses and favorable climate, BiH provides favorable opportunities for leptospirosis to be maintained in the natural environment. One of the most important factors for the control and eradication of leptospirosis is knowledge of the prevalence and distribution of the disease, including the distribution of different *Leptospira* serovars in different areas. The results of this study, for the first time, give more details about the importance of dogs and foxes in the maintenance and transmission of various *Leptospira* serovars, as well as indicating the influence of heavy rainfall on the spread of leptospirosis in populations of domestic and wild canids.

In our study, the ten serovars used were those most often registered in some parts of BiH and neighboring countries [14,17,18]. Also, when selecting serovars based on antibody prevalence data in dogs in Europe, other researchers had advised that serogroups Australis, Autumnalis, Canicola, Grippotiphosa, Icterohaemorrhagiae, Pomona and Sejroe should be included in the antigen panel test [19].

The highest seroprevalence was determined in 2014, three to four months after heavy rains and floods, which were evidently a significant factor in the spread of leptospirosis and high prevalence of seropositive dogs. Also, in 2014, more dogs were found (53.84%) with an antibody titer ≥1/800 than in the next two years (22.22% and 28.57% in 2015 and 2016, respectively). In the current study, the seroprevalence determined in 2016 was in accordance with the average seroprevalence of canine leptospirosis determined in the Federation of BiH [14,17]. In neighboring countries with similar ecological environments and socioeconomic and agricultural systems, the seroprevalence in dogs ranged from 37.7% in Croatia [19] to 5.45% in Serbia [21].

The most frequently identified serovars in our dog population were Australis, Bratislava, Sejroe, Autumnalis and Pomona during all three years, although seroprevalences varied slightly from year to year. There is an obvious difference in the prevalence of infectious serovars in relation to the situation in the Federation of BiH, where in two studies the dominant serovar was Pomona, Icterrohaemorhagiae and Sejroe. In both studies, serovars Bratislava and Australis were rarely detected [14,17] In the surrounding countries, in Croatia and Serbia, the most common serovars in the dog population were: Pomona, Grippotyphosa and Icterohaemorrhagiae [20,21].

MAT is documented to have good specificity and no cross reactivity with other bacteria. However, there is a significant serological cross-reactivity between different Leptospira serovars and serogroups. This means that an animal positive to one serovar can possess cross-reactive antibodies to other serovars, in lower concentrations [22]. For this reason, MAT results cannot be the only basis for Leptospira serovar identification in a particular region. One study indicated the cross-reactivity of serovars Bratislava and Australis belonging to the same serogroup, as well as the fact that in dogs infected with Australis serogroup Leptospira, the highest titer occurred in the case of serovar Bratislava [23]. Serovar Autumnalis was among the four most common serovars in dogs and foxes in the Republic of Srpska (45.09%), while in the Federation of BiH, it was registered in only 12.4% of dogs [14]. Infection with serovar Autumnalis is thought to occur in dogs, but in the absence of isolates, cross-reactivity with serotype Pomona is believed to be possible [24]. In our study of 16 canids positive to serovar Pomona, 81.25% of these animals were also positive to serovar Autumnalis.

The importance of cross reactivity can be discussed. Same animal species, as in our study is the dog, can be infected in the same region at different time intervals with different leptospira serovars that are present in that region. For further evaluation, there is a need to isolate the leptospira from seropositive cats, from blood, urine and tissues, and molecular antigenic structure analysis is a prerequisite to have a complete information [25].

Serovars Icterohaemorrhagiae and Canicola have been the main causes of canine leptospirosis for many years. However, in the last fifty years since the bivalent inactivated vaccine against these two serovars was introduced, the prevalences of these two serovars in dog populations have declined in many parts of the world [19]. Certainly, the established low prevalence of these serovars, could indicate the currently low importance of these serovars in the epidemiology of leptospirosis in the study area.

Studies of the seroprevalence in wild canids in Europe have shown that red foxes are often exposed to various *Leptospira* serovars [11,19,27,28]. Given the continuous increase in wildlife populations in BiH near populated areas, it is clear that monitoring of leptospirosis in the wildlife population is necessary in order to prevent the spread of leptospirosis among domestic animals and humans. The seroprevalence of leptospirosis in red foxes in BiH varies among regions. The variations are probably the result of differences in targeted serovars and their regional circulation and endemicity [10].

