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The occurrence and maintenance of *Leptospira* serovars Australis and Bratislava in domestic and wild animals in Croatia

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ABSTRACT

Over a 10-year period, from 2002 to 2011, 20,157 sera samples and 984 kidneys of wild and domestic animals were collected and tested for leptospirosis at the Laboratory for Leptospirosis of the Veterinary Faculty, University of Zagreb. Out of 19,732 sera samples of horses, 3876 (19.64%) had agglutinating antibodies against one or more *Leptospira* serovars. The highest seroprevalence in horses was found for the following serovars: sv Bratislava, sv Pomona and sv Australis. In wild boars, out of 215 samples 75 (34.88%) were positive and the most prevalent serovars were sv Australis, sv Grippotyphosa and sv Tarassovi. Out of 170 pig sera we found 66 (38.82%) positive animals. The most prevalent serovars were sv Australis, sv Ballum and sv Saxkoebing. In red foxes, out of 59 sera samples 34 (57.60%) were positive for leptospirosis. We found the highest titre for sv Australis, sv Sejroe, sv Saxkoebing and sv Grippotyphosa. Out of 151 sera samples of dogs, 26 (17.22%) had antibodies for *Leptospira* serovars. The highest seroprevalence was for sv Pomona, sv Grippotyphosa, sv Australis and sv Icterohaemorrhagiae. Out of 262 kidney samples taken from the yellow-necked field mouse (*Apodemus flavicollis*) *Leptospirae* were isolated from 32 (12.21%) samples including 13 (40.63%) isolates of *L. interrogans*, serogorup Australis, sv Bratislava; five (15.62%) isolates of *L. borgpeterseni*, serogoup Sejroe, sv Saxkoebing; four (12.5%) isolates of *L. interrogans*, serogroup Australis, sv Muenchen-FR. From 122 kidney samples of the long-tailed field mouse (*Apodemus sylvaticus*) 22 (18.03%) were positive for *Leptospira*. The most frequently isolated *Leptospira* were: seven (31.82%) isolates of *L. interrogans*, serogroup Australis, sv Bratislava; six (27.27%) isolates of *L. interrogans*, serogroup Australis, undetermined serovar; three (13.64%) isolates of *L. interrogans*, serogroup Australis, sv Muenchen-FR. Out of 96 kidney samples of the black-striped field mouse (*Apodemus agrarius*) from 29 (30.21%) we isolated *Leptospira*. The most frequent serovars were:

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L. kirschneri, serogroup Pomona, sv Mozdok in eight (27.59%) samples, *L. kirschneri*, serogroup Bataviae, sv Bataviae in two (6.9%) samples while 19 (65.52%) isolates are still undetermined. Considering the results of our and previous investigations of leptospirosis in Croatia, we can conclude that *Leptospira* serovars from the serogroups Australis, sv Bratislava, sv Australis and sv Lora are maintained among wild life animal species. The results of this and our previous studies of leptospirosis in wild animal species in Croatia strongly support the conclusion that wild carnivores and omnivores, such as the red fox, wild boar and brown bear, could also be maintaining reservoir hosts for serovars from the serogroup Australis.

Key words: *Leptospira*, Australis, Bratislava, reservoirs, Croatia

Introduction

The life cycle of *Leptospira* is maintained by circulation in nature among subclinically infected animals, which serve as maintenance hosts or reservoir hosts. These animals, which include a great number of wild and domestic animal species, shed *Leptospira* via urine and serve as a source of infection for another animal species, called accidental hosts.

Distribution of *Leptospira* serovars and the spectrum of maintaining and accidental reservoirs is very wide and heterogeneous, depending on different regions in the world and they are liable to change over time. Leptospirosis has been investigated in Croatia for more than 50 years. The collected data provide a wide and very nearly clear picture of leptospirosis epidemiology in humans and animals, but also raise a large number of questions. Previous epizootiological studies in Croatia suggest a high degree of adaptation between rats (*Rattus norvegicus*) and the serovar Icterohaemorrhagiae, house mice (*Mus musculus*) and serovar Sejroe, the common vole (*Microtus arvalis*) and serovar Grippotyphosa, the black-striped field mouse (*Apodemus agrarius*) and the serovar Pomona, the yellow-necked field mouse (*Apodemus flavicollis*) and the serovar Saxkoebing (ZAHARIJA et al., 1982; BORČIĆ et al., 1986; MILAS et al., 2002; TURK et al., 2003; ŠTRITOF MAJETIĆ, 2010). The objective of this study is to present the results of investigations of leptospirosis in Croatia over the last 10 years and the ecological and epizootiological relationships among *Leptospira* serovar Australis and Bratislava and various wild and domestic animal species.

