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Incidence of leptospiral antibodies in different game species over a 10-year period (1996–2005) in Croatia

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Abstract During the 10-year survey (1996–2005), a total of 868 blood samples from different game species in Croatia were analyzed for the presence of leptospiral antibodies. The specific antibodies (AB) were detected in 242 samples (27.88%). According to the species in red deer (*Cervus elaphus*), the antibodies against six different leptospiral serovars were found in 43 of 226 analyzed sera (19.02%). The most frequent antigen serovars in the deer population were Pomona and Ballum (with the same frequency of 23.6%), whereas the highest titer was recorded for serovar Sejroe (1:800). In the analyzed roe deer (*Capreolus capreolus*) serum samples, a low level of leptospiral antibodies (6.07%) was determined, with just two AB for antigen serovars—Australis and Sejroe. In wild boar (*Sus scrofa*), leptospiral antibodies were detected in 151 of 431 samples analyzed (35.03%), with AB for nine antigen serovars. The serovars most frequently found were Australis (48.70%) and Pomona (22.70%), and these serovars also recorded the highest titer (1:3,200). Among brown bear (*Ursus arctos*) samples, leptospiral antibodies were detected in 25.00% of the samples, with four AB for antigen serovars, of which the most frequent was Ictero-haemorrhagiae (>40%). This serovar had the highest recorded titer (1:400). From 112 analyzed red fox (*Vulpes vulpes*) samples, leptospiral antibodies were found in 35 samples (31.25%). The determined antibodies were specific for four antigen serovars, of which the most frequent (46.2%) and with highest titer (1:1600) was serovar Australis. No antibodies (28/0) were recorded in mouflon (*Ovis musimon*). The most important game species from an epizootiological point of view in the studied area were certainly wild boar and red foxes. With strong serological reactions, these two species could be emphasized as important hosts for *Leptospira interrogans* sv. Australis in Croatia, but for their declaration as 'maintaining hosts,' isolation of sv. Australis is needed. According to aerial distribution, the highest number of positive samples from different game species was recorded in the central and eastern parts of Croatia, known as the 'historical natural foci' of leptospirosis—the regions of Posavina, Podravina, Slavonija, and Baranja. In contrast, the areas of Kordun and Gorski Kotar are declared as leptospira low-risk regions for the game species studied.

Keywords Leptospirosis · Game · Serology · Epidemiology · Croatia

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Introduction

Leptospirosis is a bacterial zooantroponosis of worldwide distribution that affects domestic and wild animals and can be caused by more than 220 *Leptospira* serovars (Yasuda

et al. 1987; Kmety and Dikken 1993; Brenner et al. 1999). Understanding its complex etiology and epidemiology requires an interdisciplinary approach and establishment of permanent monitoring. The main sources (also called 'naturally' or 'secondary foci') of all leptospira serogroups are different water plants contaminated with leptospires (Karaseva et al. 1973; Mailloux 1977, 1980), whereas the primary reservoirs of leptospiral infection are microtine rodents (Songer et al. 1983; Collares-Pereira et al. 2000; Adler et al. 2002; Treml et al. 2002) and various game species (Babudieri 1958; Gillespie and Ryno 1963; Mailloux 1980; Bondarenko et al. 2002). Wildlife species are especially considered to be epidemiological relevant carriers, mainly because of their frequent reactivity to *Leptospira* serovars native to their habitat (Lins and Lopes 1984). Whereas animals serve as primary sources of different *Leptospira* serovars, it is well known that humans are the 'dead end' of the infection chain (Gillespie and Ryno 1963) because, apart from a few exceptions, there is no data about interhuman or human-to-animal transmission of leptospirosis (Johnson 1976).

The importance of wildlife in maintaining the natural foci of leptospirosis in Croatia has been evaluated in a few studies (Borčić et al. 1978, 1982, 1983, 1989; Kovačić et al. 1983, 1984, 1985a, b; Modrić and Karlović 1977; Modrić and Huber 1993). Furthermore, several studies on the epidemiological, serological, and molecular level have been performed lately on myomorph mammals to determine their role in spreading leptospirosis (Cvetnić et al. 2002; Milas et al. 2002; Cvetnić et al. 2003), and at the same time, new serovars (Tsaratsovo and Lora) have been isolated from *Apodemus agrarius* and *A. flavicollis* for the first time in this region (Turk et al. 2003). The latest study analyzed and described the presence of leptospiral antibodies in foxes (Milas et al. 2006), but so far, there has been no systematic model of monitoring wildlife leptospirosis in Croatia aimed at establishing an epidemiological data base, which would serve as a reference center for chart drawing and mapping of most endangered areas. This study was conducted with the aim of gathering referral epizootiological data, and this paper reports the results of a 10-year serological survey of leptospirosis among the most important game species in Croatia.