The seroprevalence in foxes from the Republic of Srpska was 34.82% and was similar to the seroprevalence in foxes from Croatia [11], Slovenia [28] and Spain [26] while the prevalence in foxes was lower in Poland [12] Norway [27] and France [29]. As in the dog population, there was a high prevalence of antibody-positive foxes in 2015 (52.94%), but in 2016, the prevalence in foxes had dropped to 6.82%, which indicates the importance of heavy rainfall and floods in the spread of leptospirosis in wildlife populations.

In foxes as in dogs, high prevalences of serovars Sejroe, Bratislava and Australis were measured, which could indicate the active circulation of these serovars in the study area. Serovar Bratislava is rare in fox populations in Europe [28]. Serovar Icterohaemorrhagiae was the most abundant serovar in the red fox populations [26-28] while it was rarely detected in the fox population in Poland [12]. In the Republic of Srpska was registered in only one fox (Table 2).

Differences in the prevalence of serovars between studies can be due to inconsistencies in the serovars tested, climate or geographical location, and year of study [30]. What is obvious based on the conducted research is the presence of different serovars in climatically and geographically close areas. Hypothetically, any pathogenic *Leptospira* can infect domestic and wild animals, but in practice only a small number of serovars are endemic in a particular region. Despite the fact that leptospirosis is of major importance in tropical and developing countries, the incidence of leptospirosis in temperate areas can be expected to increase in the coming years as a result of climate change leading to more frequent extreme weather events such as floods and rising temperatures, but also as a result of the continuous expansion of urban areas leading to increased contact with wildlife [31].

CONCLUSION

In conclusion and based on the results obtained, it was determined that the same *Leptospira* serovars dominate both domestic and wild canids in various parts of the Republic of Srpska. Although seroprevalences were measured in a limited number of animals, the relatively high percentage of seropositive dogs and foxes found in our study indicates the importance of these animal species in the epizootiology and epidemiology of leptospirosis. The high seroprevalence of dogs and foxes in the years after heavy rainfall confirms once again the importance of such natural disasters to the spread of leptospirosis. In order to get a more clear picture of the epizootiological and/or epidemiological significance of these canids, *Leptospirae* must be isolated from blood, urine and tissues and the molecular antigenic structure be determined, which would provide prerequisite data for more complete information. In further research, it is necessary to examine different species of wild animals in order to clarify their importance in the epizootiology of leptospirosis. Knowing the presence and distribution of dominant *Leptospira* serovars in natural hosts is important for understanding the epidemiology of the disease in any region.

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Authors' contributions

JM writing, carried out the laboratory tests, helped to draft the manuscript; DN epidemiological investigations and helped to draft the manuscript; BV stastical data analyses; LV epidemiological investigations; SO conceptualisation, writing-review and editing manuscript supervision, validation. All authors have read and agreed to the published version of the manuscript.

Competing interests

The authors declare that they have no competing interests.

Institutional Review Board Statement

No ethical approval was required for the sample types collected in this study. The authors declare that no animals were killed for the purpose of this study and that all procedures that contributed to this work meet the ethical standards of the relevant national and European regulations on the care and use of animals (Directive 2010/63/EC).