Materials and methods

In a 10-year period, from 2002 to 2011, 20,157 sera samples and 984 kidneys of wild and domestic animals were collected or received and tested for leptospirosis (Table 1) at the Laboratory for Leptospirosis of the Faculty of Veterinary Medicine, University of Zagreb.

Sera samples of 19,732 horses were taken by local veterinary practitioners across Croatia, as part of routine measures of leptospirosis control, ordered by the Veterinary Directory of the Ministry of Agriculture of the Republic of Croatia. Wild boars and foxes were trapped during regular hunting seasons throughout northwest Croatia. The blood

samples of wild boars were taken from the heart, immediately after shooting and from red foxes after necropsy and testing for rabies at the Croatia Veterinary Institute. A total of 215 sera from wild boars (*Sus scrofa*) and 59 of red foxes (*Vulpes vulpes*) from northwest Croatia were collected. Serum samples from 151 dogs were submitted to the Laboratory for Leptospirosis, during a period of four years. The majority of samples originated from dogs with some form of clinical disease, mostly hepato-renal lesions.

Table 1. Samples of animals collected for testing to leptospirosis

	Sample	
Animal	Blood	Kidney
Horse	19732	-
Wild boar	215	215
Red fox	59	59
Small rodents	-	710
Dog	151	-
Total	20157	984

Serological tests were conducted by the microscopic agglutination test (MAT) (DIKKEN and KMETY, 1978; HARTSKEERL et al., 2006) with 12 *L. interrogans* serovars: Grippotyphosa, Sejroe, Australis, Pomona, Canicola, Icterohaemorrhagiae, Tarassovi, Saxkoebing, Ballum, Bataviae, Poi and Hardjo. In 2010 sv Bratislava was added to the standard panel of antigens. Blood sera were examined at a basic dilution of 1:100 for listed *Leptospira* serovars, whereas positive sera were examined for corresponding *Leptospira* serovars up to a final dilution titre of 50% agglutination. Microagglutination antibody titre ≥1:100 was considered as an indicator of earlier infection. If the serum was positive for two or more *Leptospira* serovars, the serovar with the highest antibody titre was considered as the infective serovar. Whilst evaluating these findings, the likelihood was discovered that occasional cross-reactivity among serovars may result in an equal or even higher antibody titre (MODRIĆ et al., 1985).

For the purpose of *Leptospira* isolation, kidney tissue was immediately inoculated into home made Korthof's medium (FAINE et al., 1999) and sampled for DNA extraction. Positive cultures were subcultured in Korthof's medium until they reached stable growth, then subcultured to Ellinghausen-McCullogh-Johnson-Harris (EMJH) liquid medium (ELLINGHAUSEN and McCULLOUGH, 1965; JOHNSON and HARRIS, 1967) and grown to obtain a density of $2\text{-}4\times10^8$ leptospires per millilitre, suitable for serological and molecular identification procedures.

To identify the obtained isolates to the serogroup level, the microscopic agglutination test (MAT) was performed, following the standard procedure, using a panel of 18 rabbit

anti-*Leptospira* reference antisera (DIKKEN and KMETY, 1978). The reference rabbit antisera used in this study were from the Koninklijk Instituut voor de Tropen (KIT), Amsterdam, the Netherlands. Reference strains and isolates were grown at 30 °C on EMJH medium and harvested by centrifugation during the late logarithmic phase. Genomic DNA from animal kidneys and *Leptospira* isolate cultures was extracted using a QIAamp DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions, and stored at -20 °C.

Polymerase chain reaction was performed on all obtained isolates and kidneys of related animals with three primer pair sets. PCR with primers LeptoA (5'-GGCGGCGCGTCTTAAACATG-3') and LeptoB (5'-TTCCCCCCATTGAGCAAGATT-3') as described by MERIEN et al. (1992), was used to confirm the presence of *Leptospira* in cultures and kidney tissue. Semi-nested PCR with two primer sets, L3 (5'-TGAGGGTTAAAACCCCCAAC-3') and L4 (5'-GATTTTTCGGGTAAAGATT-3') followed by L4 and Lepat2 (5'-TCACAT(CT) GCTGCTTATTTT-3') as described by GRAVEKAMP et al. (1993) was performed to confirm the pathogenicity of the *Leptospira* present in cultures and kidney tissue. All products were electrophoresed in 1% agarose gel and compared to molecular size marker. Primers were synthesized by Tib Molbiol, Berlin, Germany.