Materials and methods

Sampling area and sample origin

Samples from different game species were collected over a 10-year period (1996–2005). The sampling area is marked on Fig. 1, going from point A (Varaždin, $\lambda = 46°18'N$, $\varphi =$

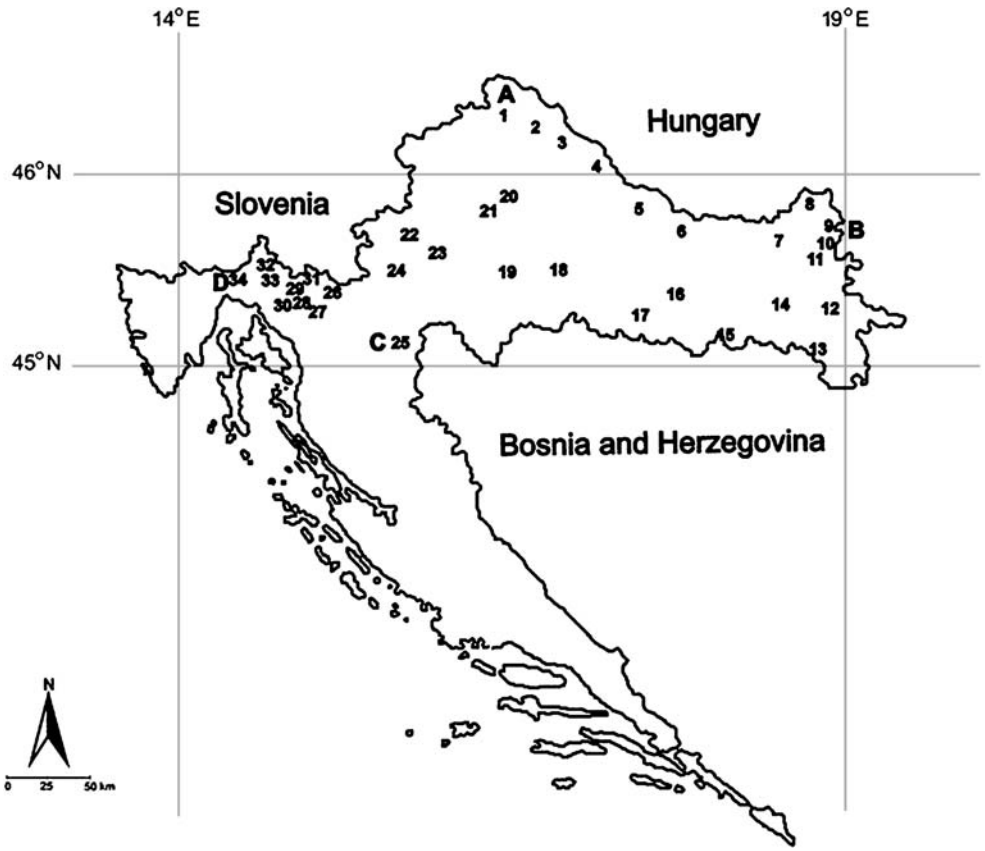

Fig. 1 Location of sampling over the 10-year period

16°21′E—marked on map as 1) in the north to point B (Zlatna Greda, λ=*45°43′N,* φ=18°52′E—marked on map as 9) in the east and from point C (Slunj, λ=*45°06′N,* φ=18°42′E—marked on map as 25) in the south to point D in the west (Klana, λ=*45°26′N,* φ=14°22′E—marked on map as 34). Samples were collected at a total of 34 locations in coordination with field veterinarians, foresters, and hunters. The micro-locations were chosen to include all three types of habitats in Croatia (lowland, hilly–karst, and karst), with emphasis on lowland habitats as the most important areas for maintaining leptospirosis (Borčić et al. 1978). More than 75% of the total of 868 samples was collected near Croatia's major water courses (the Rivers Sava, Drava, and Danube). The sampling area was divided into five main regions: the Podravina region (marked on map from point 1 to 6); the Slavonija and Baranja region (from point 7 to 14), the Posavina region (from point 15 to 21), the Kordun region (from point 22 to 25), and Gorski kotar (from point 26 to 34). During the study period, blood samples of eight game species were collected including red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), mouflon (*Ovis musimon*), wild boar (*Sus scrofa*), brown bear (*Ursus arctos*), red fox (*Vulpes vulpes*), stone marten (*Martes foina*), and Eurasian lynx (*Lynx lynx*), as shown in details in Table 1.