REFERENCES

1. Faine S, Adler B, Bolin C, Perolat P: Leptospira and Leptospirosis, 2nd Edition; Medisci Press: Melbourne, Australia; 1999, 272.
2. Ward MP: Seasonality of canine leptospirosis in the United States and Canada and its association with rainfall. Prev Vet Med 2002, 56: 203-213.
3. Ganoza CA, Matthias MA, Saito M, Cespedes M, Gotuzzo E, Vinetz JM: Asymptomatic renal colonization of humans in the peruvian Amazon by Leptospira. PLoS Negl Trop Dis 2010, 4: e612.
4. Andre-Fontaine G: Canine leptospirosis — do we have a problem? Vet Microbiol 2006, 117: 19-24.
5. Ghneim G, Viers J, Chomel B, Kass P, Descollonges D, Johnson M: Use of a case-control study and geographic information systems to determine environmental and demographic risk factors for canine leptospirosis. Vet Res 2007, 38: 37-50.
6. Ward MP, Guptill LF, Wu CC: Evaluation of environmental risk factors for leptospirosis in dogs: 36 cases (1997–2002). J Am Vet Med Assoc 2004, 225: 72-77.
7. Sykes JE, Hartmann K, Lunn KF, Moore GE, Stoddard RA, Goldstein RE: 2010 ACVIM small animal consensus statement on leptospirosis: diagnosis, epidemiology, treatment, and prevention. J Vet Intern Med 2011, 25: 1-13.
8. Gay N, Soupé-Gilbert ME, Goarant C: Though not Reservoirs, Dogs might Transmit Leptospira in New Caledonia. Int J Environ Health Res 2014, 11: 4316-4325.
9. Schuller S, Francey T, Hartmann K, Hugonnard M, Kohn B, Nally JE, Sykes J: European consensus statement on leptospirosis in dogs and cats. J Small Anim Pract 2015,56: 159-179.
10. Alić A, Šupić J, Goletić T, Rešidbegović E, Lutvikadić I, Hodžić A: A unique case of fatal coinfection caused by *Leptospira spp*. and *Hepatozoon canis* in a Red fox cub (*Vulpes vulpes*). Pathogens 2021, 11: (1).
11. Slavica A, Dezdek D, Konjevic D, Cvetnic Z, Sindicic M, Stanin D, Turk N: Prevalence of leptospiral antibodies in the red fox (Vulpes vulpes) population of Croatia. Vet Med (Praha) 2011, 56: 209-213.
12. Żmudzki J, Arent Z, Jabłoński A, Nowak A, Zębek S, Stolarek A, Pejsak Z: Seroprevalence of 12 serovars of pathogenic Leptospira in red foxes (*Vulpes vulpes*) in Poland. Acta Vet Scand 2018, 60: 1-9.
13. Naing C, Reid SA, Aye SN, Htet NH, Ambu S: Risk factors for human leptospirosis following flooding: A meta-analysis of observational studies. PloS One 2019, 14: e0217643
14. Lindtner Knific R, Ćutuk A, Gregurić Gračner G, Dovč A: Seroprevalence of leptospirosis in various categories of dogs in Bosnia and Herzegovina. Vet Arh 2019, 89: 627-640.

