Small rodents reservoires of leptospiroses in the forests of Posavina in Croatia

Margaletić, Josip; Glavaš, Milan; Turk, Nenad; Milas, Zoran; Starešina, Vilim

Source / Izvornik: **Glasnik za šumske pokuse: Annales Experimentis Silvarum Culturae Provehendis, 2002, 39, 43 - 65**

Journal article, Published version
Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:108:516695

UDK: 630*151

Original scientific paper
Izvorni znanstveni članak

SMALL RODENTS RESERVOIRES OF LEPTOSPIROSES IN THE FORESTS OF POSAVINA IN CROATIA

SITNI GLODAVCI KAO IZVOR LEPTOSPIROZA U POSAVSKIM ŠUMAMA U HRVATSKOJ

JOSIP MARGALETIĆ*, MILAN GLAVAŠ*, NENAD TURK**, ZORAN MILAS**, VILIM STAREŠINA**

* Department of Forest Protection and Wildlife Management, Faculty of Forestry, University of Zagreb, P. O. Box 422, HR – 10002 Zagreb
** Department of Microbiology and Infectious Diseases with Clinic, Veterinary Faculty, University of Zagreb, P. O. Box 466, HR – 10002 Zagreb

Received – *Prispjelo*: 22. 7. 2002. Accepted – *Prihvaćeno*: 7. 12. 2001.

Small rodents from the subfamilies *Murinae* and *Arvicolinae* are a reservoires of infectious diseases affecting humans, domestic and wild animals. The paper presents the results of research on these mammals as the reservoirs of leptospiroses. The samples were collected from April to November 2000 in the forests of Posavina in Croatia. The forests are managed by 11 forest administrations of the public enterprise "Croatian Forests", Ltd. The following species of small rodents were detected: *Apodemus agrarius, A. flavicollis, A. sylvaticus, Arvicolla terrestris, Clethrionomys glareolus, Microtus agrestis, M. arvalis* and *Mus musculus*. The dominant species in the studied plots (44) were *A. flavicollis* and *A. agrarius*. Most individuals were captured in the forests managed by forest administrations Popovača (25.86 %) and Nova Kapela (17.94 %). 83.82 % of the animals were caught in the forest communities of *Genisto elatae - Quercetum roboris caricetosum remotae, Genisto elatae - Quercetum roboris caricetosum brizoides* and *Carpino betuli - Quercetum roboris typicum*. The majority of them were captured during autumn months. A total of 379 animals were analysed with the method of renoculture were down in Korthof's liquid medium and serological reaction to microscopic agglutination with 12 serological variants of the *Leptospira*. A total of 17 *Leptospira* strains were isolated with renoculture from the individuals of three rodent species (*M. musculus, A. flavicollis* and *A. agrarius*) caught in the localities in Kutina, Velika Gorica, Popovača and Nova Kapela. The antibody titre to *Leptospira* was identified in 48 animals of the following species: *A. agrarius, A. flavicollis, M. musculus, C. glareoulus* and *A. sylvaticus*.

Key words: small rodents, forest ecosystems, leptospiroses, Posavina, Croatia

INTRODUCTION
UVOD

Forests are complex ecosystems supporting a large variety of organisms. Biodiversity, sustainable management and natural regeneration are the basic postulates of past, present and future development of forests and forestry (Gračan *et al.* 1998). In Croatia, forests cover about two million hectares. The total forest area with pedunculate oak (*Quercus robur* L.) as the dominant species accounts for 201,739 ha, which is 10 % of the overall forest area (Klepac 1996, Klepac & Fabijanić 1996).

Small rodents from the subfamilies *Murinae* (the true mice) and *Arvicolinae* (the voles) directly influence forest regeneration by damaging forest seeds, seedlings and young plants (Margaletić 1998). Damage is particularly high in the years of their overproliferation. Croatia's lowland forests are inhabited by the following species of small rodents: bank vole (*Clethrionomys glareolus* Schreib.), water vole (*Arvicola terrestris* L.), common pine vole (*Microtus subterraneus* de Sel.), common vole (*M. arvalis* Pall.), field vole (*M. agrestis* L.), Alpine pine vole (*M. multiplex* Fat.), striped field mouse (*Apodemus agrarius* Pall.), wood mouse (*A. sylvaticus* L.) and yellow-necked mouse (*A. flavicollis* Melch.) (Margaletić 1998, Glavaš & Margaletić 2001, Margaletić & Glavaš 2001). The high population densities and width of ecological valence make small rodents an important part of almost any forest ecosystem. They represent a significant group of animals which link the primary producers with higher trophic levels. These mammals have exceptionally sharp, characteristic front incisors (Delany 1974). Their breeding potential is very high (Kowalski 1976).

Small rodents are reservoires of various infectious diseases (zoonoses) of human, as well as domestic and wild animals and they play an important role in spreading zoonoses, infectious and parasitic diseases (trichinosis, leptospirosis, tick encephalitis, Lyme disease, hemorrhagic fever with kidney syndrome and others) (Bäumler 1975, Geisel *et al.* 1979, Barrow 1981, Borčić *et al.* 1982a, 1982b, 1983). This depends on the rodent population size, their distribution, mobility, feeding intensity, habitat conditions and breeding potential, as well as the number and distribution of wild and domestic animals susceptible to infectious diseases. Small rodents transmit the causes of infectious diseases actively (secretions or excretions) or passively (ectoparasites and endoparasites). The spread of diseases transmitted by mice and voles can seriously endanger the health and the number of receptive wild animal species, disturb the balance of the forest ecosystem, or inflict vast damage to organised management of good-quality hunting game. In lowland oak forests, which are the most widespread in the Posavina region of Croatia, zoonoses particularly affect people who work there or who occasionally spend some time there (foresters, gamekeepers, hunters, natural scientists, soldiers, hikers and others). As germ carriers, small rodents may occasionally or permanently secrete the causes of infectious diseases and contaminate their living environment with their secretions or excretions, turning it into intermediary and secondary sources of infectious diseases (Zaharija 1980, Cvetnić 1993).

This paper deals with leptospirosis. It is an acute septicaemic infectious disease affecting a wide range of domestic and wild animals, as well as human, which usually occurs enzootically. It is clinically manifested in icterus, sometimes in haemoglobinuriam, and in abortion in cattle

and pigs. Leptospirosis is caused by pathogenic spiral-shaped bacteria of the genus Leptospira (Noguchi 1918) which comprises several genomospecies (Ellis 1955, Brenner *et al.* 1999), 24 serogroups and more then 250 serovars (Kmety & Dikken 1993). This research gives new insights into the representation of some serological *Leptospira* strains (serovars) in the studied localities and identifies small rodents species infected with leptospiroses in the studied localities of Posavina.

The results of research of small rodents as the reservoirs of zoonoses (leptospirosis, tularaemia, rabies, hemorrhagic fever, etc.) in Croatia have been published in the following papers: Borčić et al. 1982a, 1982b, 1983, 1986, 1987, 1999, etc. These authors have recorded a natural focus of tularaemia and leptospirosis in the lowland part of northern Croatia, while hemorrhagic fever and rabies were identified in the localities of Plitvice, Dinara, Turopolje, Psunj, Papuk and Central Posavina.

Leptospiroses in micro mammals in the area of Croatia were also studied by Zaharija (1968). He studied nine rodent species, 1,431 animals, of which leptospiroses were detected in the following four species: *M. arvalis*, *Mus musculus* (L.), *Crocidura* sp. and *A. agrarius*. On six localities in Podravina and Posavina he isolated 28 leptospira strains of the following serological variants: *grippotyphosa* (21), *sejroe* (4) and *ballum* (2). Borčić *et al.* (1982a) studied small rodents in the River Sava valley. In seven localities 1,734 animals were caught, of which 15 species were identified, dominated by *M. arvalis* (38 %) and *A. agrarius* (32 %). A total of 675 animals were analysed with renoculture and 580 animals were analysed serologically. In the animals under study an infection with leptospires of the serologic variants *pomona* and *grippotyphosa* was identified with isolation and serology. Earlier papers were mostly concerned with small meadow and field rodents as sources of leptospiroses, while this paper deals with infected small rodents inhabiting forest stands, which are natural foci of these diseases.

RESEARCH AREA
PODRUČJE ISTRAŽIVANJA

The research was conducted in the forests of Posavina in Croatia. The forests included forest stands managed by 11 forest administrations of "Croatian Forests" Ltd (Figure 1). Several sample plots were set up in each locality (a total of 44). Small rodents were collected in between April and November 2000. In the majority of the localities samples of small rodents were collected twice a year, in spring and autumn. Samples were not taken from the end of July to the beginning of September.