Serological tests

The collection of blood samples was performed aseptically in two different ways, depending on the animal status. From live animals, blood was drawn by vacuum collection tube ("Becton–Dickinson," Meyland Cedex, France) using the technique described by Poljičak-Milas et al. (2004), whereas from shot animals, blood was taken directly from the heart using a long syringe, as modification of the Sebek method, which was described by Cvetnić et al. (2003). Blood samples were transported to the laboratory within 12 h, and all sera were tested by microscopic agglutination test (MAT) for ten leptospiral antigens (serovars: Icterohaemorrhagiae, Australis, Pomona, Grippotyphosa, Sejroe, Bataviae, Hardjo, Ballum, Saxkoebing, and Tarassovi). The minimum sera dilution was at 1:100 titers (basic dilution, BD) and positive samples were examined for particular leptospira serovars up to a final dilution titer of 50% agglutination (maximum dilution, MD). The differences between positive reactions and the number of positive samples were noted because of the high possibilities of cross-reaction among several antigens of *L. interrogans* (Krawczyk 2005). If one sample was positive for two or more leptospira antigens, serovar with highest titer was considered as the infective agent (Milas et al. 2002). For data analysis, the standard statistical package Sigma Stat for Windows (Statistical Package for the Social Sciences (SPSS)/PC version 3.0) was used (data distribution, Pearson's χ^2 test).

Results

From the total number of 868 samples, positive findings, according to respective species, were found as follows (Table 2): roe deer, 2 positive of 33 samples (6.07%, two serovars recorded); red deer, 43 positive of 226 samples (19.02% including six *Leptospira* serovars); brown bear (25.00%, four serovars recorded); red fox (31.25%, four serovars recorded); wild boar, 151 positive of 431 samples (35.03%, nine serovars recorded); and stone marten (62.50%, three serovars recorded). Leptospiral antibodies (AB) were not found in the blood samples of mouflon (28/0) and Eurasian lynx (3/0). Altogether, we recorded 311 positive serological reactions, and 242 samples were found to be positive (27.88% of all tested sera). These results are summarized in Fig. 2.

From the positive serological reactions, 190 sera (60.32%) had reciprocal antibody titers 1:100, 43 (13.65%) had titers of 1:200, 45 (14.29%) had titers of

Table 1 Total number of blood samples collected from game animals during the 10-year survey (1996–2005) of leptospiral antibodies

Year	Wild boar	Red deer	Red fox	Roe deer	Mouflon	Brown bear	Stone marten	Euroasian lynx	Total
1996	10	45	8	5	–	6	1	–	75
1997	22	35	1	8	–	11	1	–	78
1998	4	2	–	2	6	5	–	1	20
1999	44	43	2	1	2	–	–	1	93
2000	8	10	18	4	1	4	–	1	46
2001	–	42	14	7	8	2	–	–	73
2002	162	12	21	–	9	–	–	–	204
2003	32	11	26	–	–	–	–	–	69
2004	84	25	8	6	1	–	1	–	125
2005	65	1	14	–	1	–	4	–	85
Total	431	226	112	33	28	28	7	3	868

Table 2 Results of positive MAT according to *Leptospira interrogans* serovars in different game species

Species	sv. Australis	sv. Pomona	sv. Sejroe	sv. Icterohaemorrhagiae	sv. Ballum	sv. Grippotyphosa	sv. Saxkoebing	sv. Tarassovi	sv. Bataviae	sv. Hardjo	Total
Wild boar	92	43	16	10	8	10	–	6	3	1	189
Red deer	7	13	6	8	13	8	–	–	–	–	55
Red fox	24	–	10	10	–	–	8	–	–	–	52
Brown bear	2	–	2	4	–	–	1	–	–	–	9
Stone marten	1	1	1	1	–	–	–	–	–	–	4
Roe deer	1	–	1	–	–	–	–	–	–	–	2
Total	127	57	36	33	21	18	9	6	3	1	311