15. Đurica R, Đurica D, Vujčić S, Delić-Jović M: Ekološke posledice poplava u Republici Srpskoj maj 2014. godine. Vještak 2014, 1: 97-102.
16. OIE (World Organisation for Animal Health). [https://www.oie.int]
17. Ćutuk A, Čengić B, Velić L, Dovč A, Knific RL, Šaljić E, Bejdić P: Seroprevalence of pathogenic Leptospira serovars in hunting dog in Bosnia and Herzegovina. Vet Stanica 2020, 51: 519-525.
18. Milas Z, Štritof Majetić Z, Habuš J, Mojčec Perko V, Starešina V, Barbić L, Turk N: The occurrence and maintenance of Leptospira serovars Australis and Bratislava in domestic and wild animals in Croatia. Vet Arch 2013, 83: 357-369.
19. Ellis WA: Control of canine leptospirosis in Europe: time for a change? Vet Rec 2010, 167: 602-605.
20. Štritof Majetić Z, Habuš J, Milas Z, Mojčec Perko V, Starešina V, Turk N. A serological survey of canine leptospirosis in Croatia-the changing epizootiology of the disease. Vet Arch 2012, 82: 183-191.
21. Vojinovic D, Bogicevic N, Vasic A, Manic M, Radovanovic M, Rogozarski D, Valcic M: Seroepidemiological survey of leptospiral infection in stray dogs in Serbia. Turkish J Vet Anim Sci 2015, 39:719-723.
22. Delaude A, Rodriguez-Campos S, Dreyfus A, Counotte MJ, Francey T, Schweighauser A, Lettry S, Schuller S: Canine leptospirosis in Switzerland — A prospective cross-sectional study examining seroprevalence, risk factors and urinary shedding of pathogenic leptospires. Prev Vet Med 2017, 141: 48–60.
23. Mayer-Scholl A, Luge, E, Draeger A, Nöckler K, Kohn B: Distribution of Leptospira serogroups in dogs from Berlin, Germany. Vector-Borne and Zoonotic Dis 2013, 13(3): 200-202.
24. Moore GE, Guptill LF, Glickman NW, Caldanaro RJ, Aucoin D, Glickman LT: Canine leptospirosis, United States, 2002–2004. Emerg Infect Dis 2006, 12 (3): 501 - 503.
25. Obrenović S, Radojičić S, Stević N, Bogunović D, Vakanjac S, Valčić M: Seroprevalence of cat leptospirosis in Belgrade (Serbia). Acta Vet-Beograd 2014, 64: 510-518.
26. Millán J, Candela MG, López-Bao JV, Pereira M, Jiménez MÁ, León-Vizcaíno L: Leptospirosis in wild and domestic carnivores in natural areas in Andalusia, Spain. Vector-Borne Zoonotic Dis 2009, 9: 549-554.
27. Åkerstedt J, Lillehaug A, Larsen IL, Eide NE, Arnemo JM, Handeland K: Serosurvey for canine distemper virus, canine adenovirus, *Leptospira interrogans*, and *Toxoplasma gondii* in free-ranging canids in Scandinavia and Svalbard. J Wildl Dis 2010, 46: 474-480.
28. Žele-Vengušt D, Lindtner-Knific R, Mlakar-Hrženjak N, Jerina K, Vengušt G: Exposure of Free-Ranging Wild Animals to Zoonotic Leptospira interrogans Sensu Stricto in Slovenia. Animals 2021, 11: 2722
29. Roquelo C, Kodjo A, Marié JL, Davoust B: Serological and molecular survey of Leptospira spp. infections in wild boars and red foxes from Southeastern France. Vet World 2021, 14: 825.
30. Grimm K, Rivera NA, Fredebaugh-Siller S, Weng HY, Warner RE, Maddox CW, Mateus-Pinilla NE: Evidence of leptospira serovars in wildlife and leptospiral DNA in water sources in a natural area in east-central Illinois, USA. J Wildl Dis 2020, 56: 316-327.
31. Wasiński B, Dutkiewicz J: Leptospirosis – current risk factors connected with human activity and the environment, Ann Agric Environ Med 2013, 20: 239–244

SEROPREVALENCIJA SEROVARA PATOGENIH LEPTOSPIRA KOD PASA I CRVENIH LISICA *(VULPES VULPES)* U BOSNI I HERCEGOVINI

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Cilj ovog istraživanja bio je da se ispita seroprevalencija leptospira kod pasa i crvenih lisica u entitetu Republika Srpska, Bosna i Hercegovina, nakon obilnih padavina i poplava 2014. godine i dve godine nakon toga. Seroepidemiološka studija je uključivala testiranje uzoraka seruma pasa (n = 98) i lisica (n = 112) korišćenjem MAT (mikroskopski aglutinacioni test).

Antitela na najmanje jedan serovar Leptospira pronađena su kod 52,04% testiranih pasa. Seroprevalencija pasa u 2014. godini (81,25%) bila je značajno veća nego u 2015. godini (51.42% p <0.0001) and 2016 (22.5% p<0.05). Najveće seroprevalencije bile su za serovare Australis (76,47%), Bratislava (70,58%), Sejroe (66,67%) i Autumnalis (45,09%). Antitela na najmanje jedan serovar Leptospira otkrivena su kod 34,82% ispitanih crvenih lisica. U 2015. godini seroprevalencija lisice je bila značajno veća (52,94%) nego u 2016. (6.82%) (p <0.0001). Najveće seroprevalencije bile su za serovare Sejroe (64,10%), Bratislava (48,72%), Australis (43,59%) i Bataviae (25,64%).

Visoka seroprevalencija Leptospira kod pasa i lisica utvrđena tokom ove studije ukazuje na značaj ovih karnivora u održavanju leptospiroze na istraživanom području, kao i na potencijalni rizik od infekcije ljudi i drugih životinjskih vrsta koje dolaze u kontakt sa ovim kanidama. Dobijeni rezultati ukazuju na to da obilne padavine i intenzivne poplave mogu uticati na povećanu infekciju Leptospira kod ovih kanida.