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Leptospiral antibodies in red foxes (*Vulpes vulpes*) in northwest Croatia

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ABSTRACT

In 2000 sera from 59 red foxes (*Vulpes vulpes*) from northwest Croatia were tested for antibodies against 12 *Leptospira interrogans* serovars: grippotyphosa, sejroe, australis, pomona, canicola, icterohaemorrhagiae, tarassovi, saxkoebing, ballum, bataviae, poi and hardjo. Using the microscopic agglutination test in 34 (57.6%) of the 59 sera, leptospira antibodies were found for 11 serovars, mainly for the serovar australis, then (in decreasing order) for the serovars sejroe, icterohaemorrhagiae, grippotyphosa and saxkoebing. The results showed a strong correlation with previous results of seroepidemiology and isolation of leptospira from small mammals in northwest Croatia. The role of the red fox as a maintaining leptospira reservoir in the natural foci of leptospirosis is still a matter of question and should be further investigated.

Key words: red fox, *Vulpes vulpes*, leptospira, natural foci, Croatia

Introduction

The life cycle of leptospira is maintained by circulation in nature among wild animals. Although infection by leptospira is common in a wide range of wild mammals wild murine and microtine rodents have been considered of primary importance as reservoirs in the

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chain of transmission of leptospirosis. Considering feed and feeding habits, the role of the fox as a predator in the nidality of leptospirosis becomes very interesting. There are few reports on antibodies against leptospira in foxes in Croatia. ZAHARIJA and TODOROVIĆ (1966) found antibodies for the leptospira serovars sejroe, saxkoebing, grippotyphosa and pomona in two foxes. KOVAČIĆ et al. (1985a) serologically tested blood samples of 196 foxes and found antibodies to nine leptospira serovars in 18.36% of foxes. ŠEBEK et al. (1976) found antibodies for leptospira in five of nine tested serum samples of foxes from north Tyrol in Austria. In Germany MÜLLER and WINKLER (1994) tested 1253 serum samples of foxes for the presence of leptospira antibodies and 24 serum samples (1.92%) were positive. The most common serovar was grippotyphosa. The object of this study is to explore the role of the fox as the most common predator in maintaining the natural foci of leptospirosis in Croatia, and to compare the results with previous investigations.

Materials and methods

Foxes were trapped during the regular hunting season throughout northwest Croatia, in 2000. After necropsy and testing for rabies at the Croatian Veterinary Institute, blood samples taken from the heart were delivered to the leptospirosis laboratory within a few hours, 12 hours at most. In total 59 sera were examined. Serological tests were performed by the microscopic agglutination test (GOCHENOUR et al., 1958) with 12 *L. interrogans* serovars: grippotyphosa, sejroe, australis, pomona, canicola, icterohaemorrhagiae, tarassovi, saxkoebing, ballum, bataviae, poi and hardjo. 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 $\geq$1:100 was considered as an indicator of earlier infection. If the serum was positive to two or more leptospira serovars, the serovar with the highest antibody titre was considered the infective agent. It was taken into account in evaluating these findings that occasional cross-reactivity among serovars may result in an equal or even higher antibody titre (MODRIĆ et al., 1985).

Results

The results obtained are shown in Table 1.

We found antibodies to 11 leptospira serovars in 34 (57.6%) of 59 red fox sera with titres ranging from 1:100 to 1:4000. The highest antibody titres were to serovar australis in 17 foxes, sejroe and icterohaemorrhagiae each in three foxes, grippotyphosa in two foxes and saxkoebing in one fox. We found the same antibody titer for two to three serovars in eight sera. Of the 34 seropositive animals, 16 were female and 18 male foxes.

Table 1. Titres of *Leptospira interrogans* antibody in sera of red foxes and the frequency of highest titres. Only positive foxes are listed.