1:400, 23 (7.30%) had titers of 1:800, 12 (3.80%) had titers of 1:1,600, and 2 (0.64%) had titers of 1:3,200. Cross-reactions between different leptospiral serovars occurred in 23% cases, mostly in red fox, red deer, and wild boar. In red foxes, cross-reactions were recorded in 32.69% cases, mostly between the serovars Icterohaemorrhagiae, Australis, and Sejroe. In red deer, cross-reactions were recorded in 21.82% of the cases, mostly between the serovars Icterohaemorrhagiae, Australis, Pomona, and Sejroe. Most serological reactions were recorded at BD (78.2%), whereas MD was detected for the serovar Sejroe (1:800). In wild boars, cross-reactions were recorded in 20.11% of the cases, mostly between the serovars Australis and Pomona. Titers of antibodies for different leptospiral serovars ranged from BD (60.4% of all reactions), 1:200 dilutions (15.4%), 1:400 dilution (14.3%), 1.800 dilution (4.8%), 1:1,600 dilution (4.0%), and 1:3,200 dilution (1.1%). Serovars Australis and Pomona showed the highest AB titers (MD= 1:3,200), which was the highest recorded titer of all the examined species. In brown bear, cross-reactions were recorded in just two cases, and of the four identified serovars (Icterohaemorrhagiae, Australis, Sejroe, and Saxkoebing), sv. Icterohaemorrhagiae was most represented (>40%) and with the highest AB titers (MD=1:400).

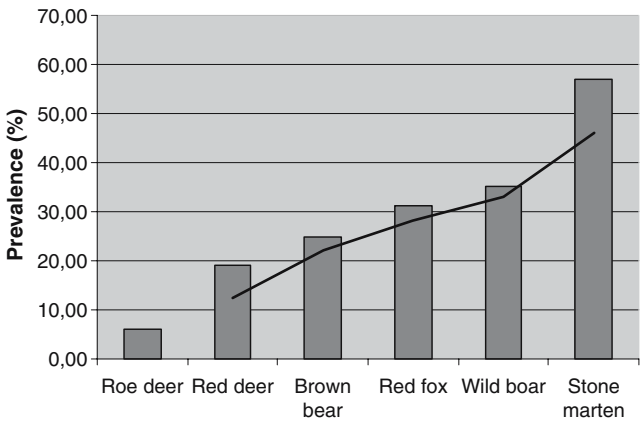

Fig. 2 Percentage of positive serological reactions in different game species

The distribution of different *Leptospira* serovars among the examined wild ruminants, boars, and carnivores is presented in Fig. 3. The obtained results indicate *Leptospira interrogans* sv. Australis as the most frequent (>45%) serovar in wild boars and carnivores, with statistically significant ($P<0.01$) determination as the infective agent in most (>75%) cases of serological cross-reactions. On the other hand, the most frequent serovars in ruminants are *Leptospira interrogans* sv. Pomona (23.6%) and *Leptospira interrogans* sv. Ballum (23.6%).

According to the aerial distribution, the majority of positive samples originated from lowland habitats, located near the Rivers Sava, Drava, and Danube. Pearson's chi quadrate test showed a very high significance (according to the number of positive samples, the number of leptospira serovars and different game species) in Posavina ($P<0.0001$), Slavonia, and Baranja regions ($P<0.0001$). In the Podravina region, a high significance was recorded ($P<0.0008$), whereas in the Kordun region and Gorski Kotar, there was no relation between habitat and the studied parameters ($P>0.05$).

Discussion and conclusions

In Croatian lowland habitats, the most important leptospiral serovars belong to the serogroups Icterohaemorrhagie, Australis, Pomona, Hebdomadis, Ballum, Grippotyphosa, Bataviae, and Tarassovi (Modrić and Karlović 1977; Kovačić et al. 1983, 1984; Borčić et al. 1989; Milas et al. 2002, 2006). For the last 25 years of research period, the prevalence of different antibodies for leptospiral serovars in wildlife species has changed through the decades, so we could follow the antigen variation line for the main game species. In the 1980s (Kovačić et al. 1983, 1984, 1985a, b), serological researches were undertaken on wild boar, red deer, and roe deer. Survey data revealed wild boars (Kovačić et al. 1984) as a main source of various antibodies for leptospiral serovars in wildlife (AB for serovars Pomona, Ballum, Sejroe, Australis, Icterohaemorrhagie,