Rodents were captured in the following forest communities: *Genisto elatae-Quercetum roboris caricetosum remotae, Genisto elatae-Quercetum roboris caricetosum brizoides, Carpino betuli-Quercetum roboris typicum, Carpino betuli-Quercetum roboris fagetosum, Carpino betuli-Quercetum roboris quercetosum cerris, Carici pilosae-Fagetum sylvaticae, Epimedio-Carpinetum betuli, Leucoio-Fraxinetum angustifoliae* and *Frangulo-Alnetum glutinosae* (Vukelić & Rauš 1998). The forest communities of *Carici pilosae-Fagetum sylvaticae, Epimedio-Carpinetum betuli, Leucoio-Fraxinetum angustifoliae* and *Frangulo-*

Alnetum glutinosae encompassed by the research, are distributed in the studied localities of state hunting grounds "Garjevica" (no. VII/4) (*Carici pilosae-Fagetum sylvaticae* and *Epimedio-Carpinetum betuli*) and "Radinje" (no. XII/16) (*Leucoio-Fraxinetum angustifoliae* and *Frangulo-Alnetum glutinosae*).

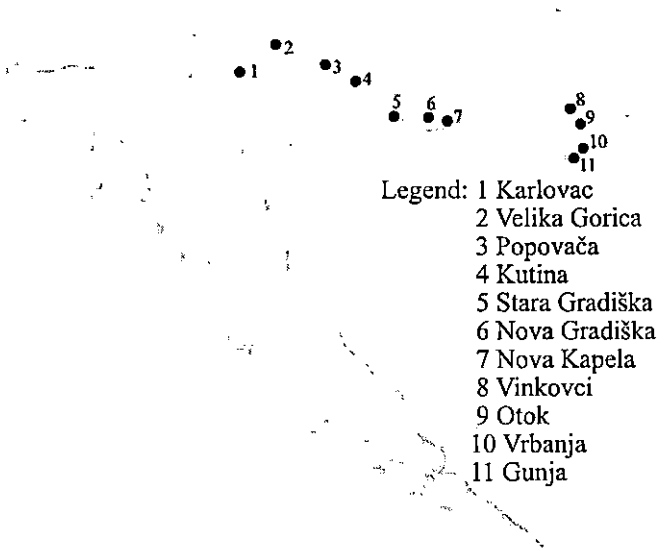

Figure 1. The localities where small rodents were collected
Slika 1. Lokaliteti uzorkovanja sitnih glodavaca

In the area managed by the Forest Administration Zagreb, research was done in the localities of the following Forest Offices: Popovača (MU „Popovačke nizinske šume", localities: „Paljevička", compartment/subcompartment 90b; "Ravnik", compartment/ subcompartment 95b and "Veliki Ravnik" compartment/subcompartment 99a), Velika Gorica (MU "Turopoljski Lug", localities: "Vratovo", compartment/subcompartment 98a; "Klenovo", compartment/ subcompartment 120a, "Rastine", compartments/subcompartments 90a and 59a, and "Jalševa Greda", compartment/subcompartment 58a) and Kutina (MU "Kutinska Garjevica", localities: "Izbjeljeno Brdo", compartment/subcompartment 102a; "Šib", compartments/subcompartments 110a and 116a, and "Poznanović Planina", compartment/subcompartment 111a).

In the area managed by the Forest Administration Karlovac, research was done in the Forest Office Karlovac, MU "Rečički Lugovi, in the localities "Stara Brajnica (compartments/ subcompartments 26a, 27b and 77a), "Prekblatnica" (compartment/subcompartment 70a) and "Mokrice" (compartment/subcompartment 33a).

In the area of the Forest Administration Nova Gradiška, research was done in the following Forest Offices: Stara Gradiška (MU "Ljeskovače", locality "Ljeskovača", compartments/subcompartments 24a, 25b, 26a, 26b, 29a and 30a), Nova Gradiška (MU "Ključevi", locality "Ključevi", compartments/subcompartments 27a, 32a and 35a) and Nova Kapela (MU "Radinje", localities: "Rastovica", compartment/subcompartment 49a; "Hajdučka Greda", compartments/subcompartments 12b and 13b; "Vlakanac", compartment/subcompartment 16d; "Rušćica", compartment/subcompartment 17a; "Vrapča", compartment/subcompartment 22a and "Lukovo", compartment/subcompartment 50a).

In the area of the Forest Administration Vinkovci, rodents were caught in the localities of the following Forest Offices: Vinkovci (MU "Kunjevci", locality "Kunjevci", compartments/subcompartments 29a and 32a), Otok (MU "Slavir", locality "Tikar", compartments/subcompartments 152a and 152b), Vrbanja (MU "Vrbanjske Šume", locality "Svenovo", compartments/subcompartments 147c, 150a, 150c and 150d) and Gunja (MU "Trizlovi-Rastovo", locality "Rađenovci", compartments/subcompartments 44a, 45a, 46a and 461).

Figure 2 shows the number of sample plots set up in the forest communities in which animal samples were taken.

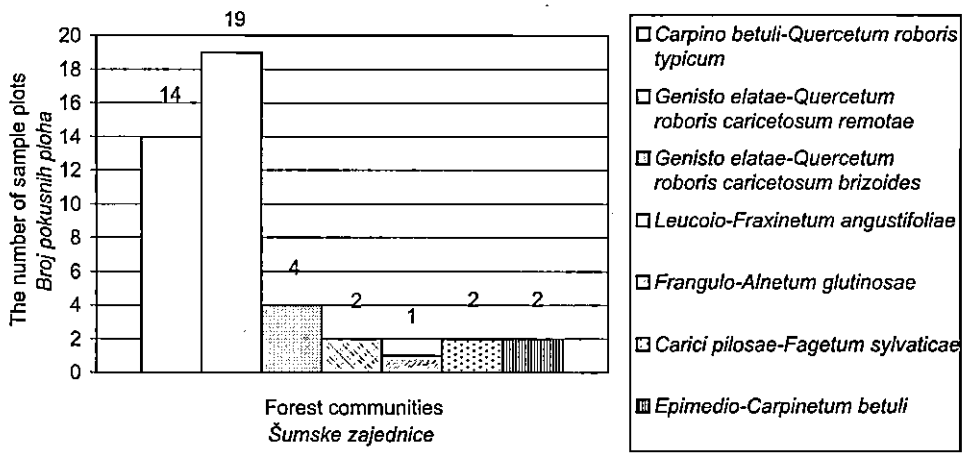

Figure 2. The number of sample plots by forest communities in which samples of small rodents were trapped

Slika 2. Broj pokusnih ploha po pojedinim šumskim zajednicama na kojima su uzorkovani sitni glodavci

METHODS
METODE

Small rodents were trapped with Sherman snap traps and live-traps. The traps were set up in transects at a distance of 5 - 7 m and were baited with apples, as well as a mixture of oatmeal and sardines in oil. The collected individuals were identified according to Niethammer & Krapp (1978, 1982) and deposited to Faculty of Forestry from University of Zagreb.

A total of 379 rodents were analysed serologically and bacteriologically in the Institute of Microbiology and Infective Diseases at the Faculty of Veterinary Science in Zagreb. A method of microscopic agglutination was used as a standard method of serologic diagnostics and leptospira classification. In case of the presence of antibodies in the basic dilution 1:100, the samples were tested in higher serum dilutions (1:500, 1:1000, etc.). The highest antibody titre to an individual leptospira antigen or serovar in the serum indicates a "possible" serovar responsible for the infection (Johnson 1976). Each blood sample was tested with the antigens *L. interrogans* sv: *icterohaemorrhagie, ballum, australis, pomona, grippotyphosa, sejroe, saxkoebing, bataviae, tarassovi, canicola, poi* and *hardjo*. To isolate leptospira, the positive individuals were treated with the renoculture weredown in Korthof's liquid medium.

A total of 17 leptospire strains isolated from *A. flavicollis, A. agrarius* and *M. musculus*, which were collected in Kutina, Popovača, Velika Gorica, Nova Kapela and Otok, were sent to Pasteur Institute (Paris, France), where the PFGE– pulsed-field gel electrophoresis was done. The PFGE analysis consists of the following: after restricting a chromosome DNA with the restriction enzymes "NotI" and "Srg AI", the restriction profiles of the tested serovars (serologic variants) are compared with the referent serovar profiles. This method may determine leptospires at the serovar level, that is, establish their physical map (Baril & Saint Girons 1990). The PFGE analysis with the restriction enzyme "NotI" isolates the serovars of "*sejroe*" and "*pomona*" serologic groups (serogroups), while the analysis with the restriction enzyme "Sgr AI" isolates the serovars of the "*australis*" serologic group.

RESULTS
REZULTATI

A total of 445 small rodents were collected in all the localities, and 379 were analysed in the laboratory (85.17 %). The remaining 66 individuals were not analysed due to predator damage or tissue decomposition caused by atmospheric occurrences (high temperature). The results of analysed individuals by species and localities are given numerically in Table 1. The animals were collected in the area of 10 different forest offices in Upper, Central and Lower Posavina. No samples of small rodents were caught in the area of the Forest Office Vinkovci, and therefore there are no results of the leptospira infection from this locality.

