SMALL TERRESTRIAL MAMMALS AS RESERVOIRS OF LEPTOSPIRES IN THE SAVA VALLEY (CROATIA)

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Abstract. Small mammals trapped in seven sites along the river Sava in Croatia were examined for leptospiral infection by renalculture and serological methods. Of the 1749 animals caught 702 were examined by renoculture method and 628 were tested by microagglutination-lysis. The dominant animal species caught were Microtus arvalis (672 animals) and Apodemus agrarius (503 animals). Of the 115 strains of leptospires isolated the dominant serotypes were L. grippotyphosa (40) and L. pomona (35). Five strains of L. sejroe, 9 strains of L. autumnalis, 1 strain of L. bataviae and 35 undetermined strains were isolated, the infection rate ranging from 3 to 32%. Serological evidence of infection was demonstrated in 91 animals, the prevalence ranging from 0 to 43% of animals depending on the trapping site. Specific agglutinins most frequently encountered were those against L. pomona (50) and L. grippotyphosa (20), though agglutinins against L. jolana (8), L. bataviae (4), L. bratislava (3) etc., were also found. A high degree of association between L. grippotyphosa and the common vole as well as between L. pomona and the field mouse was found indicating that as far as these two types of leptospires are concerned the Sava valley may be considered as their vast natural focus. It was also concluded that wild small mammals in this region of Croatia may be a source of leptospiral infection for the local population as well as for outdoor-reared domestic animals (swine, bovines).

Leptospiroses are the most frequently reported zoonoses in the continental part of the Socialist Republic of Croatia. The average annual incidence in the years 1969–1978 was 145 with the annual maximum and minimum of 209 (1970) and 76 (1978) respectively. In regard of the 10-year interval preceding the mentioned decade (1959–1968), there had been a significant decrease in the average incidence (354). This is probably not accidental, but could be related to the drainage work (Chernukha and Ėvédokimova 1976) in marshy areas of the Panonian lowlands, otherwise well-known endemic foci of leptospirosis. Although on a decrease, leptospiral infection in those areas is much more common than is recorded. In fact, serological screenings have demonstrated that up to 50% of the endemic area population have traces of infection (Tomasić 1964). With regard to the total number of cases in Yugoslavia, about three quarters (75%) are registered in Croatia. Among hospitalized patients the infection with Leptospira sejroe is predominant (34%) and is followed by L. pomona (25%), L. icterohaemorrhagiae (13%) and L. grippotyphosa (13%). All the other types (L. autumnalis, L. canicola, L. hyos, L. sejroe, L. canicola, L. autumnalis, L. miius, L. bataviae, L. ballum and L. tarassovi) account for only 16% of the cases. However, there are certain regional differences in the distribution of types of leptospires. While in Varadžin, Čakovec and Koprivnica the most frequently recorded infection is that with L. icterohaemorrhagiae, in the areas of Slavonski Brod, Slavonska Požega and Nova Gradiška it is the infection with L. pomona. L. sejroe is dominant in other parts of Slavonia. As there are very slight differences between the said areas with regard to the most common sources of human infection (swine, bovines, rat and dog) the observed differences could be ascribed to, it was of interest to carry out a pilot study on wild reservoirs of leptospires i.e. small
terrestrial mammals. They are therefore the object of the present study. According to the initial hypothesis regional differences in the wild fauna pattern, i.e., the carriage of leptospires are the cause of the differences in the type of infection, allowing that the already indisputably demonstrated basic maintenance cycle of numerous types of leptospires takes place among those animals (Babudieri 1938). This paper presents the results of the research carried out so far in Posavina.

**MATERIAL AND METHODS**

The study has been focused on the Posavina region, a lowland 10–50 km wide and about 300 km long stretching from the Slovenia to the Serbia-Vojvodina border in a west-east direction. It is a lowland valley, an artificial lake, with a minor influence of the Rhine and subject to periodic flooding. Edaphically, it is dominated by moderate-to-heavy and up to very heavy soils, neutral or slightly alkaline, with a low coefficient of filtration. The central part of Posavina, where the irrigation on a larger scale has not been carried out yet, is characterized by meadows, pastures and forests while elsewhere it has more or less been transformed into culturecones. Climatically, Posavina persists to the zone of moderate continental climate with noticeable differences going from east to west: the average precipitation decreases while mean temperature increases (Zagreb 500 mm; 91.4°C; Slavonski Brod 800 mm; 10.6°C).