Subsequent typing of the isolates to the serovar level was performed by pulsed-field gel electrophoresis (PFGE). Preparation of agarose plugs was performed as described by GALLOWAY and LEVETT (2008). Genomic DNA of isolates from serogroups Pomona and Grippotyphosa was restricted with endonuclease *Not*I and subjected to PFGE for 18 hours at 14 ºC, with circulating 0.5X TBE buffer. Electrophoresis conditions were as follows: switch times of 2.16 and 35.07 seconds, angle of 120 ºC, gradient of 6V/cm, temperature of 14 ºC, and linear ramping factor. Genomic DNA of isolates from serogroup Australis was restricted, with endonuclease *Sgr*AI, and subjected to PFGE for 22 hours at 14 ºC, with a circulating 0.5X TBE buffer. Electrophoresis conditions were as follows: switch times of 5 and 30 seconds, angle of 120 º, gradient of 6 V/cm, temperature of 14 ºC, and linear ramping factor. Gels were stained with ethidium bromide and analysed by the Gel Doc 2000 System (Bio-Rad Laboratories, Richmond, California, USA).

Results

The results of the investigations are shown in Tables 2-3.

Table 2 shows the *Leptospira* antibodies findings in the sera of the examined animals. Out of 19,732 sera samples of horses 3876 (19.64%) had agglutinating antibodies against one or more *Leptospira* serovars. The highest seroprevalence for horses were found for sv Bratislava in 1093 (28.20%) samples, sv Pomona in 574 (14.81%) and sv Australis in 479 (12.36%). In wild boars, out of 215 samples 75 (34.88%) were positive and the

most prevalent serovars were sv Australis with 28 (37.33%) samples, sv Grippotyphosa with eight (10.67%) and sv Tarassovi with five (6.67%). From 170 pig sera we found 66 (38.82%) positive animals. The most prevalent serovars were sv Australis with 23 (34.85%) samples, sv Ballum with nine (13.64%) and sv Saxkoebing with four (6.06%). In red foxes, out of 59 sera samples 34 (57.60%) were positive for leptospirosis. We found the highest titre for sv Australis in 17 (50.00%), for sv Sejroe and Saxkoebing in three (8.82%) each and sv Grippotyphosa in two (5.88%). Out of 151 sera samples of dogs, 26 (17.22%) had antibodies for *Leptospira* serovars. The highest seroprevalence was for sv Pomona in eight (30.77%) samples, sv Grippotyphosa in five (19.23%) and sv Australis and Icterohaemorrhagiae in four (15.38%) each. The prevalence of other *Leptospira* serovars for the examined animals is presented in Table 2.

Table 2. Serological findings of *Leptospira* antibodies in different animal species

	Animal									
	Horse		Wild boar		Pig		Red Fox		Dog	
	No. of tested sera: 19732		No. of tested sera: 215		No. of tested sera: 170		No. of tested sera: 59		No. of tested sera: 151	
Leptospira serovar	Positive sera	%	Positive sera	%	Positive sera	%	Positive sera	%	Positive sera	%
Grippotyphosa	353	9.11	8	10.67	2	3.03	2	5.88	5	19.23
Sejroe	158	4.08	1	1.33	-	-	3	8.82	-	-
Australis	479	12.36	28	37.33	23	34.85	17	50.00	4	15.38
Bratislava	1093	28.20	-	-	-	-	-	-	-	-
Pomona	574	14.81	3	4.0	3	4.55	-	-	8	30.77
Canicola	65	1.68	-	-	-	-	-	-	-	-
Icterohaemorrhagiae	416	10.73	2	2.67	1	1.52	3	8.82	4	15.38
Tarassovi	18	0.46	5	6.67	-	-	-	-	-	-
Mozdok	2	0.05	-	-	-	-	-	-	-	-
Saxkoebing	153	3.95	1	1.33	4	6.06	1	2.94	1	3.85
Ballum	1	0.03	2	2.67	9	13.64	-	-	-	-
Bataviae	3	0.08	1	1.33	-	-	-	-	-	-
Poi	4	0.10	2	2.67	1	1.52	-	-	-	-
Hardjo Prajitno	7	0.18			2	3.03	-	-	1	3.85
Undetermined	550	14.20	22	29.33	21	31.82	8	23.53	3	11.54
Total	3876	19.64	75	34.88	66	38.82	34	57.6	26	17.22