Table 1. Analysed small rodents by locality of collection and sex of the animal. *First row – number of analysed animals. Second row – the first number are males and the second females

*Tablica 1. Prikaz pretraženih sitnih glodavaca prema lokalitetu uzorkovanja i spolu životinje. *Prvi red – broj analiziranih životinja. Drugi red – prvi broj predstavlja muški, a drugi broj ženski spol*

Animal species *Vrsta životinje*	Kutina	Velika Gorica	Popovača	Vrbanja	Gunja	Stara Gradiška	Nova Kapela	Otok	Nova Gradiška	Karlovac	Σ	%
Apodemus agrarius	0	2 (2/0)*	33 (6/27)	9 (2/7)	1 (1/0)	12 (6/6)	33 (8/25)	5 (2/3)	8 (0//8)	5 (2/3)	108 (26/82)	28.5
Apodemus flavicollis	8 (3/5)	10 (6/4)	38 (9/29)	6 (3/3)	2 (1/1)	1 (0/1)	11 (5/6)	6 (4/2)	5 (1/4)	24 (11/13)	111 (43/68)	29.3
Apodemus sylvaticus	2 (2/0)	9 (3/6)	11 (5/6)	7 (0/3)	7 (2/5)	0	4 (1/3)	6 (2/4)	3 (1/2)	13 (3/10)	62 (23/39)	16.4
Arvicola terrestris	0	0	1 (0/1)	0	0	0	0	0	0	0	1 (0/1)	0.3
Clethrionomys glareolus	1 (0/1)	5 (4/1)	12 (0/12)	4 (2/2)	6 (1/5)	1 (1/0)	19 (9/10)	1 (0/1)	1 (0/1)	3 (0/3)	53 (17/36)	13.9
Microtus agrestis	0	2 (1/1)	2 (2/0)	0	0	0	0	0	0	2 (1/1)	6 (4/2)	1.6
Microtus arvalis	0	3 (1/2)	0	1 (0/1)	0	0	1 (1/0)	1 (0/1)	0	0	6 (2/4)	1.6
Mus musculus	30 (17/ 13)	0	1 (0/1)	0	0	1 (1/0)	0	0	0	0	32 (18/14)	8.4
Σ	41 (22/9)	31 (17/4)	98 (22/76)	27 (11/16)	16 (5/11)	15 (8/7)	68 (24/44)	19 (8/11)	17 (2/15)	47 (17/30)	379 (136/243)	100.0

The following species of small rodents were trapped: *A. agrarius, A. flavicollis, A. sylvaticus, A. terrestris, C. glareolus, M. agrestis, M. arvalis* and *M. musculus*. The dominant species were *A. flavicollis* (111 individuals) and *A. agrarius* (108 individuals), while the most numerous vole was *C. glareolus* (53 individuals) (Figure 3). The highest number of collected individuals was in Popovača (100 animals or 25.86 %). In the total of 379 analysed individuals, 136 were males and 243 females.

The majority of *M. musculus* (93.75 %) were captured in the closed facilities of the state hunting ground "Garjevica" (Forest Office Kutina).

The antibody titre to leptospires was detected in 48 animals in all the localities. The species include *A. agrarius, A. flavicollis, M. musculus, C. glareolus* and *A. sylvaticus* (Table 2). Table 3 gives detailed analysis of the presence of antibodies against serological variants of leptospires according to the highest antibody titre (quantity). The amount of 34.38 % of *M. musculus*, 14.18 % of *A. agrarius*, 10.81 % of *A. flavicollis*, 6.45 % of *A. sylvaticus*, and 9.43 % of *C. glareolus* species were found to be serologically positive. Serological variants *saxkoebing* and *hardjo* were found in *M. musculus*, while four isolates remained unidentified with the antibody titre 1:100. In *A. agrarius*, the serological variants of *grippotyphosa, sejroe, pomona, icterohaemorragiae, tarassovi* and *hardjo* were found, while the analysis of *A. flavicollis* showed the presence of antibodies against the *australis, pomona, saxkoebind* and *bataviae* serological variants. Four antibody findings remained unidentified with the antibody titre 1:100. In *A. sylvaticus*, serological variants *sejroe, pomona* and *tarassovi* were found, while the analysis of *C. glareolus*

revealed the presence of the antibodies of serological variants *sejroe, australis, saxkoebing* and *hardjo*. Five isolates remained unidentified with the antibody titre 1:100 (4) and 1:500 (1).

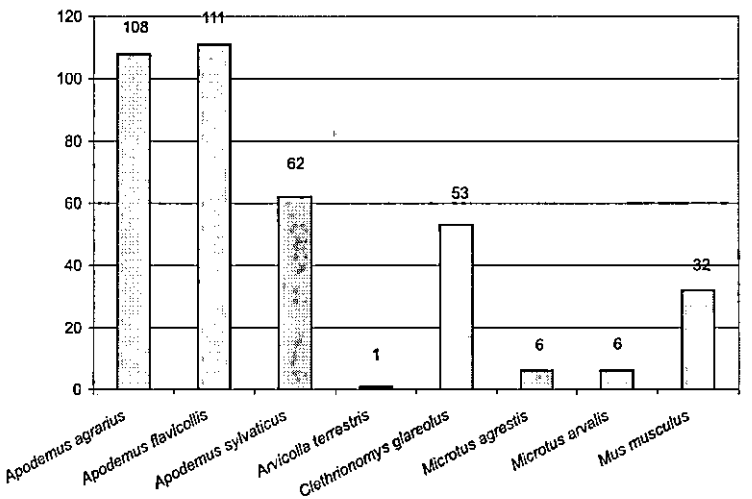

Figure 3. The number of small rodents according to species tested for leptospirosis during the year 2000.

Slika 3. Broj sitnih glodavaca po vrstama obrađenih na leptospirozu tijekom 2000. godine

Table 2. The presence of antibody titres to leptospires according to small rodent species

Tablica 2. Prisutnost titara protutijela na leptospire po vrstama sitnih glodavaca

Small rodent species *Vrsta sitnih glodavaca*	Total number captured *Ukupno ulovljeno*	Total number analysed *Ukupno pretraženo*	Antibody titre *Titar protutijela*	% infected *% inficiranih*	% analysed *% pretraženih*
Apodemus agrarius	135	108	16	14.81	80.00
Apodemus flavicollis	133	111	12	10.81	83.46
Mus musculus	36	32	11	34.38	88.89
Clethrionomys glareolus	61	53	5	9.43	86.89
Apodemus sylvaticus	62	62	4	6.45	100.00
Microtus agrestis	7	6	0	0.00	85.71
Microtus arvalis	10	6	0	0.00	60.00
Arvicola terrestris	1	1	0	0.00	100.00
Total - *Ukupno*	445	379	48	12.66	85.17

Table 3. Antibodies against leptospires in the serum of analysed animals by the highest titre

Tablica 3. Nalaz protutijela za leptospire u serumu pretraženih životinja prema najvišem titru

Animal species *Vrsta životinje*	Total number analysed *Ukupno pretraženo*	Antibody titre *Titar protutijela*	Serological leptospires variant														% serologically positive *% serološki pozitivnih*
			grippotyphosa	*sejroe*	*australis*	*pomona*	*canica*	*icterohaemorrhagiae*	*tarassovi*	*saxkoebing*	*ballum*	*bataviae*	*poi*	*hardjo*	Unidentified Neodređeno	Total number of positive *Ukupno pozitivnih*	
Mus musculus	32	1:100								3				1	4	3	34,38
		1:500								2				1		3	
		Σ								5				2	4	11	
Apodemus agrarius	108																14,81
		1:100	2	1		4		1	2					3		13	
		1:500				3										3	
		Σ	2	1		7		1	2					3		16	
Apodemus flavicollis	111																10,81
		1:100			3					1		1			4	9	
		1:500			1											1	
		1:1000				1										1	
		1:2000			1											1	
		Σ			5	1				1		1			4	12	
Apodemus sylvaticus	62																6,45
		1:100		1		1			1							3	
		1:500				1										1	
		Σ		1		2			1							4	
Clethrionomys glareolus	53																9,43
		1:100		1						1				1	1	4	
		1:500			1											1	
		Σ		1	1					1				1	1	5	
Σ	366																13,11

Table 4. Antibodies against leptospires with the microscopic agglutination reaction and leptospire isolation with renoculture in dependence on the locality and small rodent species. *First row–number of analysed animals. Second row–the first number represents antibodies against leptospires and the second leptospire isolation

*Tablica 4. Nalaz protutijela za leptospire reakcijom mikroskopske aglutinacije i izolacija leptospira renokulturom ovisno o lokalitetu i vrsti sitnih glodavaca. * Prvi red – broj pregledanih životinja. Drugi red – prvi broj predstavlja nalaz protutijela na leptospire, a drugi broj izolata leptospira*