In the Posavina region 7 trapping areas (Donja Kupčina, Gukić, Struđe, Stara Gradiska, Kanjiža, Babina Greda, and Strizivoja) have been selected partly with definite intention (3 sites because of the occurrence of local epidemics or epizootics) and partly at random. The studies have covered wild small terrestrial mammals of the Rodentia and Insectivora orders. The animals were collected from 1976 to 1978, the trapping taking place every year at the same time (November–December). Different biotopes have been covered with trapping to obtain as a representative a picture of the present therioflora as possible. The only exception was Stara Gradiska where the trapping had to be done only in cornstaves (high snow). An effort was made to maintain the greatest possible similarity between trapping biotopes.

The animals were examined within 6–14 hours after being caught (and cooled in a freezer). The animals were examined in two directions: toward leptospirosis isolation from kidneys by using the standard renoculture method (Babudieri 1931), and toward demonstrating the presence of spore agglutinins in their sera by microagglutination-lysis (Sachek 1964). The technique of isolated leptospires was done with antisera for 12 most frequently isolated leptospires in this country, while agglutination was done with antisera for an equally large number of leptospires with insignificant differences in serotypes used. It was accepted that agglutinin titers of 1:100 and higher stand for an infection with a corresponding type of leptospires.

**RESULTS**

A total of 1749 animals were caught. Their distribution and specification is shown in Table 1. It is evident that the two ubiquitously dominant species are the common vole (Microtus arvalis) (38% of all animals caught) and the field mouse (Apodemus agrarius (32%)). They are followed by the wood mouse (A. sylvaticus (13%)) and the bank vole (Clethrionomys glareolus (4.6%)), while all the other species (11%) account for only 12.5%. The dominant position of the common vole is only disturbed in Stara Gradiska, where it is not. In the Stara Gradiska vole was caught as an "irregular" trapping such a composition of animal population cannot be regarded as representative of that area, and cannot be compared with the findings from other localities. Comparisons of animal populations are also hindered by the fact that they were not collected simultaneously, i.e. in the same year but over a number of years: Donja Kupčina, Gukić and Struđe in 1976, Stara Gradiska in 1976 and 1977, Babina Greda, and Strizivoja in 1979. Population density, assessed by the number of animals caught per number of traps set (a calculation having orientational value only), ranged from 30% (D. Kupčina) to 105% (Babina Greda). The number and species of animals submitted to renoculture and to MAL are also shown in Table 1. This provides an insight into the representativeness of the samples of animals examined, as it was not feasible to examine them all. Animal samples from the areas of Donja Kupčina and Gukić were not examined by the second method (MAL). The leptospiral circulation intensity, in regard to the trapping site and measured by confirmed leptospiral carrier rate, ranged from 2.8% (Strizivoja) to 32% (Stara Gradiska), while serological traces of infection were present from 0% (Kanjiža, Strizivoja) to 42.7% of animals examined. Both methods show a relatively high degree of compatibility. Tables 2 and 3 show the species of animals from which leptospires were isolated and the number of times they were demonstrated as well as the number of times specific agglutinins were demonstrated for which species leptospires were demonstrated. It is evident that leptospiral carrier rate ranges between 6.6% (C. suaveolens) to 42.8% (A. flavicollis), while the presence of agglutinins ranges from 3.8% (A. sylvaticus) to 31.4% (A. agrarius). Because of the still relatively small number of analyzed animals these findings are not particularly indicative. The mean values for leptospiral carrier rate (15.8%) and that for agglutinins (15.1%) are of orientational character only (those species in which infection was not demonstrated are not included in the calculation) (those species are certainly dependent but to an unknown extent) on the selection of the trapping sites.

**Table 1. Specification of small terrestrial mammals in relation to the trapping area, species and number of animals examined by renoculture and microagglutination-lysis**

<table>
<thead>
<tr>
<th></th>
<th>Donja Kupčina*</th>
<th>Gukić*</th>
<th>Struđe</th>
<th>Stara Gradiska</th>
<th>Kanjiža</th>
<th>Babina Greda</th>
<th>Strizivoja</th>
<th>Total</th>
<th>%</th>
<th>Renoculture</th>
<th>Microagglutination-lysis</th>
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<td></td>
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</tr>
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<td>39</td>
<td>255</td>
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<td>102</td>
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<td>26</td>
<td>4</td>
<td>42</td>
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Table 2. Distribution of isolated leptospires in relation to their animal-host species