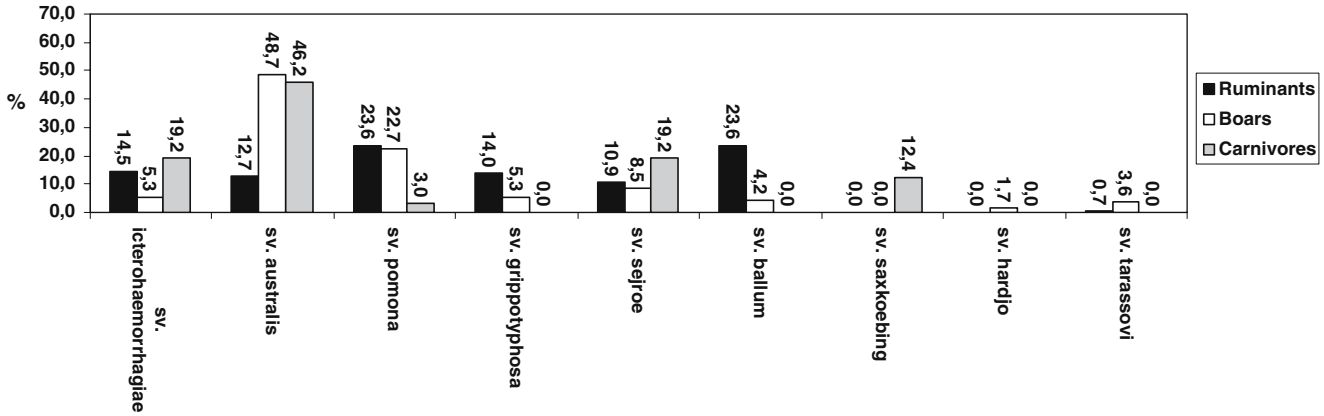

Fig. 3 Presence of different leptospiral serovars in wild ruminants, boars, and carnivores

Tarrasovi, and Grippotyphosa were found). At that time, the serovar Ballum was the most common serological antigen in boars. In the early 1990s (Borčić et al. 1989), serological tests showed antibodies for just four detected serovars, and the most common serovar in wild boars was *Leptospira interrogans* sv. Pomona.

In contrast, the results obtained through this survey, together with the latest studies on wild boar (Cvetnić et al. 2003), showed a significant variation in the appearance frequency of leptospiral serovars (AB for nine antigens were recorded). Over the last 7 years, all serological analysis conducted revealed sv. Australis as the most commonly recorded serovar in the wild boar population. In our study, the same serovar showed the highest AB prevalence (>45%) and the highest AB titer (1:3,200), as well as a high percentage of cross-reactions recorded (20.11% of tested sera). It is further important to emphasize the stability of the AB titer for sv. Australis in serological reactions during the research, whereas the intensity of specific reactions of other serovars is changing with time (Yasuda et al. 1987). With the highest prevalence, sv. Australis participates in almost half (48.7%) of the positive reactions in wild boars (Fig. 3), and in the eastern lowland parts of Croatia, this percentage was even more obvious (>70%). The specific habits of wild boar (wallowing) and the possibility of cohabitation with domestic swine (masting and grazing) during the still-present nomadic method of swine rearing in some rural parts of the country, together with data on long-term germ carrying (Borčić et al., 1978), are also very important for the local epidemiological situation. Considering the fact that the wild boar population in Croatia shows constant tendencies to grow, we can conclude that wild boars have the main role in transmission of leptospirosis in the wild in the regions researched, but to declare them as maintaining hosts for *L. interrogans* sv. Australis, isolation of the infective agent is necessary.

The registered prevalence of antibodies to different leptospira serovars in the red fox population in this serological survey was 31.25%. Recently, Milas et al. (2006) found very high leptospira antibodies prevalence (57.6%) in red foxes from northwest Croatia. The authors found an explanation for such a high antibody rate in the fact that the populations of myomorphous mammals (as the main food sources for red fox) were overabundant during the research period. They reported sv. Australis as a most prevalent serovar in red fox, followed by sv. Sejroe and sv. Icterohaemorrhagiae. In our study, we recorded very similar results, with the highest AB prevalence for sv. Australis (46.2%) and same percentage of seropositive reaction for sv. Sejroe (19.2%) and sv. Icterohaemorrhagiae (19.2%). With a long-term period of germ carrying (Borčić et al. 1982), red foxes are presumed to be the main 'missing link' in the transmission of leptospirosis between domestic and wild species (Gillespie and Ryno 1963; Hathaway et al. 1983). Partial vaccination of red foxes against rabies in central parts of Croatia resulted in a population boom of foxes, with the consequent higher percentage of seropositive individuals. According to the results obtained from this study, red foxes have the second highest antibody prevalence (only 3.78% behind wild boars) for leptospirosis among the examined wild species, with the exception of stone martens. The observed high prevalence of leptospiral antibodies in stone marten (Fig. 2) could not be applied to the whole population level in Croatia because of the statistically insufficient number of samples (seven), but it could be a good indicator of this carnivores' importance in the transmission of leptospirosis.