Small rodent species *Vrste sitnih glodavaca*	Kutina	Velika Gorica	Popovača	Vrbanja	Gunja	Stara Gradiška	Nova Kapela	Otok	Nova Gradiška	Karlovac	Σ	%
Apodemus agrarius	0	2	33 (2/0)	9	1	12 (3/0)	33 (9/4)	5 (1/0)	8	5 (1/0)	108 (16/4)	28.3
Apodemus flavicollis	8	10 (4/1)*	38 (3/2)	6	2	1 (1/0)	11 (2/0)	6	5 (1/0)	24 (1/0)	111 (12/3)	29.1
Apodemus sylvaticus	2	9	11 (2/0)	7 (1/0)	7 (1/0)	0	4	6	3	13	62 (4/0)	16.3
Arvicola terrestris	0	0	1	0	0	0	0	0	0	0	1 (0/0)	0.3
Clethrionomys glareolus	1	5 (2/0)	12	4	6	1 (1/0)	19 (2/0)	1	1	3	53 (5/0)	13.9
Microtus agrestis	0	2	2	0	0	0	0	0	0	2	6 0/0	1.6
Microtus arvalis	0	3	0	1	0	0	1	1	0	0	6 (0/0)	1.6
Mus musculus	30 (11/10)	0	1	0	0	1	0	0	0	0	32 (11/10)	8.4
Σ	41 (11/10)	31 (6/1)	98 (7/2)	27 (1/0)	16 (1/0)	15 (5/0)	68 (13/4)	19 (1/0)	17 (1/0)	47 (2/0)	379 (48/17)	99.9

Table 4 gives the results of captured individuals, and antibodies against leptospires and leptospire isolation by small rodent species and locality. Leptospira antibodies were detected in 48 individuals, while leptospira isolation was positive in 17 individuals. In 108 individuals of *A. agrarius* (28.3 %), antibodies against leptospires were found in 16 animals, while in four animals the isolate was obtained. In 111 individuals of *A. flavicollis* (29.1 %), the leptospires antibodies were identified in 12 cases, and in three cases the strain was isolated. In 62 *A. sylvaticus* the microscopic agglutination reaction revealed leptospires antibodies in four animals, while no isolate was obtained. In *A. terrestris*, *M.* and *M. arvalis*, no leptospira antibodies were found, nor isolates obtained. In 53 individuals of *C. glareolus*, the leptospires antibodies were found in five, while no isolates were obtained. In 32 *M. musculus* the leptospire antibodies were identified in 11 individuals, while renoculture provided 10 leptospire isolates.

In the locality Kutina (the hunting ground "Moslavina") leptospires antibodies were found in 11 of 41 analysed animals, while isolation was in 10 individuals. All leptospires were found in succesfull *M. musculus*. No leptospires were found in *A. flavicollis, A. sylvaticus* and *C. glareolus.*

In the sample of 31 analysed rodents in Forest Office Velika Gorica, antibodies were found in six animals, and only one isolate was found. Approximately 50 % of *A. flavicollis* and *C. glareolus* were found to be infected with leptospires, while no leptospires were found in *A. agrarius, A. sylvaticus, M. agrestis* and *M. arvalis.*

A total of 98 animals from the stands in the Forest Office Popovača were analysed. The structure of the catch by species is given in Table 4. The antibodies for leptospires were found in seven animals, while leptospires were isolated in only two cases. Infection was found in a smaller part of the population of *A. agrarius*, *A. flavicollis* and *A. sylvaticus*, while no infection was detected in the populations of other species (*A. terrestris, C. glareolus, M. agrestis* and *M. musculus*). In Forest Office Vrbanja the antibodies to leptospires were recorded only in one *A. sylvaticus*. The same result was obtained in Forest Office Gunja.

In Forest Office Stara Gradiška the antibodies to leptospires were found in five specimens, while no leptospires were isolated. Infection was confirmed in the species *A. agrarius*, *A. flavicollis* and *C. glareolus*. A similar result was obtained with the animals captured in the Forest Offices Otok, Nova Gradiška and Karlovac. The antibodies were found in a small part of the population of *A. agrarius, A. flavicollis* and *A. sylvaticus*, while the leptospire isolation did not yield a positive result. No leptospira infection was recorded in other species of small rodents captured in the localities Otok, Nova Gradiška and Karlovac.

The antibodies to leptospires were recorded in 13 animals in the Forest Office Nova Kapela (the hunting ground "Radinje"), while leptospires were isolated in four animals. One part of the animal population belonging to the species *A. agrarius, A. flavicollis* and *C. glareolus* was found to be infected with leptospires, while infection in other species (*A. sylvaticus* and *M. arvalis*) was not recorded.

Testing individual small rodent species by research localities and forest communities is given in Table 5.

Table 5. Testing individual small rodent species by research localities and forest communities

Tablica 5. Uzorkovanje pojedinih vrsta sitnih glodavaca po lokalitetima istraživanja i šumskim zajednicama

Forest office *Šumarija*	Forest communities *Biljna zajednica*	*Apodemus flavicollis*	*Apodemus sylvaticus*	*Apodemus agrarius*	*Microtus agrestis*	*Microtus arvalis*	*Clethrionomys glareolus*	*Arvicolla terrestris*	*Mus musculus*	Σ
Kutina	*Carici pilose-Fagetum sylvaticae*	5	2	0	0	0	1	0	0	8
	Closed objects *Zatvoreni objekti*	0	0	0	0	0	0	0	35	35
	Epimedio-Carpinetum betuli	2	2	0	0	0	0	0	0	4
Stara Grad.	*Genisto. elatae - Quercetum roboris caricetosum remotae*	1	1	13	0	0	1	0	0	16
Velika Gorica	*Carpino betuli-Quercetum roboris typicum*	8	6	2	0	3	4	0	0	23
	Genisto elatae – Quercetum roboris caricetosum brizoides	4	3	3	3	0	3	0	0	16
Popovača	*Carpino betuli-Quercetum roboris typicum*	36	13	28	1	1	7	1	0	87
	Genisto. elatae - Quercetum roboris caricetosum remotae	4	1	5	0	0	2	0	1	13
Vinkovci	*Genisto. elatae - Quercetum roboris caricetosum remotae*	0	0	1	0	0	0	0	0	1
	Carpino betuli-Quercetum roboris typicum	0	0	0	0	0	0	0	0	0
Vrbanja	*Genisto. elatae-Quercetum roboris caricetosum remotae*	2	1	3	1	0	7	0	0	14
	Carpino betuli-Quercetum roboris typicum	7	2	2	0	1	2	0	0	14
Gunja	*Carpino betuli-Quercetum roboris typicum*	13	6	10	0	1	8	0	0	38

Forest office *Šuma-rija*	Forest communities *Biljna zajednica*	*Apodemus flavicollis*	*Apodemus sylvaticus*	*Apodemus agrarius*	*Microtus agrestis*	*Microtus arvalis*	*Clethrio-nomys glareolus*	*Arvicolla terrestris*	*Mus musculus*	Σ
Nova Kapela	*Genisto. elatae - Quercetum roboris caricetosum remotae*	6	3	35	0	1	13	0	0	58
	Leucoio-Fraxinetum angustifoliae	5	3	8	0	1	6	0	0	23
	Frangulo-Alnetum glutinosae	0	0	0	0	1	1	0	0	2
Otok	*Genisto. elatae-Quercetum roboris caricetosum remotae*	8	6	5	0	1	1	0	0	21
Nova Grad.	*Genisto. elatae - Quercetum roboris caricetosum remotae*	6	3	13	0	0	2	0	0	24
Karlovac	*Genisto. elatae - Quercetum roboris caricetosum brizoides*	16	10	6	2	0	2	0	0	36
	Carpino betuli-Quercetum roboris typicum	10	0	1	0	0	1	0	0	12
Total - *Ukupno*		133	62	135	7	10	61	1	36	445

The following number of small rodents was collected in all the localities per forest community: 147 individuals in *Genisto elatae-Quercetum roboris caricetosum remotae*, 52 individuals in *Genisto elatae-Quercetum roboris caricetosum brizoides*, 174 individuals in *Carpino betuli-Quercetum roboris typicum*, 23 individuals in *Leucoio-Fraxinetum angustipholiae,* two individuals in *Frangulo-Alnetum glutinosae*, eight individuals in *Carici pilosae-Fagetum sylvaticae*, and four individuals in *Epimedio-Carpinetum betuli.*

The renoculture method yielded 17 leptospire isolates from three rodent species: *M. musculus, A. agrarius* and *A. flavicollis* in the localities Kutina, Velika Gorica, Popovača and Nova Kapela. Serologic analyses of 16 *Leptospira* sp. isolate showed that the serovars were grouped in three serologic groups: *sejroe, pomona* and *australis* (Table 6). The serovars of 10 strains (from *M. musculus* in "Garjevica") show the highest similarity to the serovar *istrica* of the *sejroe* serological group, the genomic species *L. borgpetersenii.* Five strains (four from *A. agrarius* and one from *A. flavicollis* captured in the Forest Offices Otok, Nova Kapela and Popovača) belong to the serovar *tsaratsovo* of the *pomona* serologic group, the genomic species *L. kirsheneri.* One strain (from *A. flavicollis* captured in the Forest Office Velika Gorica) belongs to the serovar *lora* of the *australis* serologic group, the genomic species *L. interrogans.*

One isolate from *A. flavicollis* was not defined, but probably belongs to the serologic group *australis.*