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<th>Animal species</th>
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<th>L. pomona</th>
<th>L. sejroe</th>
<th>L. australis</th>
<th>L. batavia</th>
<th>L. shesely</th>
<th>L. bratislava</th>
<th>L. soro-zena</th>
<th>Undetermined</th>
<th>Total</th>
<th>% of &quot;Positivea&quot;</th>
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<td>33</td>
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Table 3. Distribution of specific agglutinins in relation to their animal species origin

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<th>L. batavia</th>
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<th>L. bratislava</th>
<th>L. soro-zena</th>
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<th>% of &quot;Positivea&quot;</th>
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<td>3</td>
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<td>6</td>
<td>91</td>
<td>(15.1)</td>
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* Microagglutination-lysis was performed only on animals from the trapping area Stružac, Stara Gradiska, Kanjiža, Babina Greda and Strunjavina (animals from D. Kupina and Gabe were not tested).

DISCUSSION

The results of the present study on wild small mammals in Posavina indicate that these animals probably play a decisive role in the maintenance of leptospirosis endemicity in this region of Croatia at least as far as L. pomona and L. groppelli are concerned. This assumption is supported by the fact that in all the 7 trapping sites small terrestrial animals were found to harbour leptospires to a greater or lesser degree (up to 32% of these animals). Though the findings covering 7 localities may perhaps still be on too modest a scale, it does give the impression that any random sample of these animals in the area would yield similar results. Although it is certain that the presence and intensity of leptospiral circulation in a given setting is conditioned by various known and unknown factors (Havlík et al. 1961, Antunović—Mišković 1967, Svašnikova et al. 1961, Mikhailovsky 1962, Smith and Turner 1961, Karaseva et al. 1973, Goncharova 1962, Kolochine and Mailoux 1963), the present study shows that in Posavina the leptospires (of pomona and groppelli types) may be considered as usual members of the local wild small mammal biocenosis. Actually, beside the findings of the present study, such view is supported by the fact that in Posavina the conditions are highly favourable to the survival of leptospires in external environment (humidity, pH of the soil) enabling continuous transmission of leptospires among these animals (and to people and domestic animals, too). Finally, it is a known fact that certain types of leptospires have adapted to certain wild animals, such as L. groppelli to the common mole and L. pomona to the field mouse (Babušković 1962, 1958, Svašnikova et al. 1961, Ananyin 1965, Bögg—Peterson and Fennestad 1967) so well that parasitic relationship between them has disappeared and the infection in them very easily develops into a chronic asymptomatic carriage with long-life shedding of enormous amounts of leptospires into the environment (Babušković 1965, Emanuela et al. 1964). In this respect the above studies are important because they have shown that a marked (statistically significant) association between L. pomona and A. agrarius also exists in our parts. A similar, though less pronounced, association also exists between M. arvalis and M. agrestis — two very closely related typically mesophile animal species (Feriancova—Masárová and Hanák 1965) and L. groppelli. In respect of other leptospires — L. sejroe, L. australis, L. batavia, L. szaecking, L. jaena, L. soro-zena and L. bratislava — was only established in a few sporadic cases, their significance is thus vague, although it has already been reported that rodents of the genus Apodemus could be the carriers of L. szaecking, L. batavia and L. jaena (Babušković 1958) besides L. pomona and L. sejroe, whereas L. soro-zena has been found in Sorex araneus (Kmet 1963) and L. bratislava in Erinaceus europaeus (Kmet 1965), all these animal species being habitual dwellers in the region of our study.

It follows from our findings that Posavina could be considered as a single vast focus of leptospirosis, especially of the L. pomona and the L. groppelli type. Moreover, it is certain that in the formation and the maintenance of the so-called specific climactic, edaphic and hydrologic factors — all more or less related and prevailing in Posavina, play a very important role. The significance of the presence of other leptospires (7 serotypes) in the focus examined is not yet ecologically explained, although there are indications that in that environment they, too, find favourable conditions for the survival in nature.

МЕЛИКИЕ НАЗЕМНЫЕ МЛЕКОПИТАЮЩИЕ КАК РЕЗЕРВУАРЫ ЛЕТОСПИРОС В ДОЛИНЕ Р. САВА (ХОРВАТИЯ)

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Резюме. Мелкие наземные млекопитающие, отловленные в семи местностях в долине р. Сава (Хорватия), обследованы в отношении лептоспир и методом ревакцинации в серологической реакции. Из 1749 пойманных зверьков 702 обследовали с помощью метода ревакцинации и 626 с помощью лимфо-микробиотестирования. Преобладающими видами были Microtus arvalis (672 зверька) и Apodemus agrarius (552 зверька). Из 115 штаммов выделен...
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