Over a 10-year period (1981–1991), Modrić and Huber (1993) tested 32 free-ranging European brown bears and 10 captive bears from Zagreb Zoo for evidence of antibodies for 12 different leptospiral serovars. They have reported a high prevalence of leptospiral antibodies (34.3%) in the free-ranging bear population, and the most common serovar was sv. Australis followed by sv. Sejroe and sv. Icterohaemorrhagiae. In captive bears, the most prevalent serovar was sv. Icterohaemorrhagiae (40%), and the authors

presumed that this high serological reaction to this serovar could be a consequence of contact between bears and rats (*Rattus norvegicus*), which were declared as the main reservoir of sv. Icterohaemorrhagiae in Croatia (Zaharija and Perić 1969). In our study, free-ranging bears recorded the highest AB titer for sv. Icterohaemorrhagiae (44.4%) followed by sv. Australis and sv. Sejroe. It is very probable that the reverse order of the frequency of leptospiral serovars in free-ranging bears in this serological survey is an aftereffect of brown bears adaptation to co-habitation with humans. Especially in the last decade, free-ranging bears in Croatia (Frković et al. 2001) changed their feeding habits and often looked for food in trash yards and all other kinds of waste disposals. Higher concentrations of rats have been observed at waste disposals at an international level (Pascal et al. 2004; Traweger et al. 2006), whereas locally, numerous rats were recorded in trash yards (Baklaić et al. 2007) and suburban regions (Margaletić 2004; Margaletić and Kišasondi 2007) of central Croatia near the natural habitats of brown bears. Considering the fact that rats infected with leptospirosis could be germ carriers for a lifetime (Borčić et al. 1982), connection between rats and free-ranging bears on waste disposal sites should be pointed out, and this co-habitation is going to be the subject of further research.

In the red deer population, the most prevalent serovars during this studied period were the serovars Ballum and Pomona (Fig. 3). In past serological study, sv. Ballum recorded the highest AB titer as the most common leptospiral antigen among the red deer population 24 years ago (Kovačić et al. 1983, 1985a) and 18 years ago (Borčić et al. 1989). We presume that sv. Ballum persisted in free-ranging deer over a long period because of the alcaline pH value of deer's urine and their frequent habit of wallowing in lowland terrain. Although leptospiruria was not confirmed in Croatian deer (Kovačić et al. 1983) and only intermitent germ carrying is evidenced in deer (Trainer et al. 1963), there is some evidence of bucks' sterility in swamp habitats (unpublished data) in the central part of the country. Connections between red deer hinds' abortions and leptospirosis infection are yet to be investigated and have to be clarified in the future. The results of our study confirmed the relatively high serological reaction to leptospiral antigens in the red deer population in the Croatian lowland habitats (Posavina, Slavonia and Baranja), with the highest AB titer for sv. Ballum (23.6%) and sv. Pomona (23.6%).

The game species without any positive serological reactions were mouflon (28/0) and Eurasian lynx (3/0). Mouflons prefer hilly–karst habitats, characterized by lower vegetation and smaller amounts of water, and such conditions are unfavorable for leptospiral survival. Despite the insufficient number (three) of lynx serum samples, we could emphasize the predator–prey relation of these two species sharing the same habitat conditions. It is important to notice that most blood samples of the researched none-positive animals originated from the same area of Gorski Kotar and Kordun, regions with an almost total absence of lowland terrains, and characterized by a porous karst habitat.

Based on this, we can conclude that specific climate and edaphic and hydrological factors determine the incidence of leptospirosis in different types of habitat. In Croatia, the predominant districts for leptospiral perpetuity are swamp habitats around the main rivers—the Sava, Drava, and Danube, with the highest prevalence of leptospiral serovars in Posavina and Podravina ($P<0.0001$). In the mentioned region, wildlife species are the dominant vectors of leptospirosis, and among them, the most important game species are wild boar, red fox, and in some districts, red deer. Similar to previous results of studies into wildlife leptospirosis in Croatia, in this serological survey, a high prevalence of sv. Icterohaemorrhagiae was found in red foxes (a serovar highly related to rodents), whereas sv. Pomona was frequent among deers and wild boars—a serovar highly related to wild ungulates (Leighton and Kuiken 2001). It is also important to address the significance of the spread of wild boars and foxes into rural and even suburban areas, and the consequent increase of the possibility of transmission of leptospirosis to humans. Further research work is indispensable to clarify the epidemiological importance of game in leptospiral transmission, especially in lowland regions of Croatia, characterized by numerous natural foci of leptospirosis.