Table 6. Leptospira isolation with renoculture depending on the species of analysed animals

Tablica 6. Prikaz izolacije leptospira renokulturom ovisno o vrsti pretraženih životinja

Animal species *Vrsta životinje*	Analysed renoculture *Pretraženo renokultura*	Serologic *Serološka sejroe*	Leptospira *varijanta*	Variant *leptospira*	Undefined *Nedeterminirano*	Total *Ukupno*	%
			australis	*pomona*			
Mus musculus	32	10	0	0	0	10	31.25
Apodemus flavicollis	111	0	1	1	1	3	2.7
Apodemus agrarius	108	0	0	4	0	4	3.7
Total *Ukupno*	251	10	1	5	1	17	6.77

DISCUSSION
RASPRAVA

The animals captured in the studied areas contained the majority of small rodent species that inhabit lowland forests of pedunculate oak. *M. subterraneus* and *M. multiplex* are very rare and they were not recorded. Most of analysed animals belong to of *A. agrarius* and *A. flavicollis*, while these two species were dominant in the majority of the studied areas. In terms of the number of analysed individuals from a given locality, most derive from the localities Popovača and Nova Kapela. *Mus musculus* prevail in the hunting ground "Garjevica", where the majority of animals were captured in indoor premises (roe deer shed and food storage), where they were attracted by abundant game food that they feed on. The species *A. flavicollis, A. sylvaticus* and *C. glareolus* were also captured in the stand. *A. agrarius* was not recorded there, as the stand does not suit this species. The animals were collected with traps using the methodology by Bäumler & Brunner (1988), and Margaletić (1998).

The forest communities of *Genisto elatae-Quercetum roboris caricetosum remotae, Genisto elatae-Quercetum roboris caricetosum brizoides* and *Carpino betuli-Quercetum roboris typicum* account for 83.82 % of the captured individuals. Most were captured in the autumn months, when the number of small rodent populations increases (Conley & Nichols 1978, Tapper 1979, Chudoba & Huminski 1980, Clarke 1985, Margaletić 1998, Margaletić & Glavaš 2001). The highest increase in the autumnal population was recorded in *A. agrarius*

in four localities (Popovača, Nova Kapela, Nova Gradiška and Stara Gradiška). The reason for this probably lies in the vicinity of sample plots to agricultural areas. *Apodemus agrarius* characteristically inhabits agricultural areas in the vegetation period, where it is drawn by the abundance of food, while in the autumn it migrates to the forest (Gliwicz 1980). A study of the movement of the species *A. agrarius*, made by Liro & Szacki (1987), showed that 60 % of the analysed population travelled over 100 m away from the marking place. The same authors noted that the longest distances of travelling exceeded 1000 m. In his study of the movement of *A. sylvaticus*, Watts (1970) assumed that the upper boundary for this species within its home range was 130 m. Korn (1986) proved that the home range of *A. sylvaticus* was usually less than 10000 m², which means that the diameter of the range was less than 100-150 m. Andrzejewski & Bibinska-Werka (1986) recorded much larger movement of small rodents.

The study of small rodents as a reservoir of leptospiroses yielded positive results. 17 leptospire strains were isolated from three different species of small rodents in the localities Kutina, Popovača, Nova Kapela and Velika Gorica. Of 379 analysed specimens, the leptospires antibodies were found in 48 cases. Most infections where detected in *A. agrarius, M. musculus* and *A. flavicollis*. The majority of small rodent detected leptospires antibodies (Table 4) and positive leptospira isolation were achieved in Kutina (the state hunting ground "Garjevica") and Nova Kapela (the state hunting ground "Radinje"). This fact indicates a heightened risk of infection for the game living in these localities, the consequence of which could cause heavy losses in hunting management (poorer game increment, miscarriages of fertilised females and similar) (Kovačić *et al.* 1984, Kovačić & Karlović 1984, Kovačić *et al.* 1985b, Modrić & Karlović 1977, Modrić *et al.* 1979, Modrić & Huber 1993).

The results obtained by microscopic agglutination, isolation of leptospires with renoculture and macro-restriction of chromosomal DNA show that serovar identification could be a useful method of gaining better insights into the epizootiology and epidemiology of leptospiroses in Croatia. Awareness of these facts may be of essential importance in the management of game as possible receptors of leptospiroses whose basic reservoirs are small rodents. In the area of the rivers Sava and Drava and their tributaries in north Croatia 3,543 small rodents (subfamilies *Arvicolinae* and *Murinae*) were subjected to renoculture. 2,7 % were identified as carriers of the leptospira serovariant *grippotyphosa* (95 individuals) (Borčić *et al.* 1987). Of 2,643 animals tested with microscopic agglutination, 3.9 % were found to be agglutinin carriers for an identified leptospira variant (103 animals). *Microtus arvalis* proved to be the most common carrier of this serologic variant. Leptospires of the serovariant *grippotyphosa* were found more rarely, or were not found at all in the parts of Podravinian Slavonia and in the catchment area of the left tributaries of the river Sava (the Česma, the Ilova, the Orljava) (Borčić *et al.* 1982a, b, 1983). In the present research, the same serovariant was detected in only two *A. agrarius*. Borčić *et al.* (1986) proved statistically that the infection of *A. agrarius* with the leptospira of the *pomona* serovariant, as well as its carrier status, was considerably more frequent in this species than in other species of mouse-like rodents. The incidence of carriership and the presence of leptospira antibodies of the serovariant *pomona* (in the same animals), was found to be more frequent in *A. agrarius* than in other rodent species, as well as carriership with the presence of

these antibodies (Borčić *et al.* 1986). This supports the high degree of adaptation of these two organisms, which is generally the property of basic micro-organism reservoirs in the nature. The species *A. agrarius* is a natural reservoir of leptospira of the *pomona* variant in Croatia (Borčić *et al.* 1986). The results presented in this paper show that small rodents in northern Croatia are responsible for permanent existence of leptospires in the nature, which, bearing in mind high populations of these animals, poses a realistic risk of infection for domestic and wild animals and for human. In the past studies of leptospirosis in Croatia, identifications of the isolates were done only up to the level of serologic variants. In this paper, the data on the leptospira identification for Croatia up to the specific (species) level have been given for the first time. Previous research, combined with our results, confirm the well-known fact that a wider area of Posavina represents the natural focus of leptospiroses. The forests of Posavina can be regarded as a part of this large focus, while the specific traits and the role of this biotop in the leptospira survival should be studied more extensively.

CONCLUSIONS
ZAKLJUČCI

During the year 2000, a total of 445 animals were captured, of which the following species were identified: *A. agrarius, A. flavicollis, A. sylvaticus, M. musculus, C. glareolus, M. agrestis, M. arvalis* and *A. terrestris*. 379 animals were tested for leptospires. The highest number of the tested individuals belonged to *A. flavicollis* (29.29 % of the total number of tested animals) and *A. agrarius* (28.50 % of the total number of tested individuals). 83.82 % of the tested animals were captured in the forest communities *Genisto elatae - Quercetum roboris caricetosum remotae, Genisto elatae - Quercetum roboris caricetosum brizoides* and *Carpino betuli - Quercetum roboris typicum*. The largest proportion of the catch was obtained in autumn months, when the number of small rodent populations increases.

Seventeen leptospira strains were isolated with renoculture from three small rodent species: *M. musculus, A. agrarius*, and *A. flavicollis*, captured in the localities of Kutina, Velika Gorica, Popovača and Nova Kapela. They were found to belong to the following three serologic variants: *sejroe, pomona* and *australis*. The antibody titre to leptospira was detected in 48 animals: *A. agrarius* (16), *A. flavicollis* (12), *M. musculus* (11), *C. glareolus* (5) and *A. sylvaticus* (4).

The PFGE analysis was made to identify leptospires at the level of species. The results of this research point to the need to assess the numbers of small rodent populations in the field, to test the captured individuals for the presence of some zoonoses and to apply timely protective measures to forest ecosystems. In the long run, this will result in decreased losses in hunting management and increased care for the health of people and domestic animals. The multidisciplinary nature of this research requires close cooperation of the forestry profession and other relevant institutions.

REFERENCES
LITERATURA

Andrzejewski, R., & J. Babinska-Werka, 1986: Bank vole populations: are their densities really high and individual home ranges small?. Acta Theriol., 31: 407–420.

Baril, C., & I. Saint Girons, 1990: Sizing of the *Leptospira* genome by pulsed-field agarose gel electrophorens. FEMS Mikrobiol. Lett.,71: 95–100.

Barrow, P. A., 1981: *Corynebacterium kutscheri* infection in wild voles (*Microtus agrestis*). Br. vet. J., 137: 67–70.

Bäumler, W., 1975: Hämobartonellen und Milztumor bei der Erdmaus (*Microtus agrestis*). Anz. Schädlingskunde, Pflanzenschutz, Umweltschutz, 48(1): 1–5.

Bäumler, W., & M. Brunner, 1988: Einfluß des Nahrungsangebots auf die Konkurrenz sympatrischer Mäusearten in Forstkulturen. Anz. Schädlingskunde, Pflanzenschutz, Umweltschutz, 61(3): 3–5.