Fox no.	Sex[a]	Leptospira serovars											
		[b]gri.	[b]sej.	[b]aus.	[b]pom.	[b]can.	[b]ict.	[b]tar.	[b]sax.	[b]bal.	[b]bat.	[b]poi.	[b]har.
1	f	-	1:100	1:100	-	-	1:100	-	-	-	-	-	-
2	f	-	1:500	1:1000	-	-	1:500	-	-	-	-	1:100	-
3	f	-	1:100	1:1000	-	-	1:100	-	1:100	-	-	-	-
4	f	-	1:500	1:2000	-	-	1:100	-	-	-	-	1:100	-
5	f	-	1:100	-	-	-	-	-	-	-	-	1:100	-
6	m	-	-	1:2000	-	-	-	-	-	-	-	1:100	-
7	f	-	1:100	1:1000	-	-	-	-	1:100	-	-	1:100	-
8	f	-	1:1000	-	-	-	1:500	-	-	-	-	1:100	-
9	m	-	-	1:1000	-	-	-	-	1:100	-	-	1:100	-
10	m	-	1:500	-	-	-	-	-	1:500	-	-	1:100	-
11	m	-	1:500	-	-	-	1:100	-	-	-	-	-	-
12	m	1:100	1:500	1:1000	-	-	1:500	1:100	1:500	1:100	-	1:500	-
13	f	-	1:100	-	-	-	1:100	-	-	-	-	-	-
14	m	1:100	1:100	1:500	-	-	1:100	-	1:100	-	-	-	-
15	f	-	-	1:100	-	-	-	-	-	-	-	-	-
16	f	-	-	-	-	-	1:100	-	-	-	-	-	-
17	m	-	-	1:100	-	-	-	-	-	-	-	1:100	-
18	m	-	-	1:2000	-	-	-	-	-	-	-	-	-
19	m	1:500	1:100	1:100	1:500	-	1:500	-	-	-	-	-	-
20	m	-	1:2000	1:4000	-	-	1:2000	-	-	-	-	1:1000	-
21	f	-	1:100	-	-	-	-	-	-	-	-	-	-
22	f	-	-	-	-	-	-	-	1:100	-	-	-	-
23	f	1:500	-	1:1000	-	-	-	-	-	1:100	-	1:100	-
24	m	1:500	-	-	-	-	-	-	-	-	-	-	-
25	m	-	-	1:500	-	-	-	-	-	-	-	-	-
26	f	-	-	1:500	-	-	-	-	-	-	-	1:500	-
27	m	-	-	1:500	-	-	-	-	-	-	-	-	-
28	m	-	-	-	-	-	-	-	-	1:100	1:100	-	-
29	m	1:500	-	-	-	-	-	-	-	1:100	-	-	-
30	m	-	1:100	1:2000	-	-	1:500	-	-	-	-	-	-
31	m	-	-	1:100	-	-	-	1:100	-	-	-	-	1:100
32	m	-	1:100	-	-	-	1:500	-	-	-	-	1:100	-
33	f	-	-	1:1000	-	-	-	-	1:100	-	-	-	-
34	f	-	-	-	-	-	1:100	-	-	-	-	-	-
Frequency of having the highest titer		2	3	17			3		1				

[a] f = female; m = male

[b] gri. = grippotyphosa, sej. = sejroe, aus. = australis, pom. = pomona, can. = canicola, icth. = icterohaemorrhagiae, tar. = tarassovi, sax. = saxkoebing, bal. = ballum, bat. = bataviae, poi. = poi, har. = hardjo.