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References

Adler H, Vonstein S, Deplazes P, Steiger C, Frei R (2002) Prevalence of *Leptospira* spp. in various species of small mammals in an inner-city area Switzerland. Epidemiol Infect 128:107–109

Baklaić Ž, Jeličić P, Capak K, Ljubičić M (2007) Vectors of infectious diseases, real health threat in Croatia. Proceedings of DDD and ZUPP 19th Meeting—Disinfection, Disinfestation, Deratization and Protection of Stored Agricultural Products, 28–30 March, Dubrovnik, Croatia, pp 123–132

Babudieri B (1958) Animal reservoirs of leptospires. Ann N Y Acad Sci 70:393–399

Bondarenko AL, Utenkova EO, Russkikh GA, Khmeleveskaia NS (2002) Epidemiology of leptospirosis in the Kirov region. Zh Microbiol Epidemiol Immunobiol 3:27–30

Borčić B, Kovačić H, Tvrtković N, Šebek Z, Aleraj B (1978) Small rodents as reservoirs of leptospires in the lowlands of the Republic of Croatia. Lij Vijesn 100:465–470

Borčić B, Kovačić H, Šebek Z, Aleraj B, Tvrtković N (1982) Small terrestrial mammals as reservoirs of leptospirosis in the Sava Valley (Croatia). Fol Parasitol 29:177–182

Borčić B, Kovačić H, Šebek Z, Aleraj B, Tvrtković N (1983) Small terrestrial mammals as reservoirs of leptospires in the Drava Valley. Veterinarski Arh 53:41–49

Borčić B, Raos B, Šebek Z, Kranželić D, Abu Eldan J, Filipović V (1989) Detection of antibodies for leptospires in large game animals of northern Croatia. Veterinarski Arh 59:117–123

Brenner DJ, Kaufmann AF, Sulzer KR, Steigerwalt AG, Rogers FC, Weyant RS (1999) Further determination of DNA relatedness between serogroups and serovars in the family *Leptospiraceae* with proposal for *Leptospira alexanderi* sp. nov. and four new *Leptospira* genomo-species. Int J Syst Bacteriol 49:839–858

Collares-Pereira M, Mathias ML, Santos-Reis M, Ramalhinho MG, Duarte-Rodrigues P (2000) Rodents and *Leptospira* transmission risk in Terceira Island (Azores). Eur J Epidemiol 16:1151–1157

Cvetnić Ž, Margaletić J, Dikić M, Glavaš M, Djikić D, Špičić S, Jurić I, Salajpal K (2002) Rodents as possible reservoirs of leptospirosis in extensive swine breeding. Acta Agric Kapos 6:77–82

Cvetnić Ž, Margaletić J, Tončić J, Turk N, Milas Z, Špičić S, Lojkić M, Terzić S, Jemeršić L, Humski A, Mitak M, Habrun B, Krt B (2003) A serological survey and isolation of leptospires from small rodents and wild boars in the Republic of Croatia. Vet Med Czech 48:321–329

Frković A, Huber Đ, Kusak J (2001) Brown bear litter sizes in Croatia. Ursus 12:103–106

Gillespie RWH, Ryno J (1963) Epidemiology of leptospirosis. Am J Publ Health 53:950–959

Hathaway SC, Little TWA, Headlam SA, Stevens AE (1983) Infection of free-living carnivores with leptospires of the Australis serogroup. Vet Rec 113:233–235

Johnson RC (1976) The biology of parasitic spirochetes. Academic, New York

Karaseva EV, Chernukha YG, Piskunova LA (1973) Results of studying the time of survival of pathogenic leptospira under natural conditions. J Hyg Epidemiol Microbiol Immunol 17:339–343

Kmety E, Dikken H (1993) Classification of the species *Leptospira interrogans* and history of its serovars. University Press, Groningen

Kovačić H, Karlovic M, Poznanovic B (1983) Istraživanje proširenosti protutijela leptospira u jelena. Veterinarski Arh 53:35–39

Kovačić H, Karlovic M, Poznanovic B (1984) Detection of leptospira antibodies in wild boars from Baranja. Veterinarski Arh 54:77–81

Kovačić H, Lipej Z, Lacković M (1985a) Daljnja istraživanja o učestalosti protutijela za pojedine tipove leptospira u jelena (*Cervus elaphus*). Vet Glas 39:733–735