Brenner, D., A. Kaufmann, K. Sulzer, A. Steigerwalt, F. Rogers & R. Weyant, 1999: Further determination of deoxyribonucleic acid relatedness between serogroups and serovars in the family Leptospiraceae with a proposal for *Leptospira alexanderi* sp. nov. and four new *Leptospira* genomospecies. Int. J. Syst. Bacteriol., 49: 853–858.

Borčić, B., H. Kovačić, Z. Šebek, B. Aleraj & N. Tvrtković, 1982a: Mišoliki sisavci kao rezervoari leptospira u savskoj nizini. Leptospiroze – 30-godišnje istraživanje i izučavanje u SR Hrvatskoj, Zagreb, pp. 149–154.

Borčić, B., H. Kovačić, Z. Šebek, B. Aleraj & N. Tvrtković, 1982b: Small terrestrial mammals as reservoirs of leptospires in the Sava Valley. Folia Parasitol., 29: 177–182.

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

Borčić, B., H. Kovačić, Z. Šebek, B. Aleraj & N. Tvrtković, 1986: Poljski miš (*Apodemus agrarius* Pall.) naš prirodni rezervoar leptospire serotipa *pomona*. Veterinarski arhiv, 56(4): 169–178.

Borčić, B., H. Kovačić, Z. Šebek, B. Aleraj & N. Tvrtković, 1987: *Leptospira interrogans* serološke varijante *grippotyphosa* u mišolikih sisavaca sjeverne Hrvatske. Veterinarski arhiv, 57(6): 319–329.

Borčić, B., B. Kaić & V. Kralj, 1999: Some epidemiological data on TBE and *Lyme borreliosis* in Croatia. Zbl. Bakt-Int. J. Med. Microbiol., 289: 540–547.

Chudoba, S., & S. Huminski, 1980: Estimating numbers of rodents and edge effect using a modified version of the standard minimum method. Acta theriologica, 25: 365–376.

Clarke, J. R., 1985: The reproductive biology of the bank vole (*Clethrionomys glareolus*) and wood mouse (*Apodemus sylvaticus*). Symp. Zool. Soc. Lond., 55: 33–59.

Conley, W., & J. D. Nichols, 1978: The use of models in small mammal population studies. Spec. Publs Pymatuning Lab. Ecol., 5: 14–37.

Cvetnić, S., 1993: Opća epizootiologija. Školska knjiga, Zagreb, pp. 72–89.

Delany, M. J., 1974: The ecology of small mammals. Studies in biology, 51 Edward Arnold, London, 60 pp.

Ellis, W. A., 1995: International Commitee on Systemic Bacteriology Subcomittee on the Taxonomy of Leptospira. Minute of the Meetings, 1 and 2 July 1994, Prague, Czech Republic, Int. J. Syst. Bacteriol., 45: 872–874.

Geisel, O., E. Kaiser, O. Vogel, H. E. Krampitz & M. Rommel, 1979: Pathomorphologic findings in short-tailed voles (*Microtus agrestis*) experimentally-infected with *Frenkelia microti*. J. Wild. Diseases, 15: 267–270.

Glavaš, M., & J. Margaletić, 2001: Relativna brojnost sitnih glodavaca i njihovo suzbijanje u GJ "Požeška gora". 4. znanstveno-stručni skup iz DDD-a s međunarodnim sudjelovanjem "Zdravo očuvati zdravim u novom tisućljeću", pp. 253–267.

Gliwicz, J., 1980: Ecological aspect of synurbanization of the striped field mouse, *Apodemus agrarius*. Wiadomosci Ekologiczne, 26: 117–124.

Gračan, J., I. Anić & S. Matić, 1998: Potrajno gospodarenje i očuvanje biološke raznolikosti hrvatskih šuma. Šumarski list, 122(9–10): 437–442.

Klepac, D., 1996: Uvod. In: D. Klepac (ed.), Hrast lužnjak (*Quercus robur* L.) u Hrvatskoj, HAZU Centar za znanstveni rad Vinkovci & "Hrvatske šume", p. o. Zagreb, Zagreb, pp. 9–12.

Klepac, D., & G. Fabijanić, 1996: Uređivanje šuma hrasta lužnjaka. In: D. Klepac (ed.), Hrast lužnjak (*Quercus robur* L.) u Hrvatskoj, HAZU Centar za znanstveni rad Vinkovci & "Hrvatske šume", p. o. Zagreb, Zagreb, pp. 257–272.

Kmety, E., & H. Dikken, 1993: Classification of the species Leptospira interrogans and the history of its serovars. A history of the publication of the serovars of leptospires and a catalogue of their relationships. University Press Groningen, Groningen, Netherlands.

Korn, H., 1986: Changes in home range size during growth and maturation of wood mouse (*Apodemus sylvaticus*) and the bank vole (*Clethrionomys glareolus*). Oecologia, 68: 623–628.

Kovačić, H., & M. Karlović, 1984: Utvrđivanje prisutnosti protutijela leptospira u divljih svinja na području Baranje. Veterinarski arhiv, 54: 77–81.

Kovačić, H., M. Karlović & B. Poznanović, 1984: Istraživanje proširenosti protutijela leptospira u jelena. Veterinarski arhiv, 53: 35–39.

Kovačić, H., Z. Lipej & M. Lacković, 1985b: Učestalost nalaza protutijela za pojedine serotipove leptospira u lisica. Vet. glasnik, 39: 133–150.

Kowalski, K., 1976: Mammals An Outline of Terriology PWN, Polish scientific Publishers Warszawa, pp. 408–419.

Liro, A., & J. Szacki, 1987: Movements of field mice *Apodemus agrarius* (Pallas) in a suburban mosaic of habitats. Oecologia, 74: 438–440.

Margaletić, J., 1998: Rodents and their harmful effects on Turopoljski lug (Turopolje Grove) and on Croatian forests. Glas. šum. pokuse, 35: 143–189.

Margaletić, J., & M. Glavaš, 2001: Istraživanje dinamike populacija sitnih glodavaca metodom minimalnoga kvadrata u Gospodarskoj jedinici "Slatinske nizinske šume". In: S. Matić, A. P. B. Krpan & J. Gračan (eds.), Znanost u potrajnom gospodarenju hrvatskim šumama, Zagreb, pp. 317–326.

Modrić, Z., & M. Karlović, 1977: Leptospiroza u zeca u Hrvatskoj. Veterinarski arhiv, 47: 251–256.

Modrić, Z., M. Herceg, P. Ramadan & M. Potočić, 1979: Leptospiroza u kooperativnom tovu teladi u okolici Zagreba. Veterinarski arhiv, 49: 291–298.

Modrić, Z., & Đ. Huber, 1993: Serologic Survey for Leptospirae in European Brown Bears (*Ursus arctos*) in Croatia. Journal of Wildlife Diseases, 29(4): 608–611.

Niethamer, J., & F. Krapp, 1978: Handbuch der Säugetiere Europas: Nagetiere 1/1. Akad. Verlag Wiesbaden, 1: 281–381.

Niethamer, J., & F. Krapp, 1982: Handbuch der Säugetiere Europas: Nagetiere 2/1. Akad. Verlag Wiesbaden, 2: 51–491.

Noguchi, H., 1918: Morphological characteristics and nomenclature of *Leptospira* (Spirochaeta) *icterohaemorrhagiae* (Inada & Ido), J. Exp. Med., 27: 575–595.

Tapper, S. C., 1979: The effect of fluctuating vole numbers (*Microtus agrestis*) on a population of weasels (*Mustela nivalis*) on farmland. J. Anim. Ecol., 48: 603–617.

Vukelić, J., & Đ. Rauš, 1998: Šumarska fitocenologija i šumske zajednice u Hrvatskoj. Šumarski fakultet Sveučilišta u Zagrebu, Zagreb, 310 pp.

Zaharija, I., 1968: Mikromamalia Hrvatske-rezervoari leptospira (Micromammalia of Croatia-reservoirs of leptospires). Zbornik radova 3. naučnog sastanka infektologa Jugoslavije, Portorož.

Zaharija, I., 1980: Opća epizootiologija. Školska knjiga, Zagreb, pp. 24–56.

Watts, C. H. S., 1970: Long distance movement of bank voles and wood mice. J. Zool. Lond, 161: 247–256.

SITNI GLODAVCI KAO IZVOR LEPTOSPIROZA U POSAVSKIM ŠUMAMA U HRVATSKOJ

SAŽETAK

Sitni su glodavci zbog brojnosti populacija i širine ekološke valencije važan dio gotovo svakoga šumskoga ekosustava. Oni su značajna skupina životinja koja povezuje primarne proizvođače s višim trofičkim razinama. To su sisavci s karakterističnim prednjim sjekutićima (glodnjaci, incisivi) koji su vrlo oštri (Delany 1974). Potencijal razmnožavanja im je vrlo visok (Kowalski 1976).