Table 3. Isloation of *Leptospira* spp. from the kidneys of different species of small rodents

Genomic species, serologic group and serovar of isolated *Leptospira*	Animal									
	Apodemus flavicollis		*Apodemus sylvaticus*		*Apodemus agrarius*		*Myodes glareolus*		Other species	
	No. of samples: 262		No. of samples: 122		No. of samples: 96		No. of samples: 195		No. of samples: 35	
	Positive culture	%	Positive culture	%	Positive culture	%	Positive culture	%	Positive culture	%
L. borgpeterseni, Sejroe, Saxkoebing	5	15.62	2	9.09	-	-	-	-	-	-
L. kirschneri, Bataviae, Bataviae	-	-	-	-	2	6.9	-	-	-	-
L. kirschneri, Grippotyphosa, Dadas	1	3.13	-	-	-	-	-	-	1	50
L. kirschneri, Grippotyphosa, Grippotyphosa-FR	1	3.13	-	-	-	-	-	-	-	-
L. kirschneri, Pomona, Mozdok	1	3.13	1	4.55	8	27.59	-	-	1	50
L. kirschneri, Pomona*	1	3.13	1	4.55	-	-	-	-	-	-
L. interrogans, Australis, Bratislava	13	40.63	7	31.82	-	-	-	-	-	-
L. interrogans, Australis*	-	-	6	27.27	-	-	-	-	-	-
L. interrogans, Australis, Muenchen-FR	4	12.5	3	13.64	-	-	-	-	-	-
Undetermined	6	18.75	2	9.09	19	65.52	3	100	-	-
Total	32	12.21	22	18.03	29	30.21	3	1.54	2	5.71

* Isolates in procedure of serovar determination

Table 3 presents the results of *Leptospira* isolation from renoculture in relation to the examined animal species. Out of 262 kidney samples taken from the yellow-necked field mouse (*Apodemus flavicollis*) *Leptospirae* were isolated in 32 (12.21%) samples including 13 (40.63%) isolates of *L. interrogans*, serogorup Australis, sv Bratislava; five (15.62%) isolates of *L. borgpeterseni*, serogoup Sejroe, sv Saxkoebing; four (12.5%)

isolates of *L. interrogans*, serogroup Australis, sv Muenchen-FR. From 122 kidney samples of the long-tailed field mouse (*Apodemus sylvaticus*) 22 (18.03%) were positive for *Leptospira*. The most frequently isolated *Leptospira* were: seven (31.82%) isolates of *L. interrogans*, serogorup Australis, sv Bratislava; six (27.27%) isolates of *L. interrogans*, serogroup Australis, undetermined serovar; three (13.64%) isolates of *L. interrogans*, serogroup Australis, sv Muenchen-FR. Out of 96 kidney samples of the black-striped field mouse (*Apodemus agrarius*) in 29 (30.21%) we isolated *Leptospira*. The most frequent serovars were: *L. kirschneri*, serogroup Pomona, sv Mozdok in eight (27.59%) samples, *L. kirschneri*, serogroup Bataviae, sv Bataviae in two (6.9%) samples while 19 (65.52%) isolates are still undetermined. The prevalence of other *Leptospira* serovars in examined animals is presented in Table 3. We did not isolate *Leptospira* from kidney samples of wild boars and red foxes.