Discussion and conclusions

We found a prevalence of 57.6% antibodies against *Leptospira* spp., which is present, the highest antibody rate in red foxes published in literature in Croatia. In earlier investigations KOVAČIĆ et al. (1985a) reported 18.4% of 196 foxes positive, but in collecting areas which were similar to the areas in our investigation they found from 21.3% to 27.0% positive animals. Since the collecting areas of both investigations are parts of natural foci of leptospirosis, the lower prevalence in their investigation may be explained by a smaller population of small mammals and due to the fact that they used the technique of paper strips with dried blood which is a less sensitive method. Investigations throughout the world showed high differences in the spread of leptospirosis in foxes. The percentage of infected animals varied from 6.52% in Switzerland (ŠEBEK and ROSICKY, 1975), 12.0% in Ontario (KINGSCOTE, 1986), 47.0% in Wisconsin (AMUNDSON and YUILL, 1981) to 80.0% in grey fox (*Urocyon cinereoargenteus*) in California (CIRONE et al., 1978). The high percentage of seropositive foxes in our investigation is probably due to the reflection of the biotic structure of leptospirosis foci in northwest Croatia and numerous populations of myomorphous mammals in 2000. Antibody titres against serovar australis were highest in 17 foxes. Antibodies against serovars sejroe and icterohaemorrhagiae were highest in three fox sera for each serovar, for serovar grippotyphosa in two and for serovar saxkoebing in one fox sera. In northwest Croatia, 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*). Isolation of other leptospira serovars like grippotyphosa, pomona, sejroe, bataviae from different small mammals species in Croatia were reported by BORČIĆ et al. (1982; 1986), MILAS et al. (2002), and TURK et al. (2003). Thus, small wild mammals may be potential sources of infection for serovar australis, grippotyphosa, sejroe, pomona for other animals, including red foxes. In support of this statement are findings of specific antibodies for different leptospira serovars in game sera from northwest Croatia. MODRIĆ and KARLOVIĆ (1977) found antibodies in 30.7% hare sera for the serovars grippotyphosa, pomona, icterohaemorrhagiae, sejroe, australis and saxkoebing. KOVAČIĆ et al. (1985b) found antibodies for the serovars pomona, icterohaemorrhagiae, ballum, australis and grippotyphosa in 5.0% deer sera. In wild boars, antibodies were founded for serovars grippotyphosa, australis, tarassovi, sejroe, pomona, ballum and icterohaemorrhagiae (KOVAČIĆ et al., 1984). MODRIĆ and HUBER (1993) found antibodies for serovars australis, sejroe, canicola and icterohaemorrhagiae in European brown bears (*Ursus arctos*). Considering the living habits of red foxes, the antibodies against serovar icterohaemorrhagiae and sejroe found in fox sera may be due to infection from rats (*Ratus norvergicus*), the main reservoir of this serovar in Croatia (ZAHARIJA and PERIĆ, 1969) and house mice (*Mus musculus*) (MILAS et al.,

2002). Small mammals are important autumn and winter food sources for red foxes and other wild carnivores. The annual diet of the red fox includes pheasants, poultry, rabbits, small rodents, birds, snakes, turtles and grasshoppers, but the mainstay is the mouse. The amount of small mammals in the diet of foxes ranges from 27% in summer to 37% in winter (SCOTT, 1943). Thus, we suppose that the leptospiral infection in foxes depends on the spread of leptospirae among small mammals. The high prevalence of antibodies against leptospirae in foxes is evidence from the circulation of certain leptospirae serovars in their population. REILLY et al. (1970) suppose that due to food habits, the red fox as a predator is reservoir which helps leptospira to survive during the winter (when the population of small mammals decreases) and to maintain and disseminate the disease among the additional susceptible small mammal population. So, the role of the red fox as a maintaining leptospira reservoir in the natural foci of leptospirosis is still a matter of question and should be further investigated.

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SAŽETAK

Tijekom 2000. godine 59 uzoraka seruma lisica (*Vulpes vulpes*) s područja sjeverozapadne Hrvatske pretraženo je na protutijela za 12 serovara bakterije *Leptospira interrogans* i to kako slijedi: serovare

grippotyphosa, sejroe, australis, pomona, canicola, icterohaemorrhagiae, tarassovi, saxkoebing, ballum, bataviae, poi i hardjo. Metodom mikroskopske aglutinacije, 34 (57,6%) od 59 pretraženih seruma očitovalo je pozitivnu reakciju za ukupno 11 serovara, poglavito za serovar australis, a potom za serovare sejroe, icterohaemorrhagiae, grippotyphosa i saxkoebing. Polučeni rezultati pokazuju snažnu korelaciju s prethodnim nalazima seroepidemiologije i izolacije leptospira iz malih sisavaca na području sjeverozapadne Hrvatske. Uloga lisice u održanju prirodnih ognjišta leptospiroze je i nadalje upitna te nalaže dodatna istraživanja.

Ključne riječi: lisica, *Vulpes vulpes*, leptospire, prirodna ognjišta bolesti, Hrvatska