Sitni su glodavci izvor niza zaraznih bolesti čovjeka, te domaćih i divljih životinja (trihineloza, leptospiroza, krpeljni encefalitis, lyme borelioza, hemoragijska vrućica s bubrežnim sindromom i dr.) (Bäumler 1975, Geisel i dr. 1979, Barrow 1981, Borčić i dr. 1982, 1983). Kao kliconoše mogu povremeno ili trajno putem sekreta i ekskreta izlučivati uzročnike zarazne bolesti, te tako kontaminirati okoliš koji nastanjuju pretvarajući ga u intermedijarne i sekundarne izvore zarazne bolesti (Zaharija 1980, Cvetnić 1993). Od brojnih bolesti koje glodavci prenose u ovom je radu istražena leptospiroza. To je akutna septikemijska zarazna bolest različitih vrsta domaćih i divljih životinja i čovjeka koja se većinom javlja enzootski. Klinički se očituje ikterusom, ponekad hemoglobinurijom, a u goveda i svinje pobačajima. Uzročnici leptospiroze su patogene spiralne bakterije iz roda *Leptospira* (Noguchi 1918) unutar kojega je dosad otkriveno nekoliko genomskih vrsta (Ellis 1995, Brenner i dr. 1999), 24 serološke skupine i preko 250 seroloških varijanata (Kmety i Dikken 1993).

Ovim su istraživanjem dobivene nove spoznaje o zastupljenosti pojedinih seroloških varijanata (serovari) leptospira na istraživanim lokalitetima, te su determinirane određene vrste sitnih glodavaca kao izvor leptospiroza na istraživanim lokalitetima u Posavini Istraživanja su provedena u hrvatskim posavskim šumama u sastojinama kojima gospodari 11 šumarija "Hrvatske šume" d. o. o. (slika 1). Na jednom je lokalitetu bilo postavljeno po nekoliko pokusnih

ploha (ukupno 44). Ulov sitnih glodavaca obavljen je u razdoblju od travnja do studenoga 2000. godine. Na većini lokaliteta uzorkovanje je sitnih glodavaca obavljeno dva puta godišnje, u proljetnim i jesenskim mjesecima, u ovim šumskim zajednicama: *Genisto elatae-Quercetum roboris caricetosum remotae*, *Genisto elatae-Quercetum roboris caricetosum brizoides*, *Carpino betuli-Quercetum roboris typicum*, *Carpino betuli-Quercetum roboris fagetosum*, *Carpino betuli-Quercetum roboris quercetosum cerris*, *Carici pilosae-Fagetum sylvaticae*, *Epimedio-Carpinetum betuli*, *Leucoio-Fraxinetum angustifoliae* i *Frangulo-Alnetum glutinosae* (Vukelić & Rauš 1998). Šumske zajednice *Carici pilosae-Fagetum sylvaticae*, *Epimedio-Carpinetum betuli*, *Leucoio-Fraxinetum angustifoliae* i *Frangulo-Alnetum glutinosae*, koje su obuhvaćene u istraživanju, rasprostranjene su na istraživanim lokalitetima državnih lovišta "Garjevica" (br. VII/4) (*Carici pilosae-Fagetum sylvaticae* i *Epimedio-Carpinetum betuli*) i "Radinje" (br. XII/16) (*Leucoio-Fraxinetum angustifoliae* i *Frangulo-Alnetum glutinosae*). Na slici 2 je prikazan broj pokusnih ploha postavljenih u pojedinim šumskim zajednicama u kojima su uzorkovane životinje.

Za uzorkovanje jedinki upotrijebljene su mrtvolovke, te živolovke tipa "Sherman". Klopke su postavljane u lovne transekte na međusobnom razmaku 5–7 m. U Zavodu za mikrobiologiju i infektivne bolesti Veterinarskoga fakulteta u Zagrebu ukupno je serološki i bakteriološki obrađeno 379 glodavaca. Primijenjena je metoda mikroskopske aglutinacije kao standardni način za serološku dijagnostiku i klasifikaciju leptospira. U slučaju dokaza protutijela u osnovnom razrjeđenju 1:100 uzorci su pretraživani u daljnjim razrjeđenjima seruma (1:500, 1:1000 itd.). Nalaz najvišega titra protutijela za pojedini antigen leptospira odnosno serovar u serumu upućuje na "vjerojatni" serovar koji je uzrokovao zarazu (Johnson 1976). Svaki je uzorak krvi pretražen s antigenima *L. interrogans* sv: *icterohaemorrhagie*, *ballum*, *australis*, *pomona*, *grippotyphosa*, *sejroe*, *saxkoebing*, *bataviae*, *tarassovi*, *canicola*, *poi* i *hardjo*. Determiniranim jedinkama nacijepljeno je tkivo kore bubrega na Korthofovo hranilište radi izdvajanja leptospira.

Iz tijela sitnih glodavaca *A. flavicollis*, *A. agrarius* i *M. musculus*, koji su uzorkovani na lokalitetima Kutina, Popovača, Velika Gorica, Nova Kapela i Otok, izolirano je 17 izolata leptospira koji su poslani na obradu u Institut "Pasteur" (Pariz, Francuska) u kojem je analiziran gel elektroforeze u pulsirajućem polju (PFGE analiza). Ta se analiza sastoji u tome što se nakon restrikcije kromosomske DNA s restrikcijskim enzimima "NotI" i "Sgr AI" pristupa usporedbi restrikcijskih profila ispitujućih serovara (seroloških varijanata) s profilima referentnih serovara. Tom je metodom moguće odrediti leptospire na razini serovara, tj. ustanoviti njihovu fizikalnu mapu (Baril & Saint Girons 1990). PFGE analiza s restrikcijskim enzimom "NotI" razlučuje serovare seroloških grupa (serogrupa) "*sejroe*" i "*pomona*", dok analiza s restrikcijskim enzimom "Sgr AI" razlučuje serovare serološke grupe "*australis*".

Na svim lokalitetima ukupno je ulovljeno 445 jedinki, a laboratorijski ih je obrađeno 379 (85,17 %). 66 jedinki nije analizirano zbog oštećenosti uzorka izazvanoga djelovanjem predatora ili raspadanjem tkiva zbog atmosferskih utjecaja (visoka temperatura). Determinirane su ove vrste sitnih glodavaca: *A. agrarius*, *A. flavicollis*, *A. sylvaticus*, *A. terrestris*, *C. glareolus*, *M. agrestis*, *M. arvalis* i *M. musculus*. Dominantne vrste na istraživanim pokusnim plohama bile su *A. flavicollis* i *A. agrarius*. Najviše je glodavaca ulovljeno u šumama kojima gospodare šumarije Popovača (25,86 % ukupnoga ulova) i Nova Kapela (17,94 % ukupnoga ulova).

U šumskim zajednicama *Genisto elatae-Quercetum roboris caricetosum remotae*, *Genisto elatae-Quercetum roboris caricetosum brizoides* i *Carpino betuli-Quercetum roboris typicum* ulovljeno je 83,82 % uzorkovanih jedinki. Većina ih je ulovljena tijekom jesenskih mjeseci. Glavnina jedinki *M. musculus* (93,75 %) ulovljena je u zatvorenim objektima državnoga lovišta "Garjevica" (Šumarija Kutina). Rezultati obrađenih jedinki po vrstama i lokalitetima brojčano su izraženi u tablici 1.

Titar protutijela na leptospire je ustanovljen u 48 životinja sa svih lokaliteta, i to na ovim vrstama glodavaca: *A. agrarius*, *A. flavicollis*, *M. musculus*, *C. glareolus* i *A. sylvaticus* (tablica 2). Detaljna analiza prisutnosti protutijela na serološke varijante leptospira prema najvišem titru (količini) protutijela prikazana je u tablici 3. Iz nje je vidljivo da je kod vrste *M. musculus* bilo serološki pozitivno 34,38 % jedinki, kod *A. agrarius* 14,81 %, kod *A. flavicollis* 10,81 %, kod *A. sylvaticus* 6,45 %, a kod *C. glareolus* 9,43 %. Kod *M. musculus* je utvrđen nalaz seroloških varijanata *saxkoebing* i *hardjo*, dok su četiri izolata ostala neodređena uz titar protutijela 1:100. Kod vrste *A. agrarius* utvrđen je nalaz seroloških varijanata *grippotyphosa*, *sejroe*, *pomona*, *icterohaemorragiae*, *tarassovi* i *hardjo*, dok je analiza jedinki vrste *A. flavicollis* pokazala prisutnost protutijela serloških varijanata *australis*, *pomona*, *saxkoebing* i *bataviae*, a četiri su nalaza protutijela ostala neodređena uz titar protutijela 1:100. Kod vrste *A. sylvaticus* utvrđen je nalaz serloških varijanata *sejroe*, *pomona* i *tarassovi*, dok je analiza jedinki vrste *C. glareolus* pokazala prisutnost protutijela serloških varijanata *sejroe*, *australis*, *saxkoebing* i *hardjo*, a pet je izolata ostalo neodređeno uz titar protutijela 1:100 i 1:500.