Discussion

In the long history of leptospirosis investigations in Croatia, several authors have been able to establish a relationship between certain *Leptospira* serovars and their maintaining hosts. Previous epizootiological studies in Croatia suggest a high degree of adaptation between rats (*Rattus norvegicus*) and sv Icterohaemorrhagiae, the house mouse (*Mus musculus*) and sv Sejroe, the common vole (*Microtus arvalis*) and sv Grippotyphosa, the black-striped field mouse (*Apodemus agrarius*) and sv Pomona, the yellow-necked field mouse (*Apodemus flavicollis*) and sv Saxkoebing (ZAHARIJA et al., 1982; BORČIĆ et al., 1986; MILAS et al., 2002; TURK et. al., 2003; ŠTRITOF MAJETIĆ, 2010). Considering the fact that distribution of *Leptospira* serovars and the spectrum of maintaining and accidental reservoirs is very wide and heterogeneous, and they are liable to change over time, the aim of this study is to present results of investigations of the ecological and epizootiological relations between *Leptospira* sv Australis and sv Bratislava and various wild and domestic animal species. The results of our study suggest that the main reservoirs for sv Australis and Bratislava are myomorphous mammals, especially the yellow-necked field mouse and the long-tailed field mouse. The prevalence of infection with these serovars, presented in Table 3, shows that sv Bratislava is present in 40.63% *Leptospira* infections in the yellow-necked field mouse and in 31. 82% *Leptospira* infections in the long-tailed field mouse. It is also obvious that prevalence of infection for isolates of *L. interrogans*, serogroup Australis, undetermined serovar, is in second place (27.27%) in long-tailed field mouse and the prevalence of infection for sv Muenchen-FR is in third place in the long-tailed field mouse (13.64%) and in the yellow-necked field mouse (12.5%). Considering these results we may conclude that serovars from serogroup Australis are dominant in *Leptospira* infection in the mentioned species. Our findings are compatible with previous results of MILAS et al. (2002), TURK and al. (2003) and ŠTRITOF-MAJETIĆ (2010). In these investigations authors found infections in the yellow-necked

field mouse for *L. interrogans*, serogroup Australis, sv Lora. Although 19 isolates are in the process of identification, the results of *Leptospira* isolation in the black-striped field mouse (*Apodemus agrarius*) confirms previous investigations by BORČIĆ et al. (1986) and ŠTRITOF MAJETIĆ (2010) that this species is a reservoir for serovars from the serogroup Pomona.

The results of our serological investigations of leptospirosis in horses demonstrate that the most prevalent *Leptospira* serovars are sv Bratislava (28.20%), sv Pomona (14.81%) and sv Australis (12.36%). These results are in concordance with the fact that horses are a maintaining host for sv Bratislava and may be for sv Australis and sv Pomona (ELLIS et al., 1983; KISTON-PIGOTT and PRESCOTT, 1987; VAN DEN INGH et al., 1989; WILLIAMS et al., 1994; CVETNIĆ et al., 2004; ROCHA et al., 2004; BÄVERUD et al., 2009). We can assume that during evolution some serovars of *Leptospira* were adapted from small rodents to new maintaining hosts - horses. This process is still going on because horses live in a different environment, which usually combines pasture and stable, sharing these biotopes with a number of small rodents.

The results of seroepizootiology of leptospirosis in wild boars demonstrate that the most prevalent serovars are sv Australis (37.33%), sv Grippotyphosa (10.67%), sv Tarassovi (6.67%) and sv Pomona (4.0%). Considering the food composition, living habits and large migration radius of wild boars, it may be concluded that there is a great ability of transfer of *Leptospirae* from small rodents to wild boars, especially during wallowing in *Leptospira* contaminated water and mud. The role of wild boars in maintaining *Leptospira* in the environment is still unclear, but recent investigations suggest that they could serve as a reservoir host for sv Bratislava, Pomona and Australis (FIGAROLLI et al., 2012; PINTORE et al., 2012).

Testing of 170 serum samples of domestic swine to *Leptospira* antibodies showed that the most prevalent serovars were sv Australis (34.85%), sv Ballum (13.64%), sv Saxkoebing (6.06%) and sv Pomona (4.55%). In analysis of these results the fact must be taken into consideration that these swine were breeding mostly on pastures where they were exposed to an environment contaminated by the urine of free living small rodents. We consider that domestic pigs may only be incidental hosts for sv Australis. The latest reports of the seroepizootiology of leptospirosis in pigs in Croatia show that the most prevalent serovars are Pomona, Icterohaemorrhagiae, Sejroe, Australis and Grippotyphosa (RAČIĆ et al., 2012). In the past, due to traditional free ranging breeding of pigs in some parts of Croatia, sv Australis was dominant, but because of the risk of spreading classical swine fever, free ranging breeding was forbidden in 2005. Therefore domestic pigs probably cannot be a reservoir host for sv Bratislava and Australis in the future.