Kovačić H, Lipej Z, Lacković M (1985b) Učestalost nalaza protutijela za pojedine serotipove leptospira u lisica (*Vulpes vulpes*). Vet Glasnik 39:1305–1310

Krawczyk M (2005) Serological evidence of leptospirosis in animals in northern Poland. Vet Rec 156:88–89

Leighton FA, Kuiken T (2001) Leptospirosis. In: Williams ES, Barker IK (eds) Infectious diseases of wild mammals. 3rd edn. Manson, London, UK, pp 498–502

Lins ZC, Lopes ML (1984) Isolation of *Leptospira* from wild forest animals in Amazonian Brazil. Trans Roy Soc Tropic Med Hyg 78:124–126

Mailloux M (1977) Leptospiroses et climatologie. Med Mal Infect 7:428–431

Mailloux M (1980) Leptospiroses et environnement. Rev Epidemiol Sante Publique 28:323–327

Margaletić J (2004) Population dynamics of mouse and vole species in forest ecosystems of Croatia in the period from 1999 to 2003. Proceedings of DDD and ZUPP 16th Meeting—Disinfection, Disinfestation, Deratization and Protection of Stored Agricultural Products, 17–19 March, Rovinj, Croatia, pp 195–197

Margaletić J, Kišasondi A (2007) Knowledge of forest rodents' ecology and behavior for the purpose of conducting proper management. Proceedings of DDD and ZUPP 19th Meeting—Disinfection, Disinfestation, Deratization and Protection of Stored Agricultural Products, 28–30 March, Dubrovnik, Croatia, pp 431–452

Milas Z, Turk N, Starešina V, Margaletić J, Slavica A, Živković D, Modrić Z (2002) The role of myomorphous mammals as reservoirs of leptospira in the pedunculate oak forests of Croatia. Veterinarski Arh 72:119–129

Milas Z, Turk N, Janicki Z, Slavica A, Starešina V, Barbić LJ, Lojkić M, Modrić Z (2006) Leptospiral antibodies in red foxes (*Vulpes vulpes*) in northwest Croatia. Veterinarski Arh 76(Suppl):S51–S57

Modrić Z, Karlović M (1977) Leptospiroza u zeca (*Lepus europaeus*). Veterinarski Arh 47:251–256

Modrić Z, Huber Đ (1993) Serologic survey for leptospirae in European brown bears (*Ursus arctos*) in Croatia. J Wildl Dis 29:608–611

Poljičak-Milas N, Slavica A, Janicki Z, Robić M, Belić S, Milinković-Tur S (2004) Serum biochemical values in fallow deer (*Dama dama* L.) from different habitats in Croatia. Eur J Wildl Res 50:7–12

Pascal M, Lorvelec O, Borel G, Rosine A (2004) Rodent community structure in agricultural and 'natural' ecosystems of Guadeloupe and Martinique (French West Indies). Rev Ecol Terre Vie 59 (1–2):283–292

Songer JG, Chilelli CJ, Reed RE, Trauman RJ (1983) Leptospirosis in rodents from an arid environment. Am J Vet Res 44:1973–1976

Trainer DO, Hanson RP, Pope EP, Carbrey EA (1963) The role of deer in the epizootiology of leptospirosis in Wisconsin. Amer J Vet Res 98:159–167

Traweger D, Travnitzky R, Moser C, Walzer C, Bernatzky G (2006) Habitat preference and distribution of the brown rat (*Rattus norvegicus* Berk.) in the city of Salzburg (Austria): implications for an urban rat management. J Pest Sci 79(3):113–125

Treml F, Pejcoch M, Holesovska Z (2002) Small mammals—natural reservoirs of pathogenic leptospiroses. Vet Med Czech 47:309–311

Turk N, Milas Z, Margaletić J, Starešina V, Slavica A, Riquelme-Sertour N, Bellenger E, Baranton G, Postic D (2003) Molecular characterization of *Leptospira* spp. strain isolated from small rodents in Croatia. Epidemiol Infect 130:159–166

Yasuda PH, Steigerwalt AG, Sulzer KR, Kaufmann AF, Rogers F, Brenner DJ (1987) Deoxyribonucleic acid relatedness between serogroups and serovars in the family *Leptospiraceae* with proposal for seven new *Leptospira* species. Int J Syst Bacteriol 37:407–415

Zaharija I, Perić M (1969) Istraživanje leptospiroze u životinja u Hrvatskoj—VII Štakor (*R. norvegicus*) kao kliconoša u gradovima Zadru, Šibeniku, Splitu, Karlovcu, Sisku i Varaždinu. Veterinarski Arh 39:71–73