U tablici 4 izneseni su rezultati ulova jedinki, te nalaza protutijela za leptospire reakcijom mikroskopske aglutinacije i izolacija leptospira renokulturom po vrstama sitnih glodavaca i lokalitetu ulova. U 108 jedinki *A. agrarius* kod 16 životinja je utvrđen nalaz protutijela za leptospire, a kod četiri jedinke je dobiven izolat. U 111 jedinki *A. flavicollis* u 12 slučajeva je utvrđen nalaz protutijela za leptospire, a kod tri jedinke je dobiven izolat. U 62 jedinke *A. sylvaticus* protutijela za leptospire utvrđena su u četiri životinje, dok renokulturom nije dobiven ni jedan izolat. Kod vrsta *A. terrestris*, *M. agrestis* i *M. arvalis* nisu pronađena protutijela za leptospire, niti su dobiveni izolati leptospira. U 53 jedinke vrste *C. glareolus* u pet su utvrđena protutijela za leptospire, a ni jednom nije dobiven izolat. Kod 32 životinje *M. musculus* u 11 su utvrđena protutijela za leptospire, dok je renokulturom dobiveno 10 izolata leptospira.

Na području Kutine (državnio lovište "Garjevica") od 41 analizirane jedinke u 11 ih je utvrđen nalaz protutijela za leptospire, a u 10 jedinki je dobiven izolat. Svi su nalazi leptospira utvrđeni kod *M. musculus*, dok kod *A. flavicollis*, *A. sylvaticus* i *C. glareolus* leptospire nisu pronađene. U uzorku od 31 jedinke glodavaca iz Šumarije Velika Gorica u šest životinja su pronađena protutijela, a dobiven je samo jedan izolat. Približno 50 % populacije *A. flavicollis* i *C. glareolus* bilo je zaraženo leptospirama, dok kod *A. agrarius*, *A. sylvaticus*, *M. agrestis* i *M. arvalis* nije potvrđen njihov nalaz. U sastojinama Šumarije Popovača ulovljeno je i analizirano 98 životinja. Struktura ulova po vrstama prikazana je u tablici 4. U sedam je životinja potvrđen nalaz protutijela za leptospire, a u samo dva slučaja je dobiven izolat leptospira. Zaraženost je pronađena u manjem dijelu populacije *A. agrarius*, *A. flavicollis* i *A. sylvaticus*, a u populacijama ostalih vrsta (*A. terrestris*, *C. glareolus*, *M. agrestis* i *M. musculus*) nije zabilježena. Na području

Šumarije Vrbanja nalaz protutijela za leptospire potvrđen je samo kod *A. sylvaticus*. Isti je rezultat polučen i na jedinkama iz Šumarije Gunja.

Od 15 ulovljenih životinja u Šumariji Stara Gradiška protutijela za leptospire su pronađena u pet jedinki, dok izolati nisu dobiveni. Zaraženost je potvrđena kod *A. agrarius*, *A. flavicollis* i *C. glareolus*. Kod životinja s područja Šumarija Otok, Nova Gradiška i Karlovac također je dobiven sličan rezultat. Protutijela su pronađena u malom dijelu populacija *A. agrarius*, *A. flavicollis* i *A. sylvaticus*, a izolacija leptospira nije dala pozitivan rezultat. Kod ostalih vrsta sitnih glodavaca ulovljenih na lokalitetima Otok, Nova Gradiška i Karlovac nije zabilježena zaraženost leptospirama. U 13 životnja iz Šumarije Nova Kapela (lovište "Radinje") utvrđen je nalaz protutijela za leptospire, a u četiri je jedinke dobiven izolat. Zaraženost leptospirama utvrđena je u jednom dijelu populacije životinja koje pripadaju vrstama *A. agrarius*, *A. flavicollis* i *C. glareolus*, dok kod ostalih vrsta (*A. sylvaticus* i *M. arvalis*) nije potvrđena. Rezultati uzorkovanja sitnih glodavaca po lokalitetima istraživanja i šumskim zajednicama prikazani su u tablici 5. Ukupno je na svim lokalitetima uzorkovan ovaj broj sitnih glodavaca po pojedinim šumskim zajednicama: u *Genisto elatae-Quercetum roboris caricetosum remotae* 147 jedinki, u *Genisto elatae-Quercetum roboris caricetosum brizoides* 52 jedinke, u *Carpino betuli-Quercetum roboris typicum* 174 jedinke, u *Leucoio-Fraxinetum angustifoliae* 23 jedinke, u *Frangulo-Alnetum glutinosae* dvije jedinke, u *Carici pilosae-Fagetum sylvaticae* osam jedinki, te u *Epimedio-Carpinetum betuli* četiri jedinke.

Metodom renokulture izdvojeno je 17 izolata leptospira iz jedinki ulovljenih glodavaca na lokalitetima: Kutina, Velika Gorica, Popovača i Nova Kapela iz triju vrsta glodavaca: *M. musculus*, *A. agrarius* i *A. flavicollis*. Serološke analize 16 izolata *Leptospira* sp. pokazale su da su istraživani serovari razvrstani u tri serološke grupe: *sejroe*, *pomona* i *australis* (tablica 6). Determinirani serovari 10 izolata (izolirani iz *M. musculus* ulovljenih na području državnoga lovišta "Garjevica") pokazuju najveću sličnost serovaru *istrica* serološke grupe *sejroe*, genomne vrste *L. borgpetersenii*, pet izolata (četiri izolirana iz *A. agrarius* i jedan iz *A. flavicollis* ulovljenih u šumarijama Otok, Nova Kapela i Popovača) pripada serovaru *tsaratsovo* serološke grupe *pomona*, genomne vrste *L. kirshneri*, dok jedan izolat (izoliran iz *A. flavicollis* ulovljenoga na području Šumarije Velika Gorica) pripada serovaru *lora* serološke grupe *australis*, genomne vrste *L. interrogans*. Jedan izolat izoliran iz jedinke vrste *A. flavicollis* nije determiniran, a vjerojano pripada serološkoj grupi *australis*.

Najviše laboratorijski obrađenih životinja pripada vrstama *A. agrarius* i *A. flavicollis*, jer su one tijekom 2000. godine prevladavale na većini istraživanih lokaliteta. Najviše jedinki ulovljeno je na području Popovače i Nove Kapele. U državnom lovištu "Garjevica" najviše je analizirano jedinki vrste *Mus musculus*. Ta je vrsta ulovljena u zatvorenim objektima lovišta (staja za košute i skladište hrane) u koje se nastanila zbog obilja hrane za divljač koja joj služi u prehrani. Na istom lokalitetu u sastojini su ulovljene još i vrste *A. flavicollis*, *A. sylvaticus* i *C. glareolus*, dok ulov jedinki iz vrste *A. agrarius* nije zabilježen, jer sastojina u kojoj je obavljen izlov stanišno ne odgovara toj vrsti. Najveće povećanje jesenske brojnosti populacije na lokalitetima Popovača, Nova Kapela, Nova Gradiška i Stara Gradiška zabilježeno je kod vrste *A. agrarius*. Pretpostavlja se da je tomu uzrok blizina poljoprivrednih površina pokusnim plohama. Za *A. agrarius* je karakteristično da u vegetacijskom razdoblju obitava

na poljoprivrednim površinama zbog obilja hrane, a u jesen migrira u šumu (Gliwicz 1980). Istraživanja kretanja vrste *A. agrarius* koja su proveli Liro & Szacki (1987) pokazala su da je 60 % analizirane populacije prelazilo veće udaljenosti od 100 m od mjesta njihove markacije.

Zaraženost leptospirama je najviše utvrđena kod *A. agrarius*, *M. musculus* i *A. flavicollis*. Uspoređujući stanje zaraženosti sitnih glodavaca po lokalitetima ulova (tablica 4), jasno se uočava da je najviše nalaza protutijela za leptospire i pozitivnih izolacija postignuto iz populacija jedinki ulovljenih u Kutini (državno lovište "Garjevica") i Novoj Kapeli (državno lovište "Radinje"). Ta činjenica upućuje na povećanu mogućnost zaraze divljači koja obitava na spomenutim lokalitetima, a čija posljedica može biti iskazana gubitkom u lovnom gospodarenju (slabiji prirast divljači, pobačaj oplođenih ženki i sl.) (Kovačić i dr. 1984, Kovačić & Karlović 1984, Kovačić i dr. 1985b, Modrić & Karlović 1977, Modrić i dr. 1979, Modrić & Huber 1993). Mikroskopskom aglutinacijom, izolacijom leptospira renokulturom i makrorestrikcijom kromosomske DNA dobiveni su rezultati koji upućuju na to da determinacija serovara može biti koristan način za bolje upoznavanje epizoetiologije i epidemiologije leptospiroza u Hrvatskoj. Poznavanje tih činjenica bitno može pomoći u gospodarenju divljači kao mogućih primljivih vrsta leptospiroza čiji su osnovni rezervoar sitni glodavci.

Ključne riječi: sitni glodavci, šumski ekosustavi, leptospiroze, Posavina, Hrvatska