For the purpose of investigating leptospirosis in red foxes we tested 59 kidney and blood samples. We were not able to isolate *Leptospirae* from kidneys, but we found antibodies to 11 *Leptospira* serovars in 34 (57.6%) of 59 red fox sera. The highest antibody titres were to serovar Australis in 17 (50.0%) foxes, Sejroe and Icterohaemorrhagiae each in three (8.82%) foxes, Grippotyphosa in two (5.88%) foxes and Saxkoebing in one fox (2.94%). Small mammals are important autumn and winter food sources for red foxes and other wild carnivores. The amount of small mammals in the diet of foxes ranges from 27% in summer to 37% in winter (SCOTT, 1943). Considering its food habits, the role of the red fox as a maintaining *Leptospira* reservoir, especially for sv Australis, in the natural foci of leptospirosis, is still a matter of question.

Of 151 dog sera, 26 (17.22%) were positive to leptospirosis. Serovars, against which the sera tested agglutinated in the highest titre were as follows: Pomona in eight samples (30.77%), Grippotyphosa in five (19.23%), Icterohaemorrhagiae and Australis in four (15.38%), each. Traditionally, serovars Icterohaemorrhagiae and Canicola were incriminated in most cases of canine leptospirosis (FAINE et al., 1999). However, in the last decade, the *L. interrogans* serovars Bratislava and Grippotyphosa have been identified as the most prevalent in Italy (SCANZIANI et al., 2002), Grippotyphosa, Saxkoebing and Icterohaemorrhagiae in Germany (GEISEN et al., 2007) and Australis, Bratislava, Grippotyphosa and Pomona in Switzerland (FRANCEY, 2010). Dogs are often directly or indirectly exposed to environments contaminated by the urine of various small rodent species and changes in the etiology of dog leptospirosis depend directly on the etiology of leptospirosis in small rodents.

Comparing our results with previous investigations of leptospirosis in Croatia, we can see that serovar Australis was isolated from humans (ZAHARIJA, 1955), and from a clinically healthy cat (MODRIĆ, 1978). BORČIĆ et al. (1982) isolated serovar Australis from four species of small mammals (*Apodemus agrarius*, *A. sylvaticus*, *A. flavicollis* and *Clethrionomys glareolus*). MODRIĆ and HUBER (1993) found antibodies for serovars Australis, Sejroe, Canicola and Icterohaemorrhagiae in European brown bears (*Ursus arctos*). SLAVICA et al. (2007) reported about incidence of leptospiral antibodies in different game species over a 10-year period (1996-2005) in Croatia. In red deer (*Cervus elaphus*) the most prevalent serovars were Pomona and Ballum, in wild boar Australis and Pomona, in brown bear Icterohaemorrhagiae, Australis and Sejroe, and in red fox Australis, Sejroe and Icterohaemorrhagiae. Considering the living and feeding habits of wild boars, red foxes and brown bears, we can say that these species have a great ability of infection with *Leptospira*. Knowing the etiology of leptospirosis in small rodents in Croatia, it is not surprising that serovar Australis is consequently the most prevalent serovar in *Leptospira* infection in carnivore or omnivore wild species in Croatia. Serum samples of red foxes and brown bears with highest antibodies titers against serovars Icterohaemorrhagiae and

Sejroe may be result of infection from rats (*Ratus norvergicus*) and house mice (*Mus musculus*), because red foxes and brown bears very often feed in trash yards and suburban regions. Recent investigations of the seroepidemiology of leptospirosis in dogs in Croatia demonstrate that the serovars Australis, Grippotyphosa, Canicola and Pomona were the most prevalent in dogs in the Slatina and Virovitica regions (LAZIĆ, 2011; MAJETIĆ, 2011). Changes in the etiology of leptospirosis in dogs kept in urban regions in Croatia could be explained by the results of long term deratization in Croatia, which have led to a decrease in the population of rats and house mice.

Considering the results of our and previous investigations of leptospirosis in Croatia we can conclude that *Leptospira* serovars from the serogroup Australis, sv Bratislava, sv Australis and sv Lora are maintained among wild life animal species. It was undoubtedly demonstrated that myomorphous small rodent yellow-necked field mice maintain the reservoir host for *L. interrogans*, serogroup Australis, sv Bratislava, *L. interrogans*, serogroup Australis, sv Muenchen-FR and *L. interrogans*, serogroup Australis, sv Lora and the long-tailed field mouse for *L. interrogans*, serogroup Australis, sv Bratislava and *L. interrogans*, serogroup Australis, sv Muenchen-FR. It is known that the same *Leptospira* serovar could have more maintaining hosts in one biotope and the results of our and previous seroepizootiological studies of leptospirosis in wild animal species in Croatia strongly support the conclusion that wild carnivores and omnivores, such as the red fox, wild boar and brown bear, could also be maintaining reservoir hosts for serovars from serogroup Australis. The conclusion that *Leptospira* serovars from the serogroup Australis are circulating among wild animal species in Croatia is supplemented by the fact that we found a great seroprevalence of these serovars in the sera of domestic animal species such as horses and pigs, which share the same biotope as wild species. Domestic animal species, such as pigs, cows, sheep and goats, which are kept in stables, isolated from wild small rodents, could be only incidental hosts for serovars from the serogroup Australis. Speculation that wild carnivores and omnivores could also be maintaining hosts for *Leptospira* serovars from the serogroup Australis in Croatia should be confirmed in future by isolation from kidneys.

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SAŽETAK

U desetogodišnjem razdoblju, od 2002. do 2011. godine, 20157 uzoraka krvi i 984 bubrega divljih i domaćih životinja prikupljeno je i testirano na leptospirozu u Laboratoriju za leptospire Veterinarskog fakulteta Sveučilišta u Zagrebu. Od 19732 uzorka seruma konja 3876 (19,64%) imalo je aglutinirajuća protutijela protiv jednog ili više *Leptospira* serovara. Najveća seroprevalencija u konja bila je za sljedeće serovare: sv Bratislava, sv Pomona i sv Australis. U divljih svinja od 215 uzoraka, 75 (34,88%) je bilo pozitivno i najčešći serovari bili su sv Australis, sv Grippotyphosa i sv Tarassovi. Od 170 uzoraka seruma svinja pronašli smo 66 (38,82%) pozitivnih životinja. Najčešći serovari bili su sv Australis, sv Ballum i sv Saxkoebing. U crvenih lisica od 59 uzoraka seruma 34 (57,60%) je bilo pozitivno na leptospirozu. Najviši titar protutijela pronašli smo za sv Australis, sv Sejroe, sv Saxkoebing i sv Grippotyphosa. Od 151 uzorka seruma pasa 26 (17,22%) imalo je protutijela za *Leptospira* serovare. Najviša seroprevalencija bila je za sv Pomona, sv Grippotyphosa, sv Australis i sv Icterohaemorrhagiae. Od 262 uzorka bubrega uzetih od žutogrlog miša (*Apodemus flavicollis*) leptospire su izdvojene iz 32 (12,21%) uzorka uključujući 13 (40,63%) izolata *L. interrogans*, serološka skupina Australis, sv Bratislava; pet (15.62%) izolata *L. borgpeterseni*, serološka skupina Sejroe, sv Saxkoebing; četiri (12,5%) izolata *L. interrogans*, serološka skupina Australis, sv Muenchen-FR. Iz 122 bubrega šumskog miša (*Apodemus sylvaticus*) 22 (18,03%) bilo je pozitivno na leptospire. Najčešće izdvojene leptospire bile su: sedam (31,82%) izolata *L. interrogans*, serološka skupina Australis, sv Bratislava; šest (27,27%) izolata *L. interrogans*, serološka skupina Australis, nedeterminirani serovar, tri (13,64%) izolata *L. interrogans*, serološka skupina Australis, sv Muenchen-FR. Od 96 uzoraka bubrega poljskog miša (*Apodemus agrarius*) iz 29 (30,21%) su izolirane leptospire. Najučestaliji serovari bili su: *L. kirschneri*, serološka skupina Pomona, sv Mozdok u osam (27,59%) uzoraka, *L. kirschneri*, serološka skupina Bataviae, sv Bataviae u dva (6,9%) uzorka, dok je 19 (65,52%) izolata još uvijek nedeterminirano. Razmatrajući rezultate našeg i prethodnih istraživanja leptospiroze u Hrvatskoj možemo zaključiti da se *Leptospira* serovari iz serološke skupine Australis, sv Bratislava, sv Austraalis i sv Lora, održavaju između divljih životinjskih vrsta. Rezultati naših i prethodnih istraživanja leptospiroze u divljih životinja u Hrvatskoj čvrsto podupiru zaključak da divlji mesožderi i svežderi kao crvena lisica, divlja svinja i smeđi medvjed također mogu biti održavajući domaćini za serovare *Leptospira* iz serološke skupine Australis.

Ključne riječi: *Leptospira*, Australis, Bratislava, rezervoari, Hrvatska