Small mammals – natural reservoir of pathogenic leptospires

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ABSTRACT: A total of 429 specimens belonging to 9 species of small mammals, trapped in various areas of the Czech Republic during the year 2001, were examined for antibodies against leptospires. Antibodies were found in all localities under study in 50 specimens, i.e., 11.6%, belonging to 5 species. Antibodies were most often , i.e., in 42% of positives, found in *Microtus arvalis*. In *Apodemus* spp. and *Clethrionomys glareolus* it was in 34.0% and 14.0% of positives, respectively. In other specimens belonging to *Mus musculus* and *Microtus subterraneus* antibodies were found only on occasion. Antibodies against L. grippotyphosa serotype were only found. Titres varied from 100 to 12 800 and most reactions in positive individuals were at lower dilutions, i.e., up to the titre of 800 in 40 cases (80%). The results proved the already known fact of dominant persistence of leptospiral foci of the grippotyphosa type in the Czech Republic.

Keywords: free-living mammals; leptospiroses; L. grippotyphosa; serological examinations; antibodies

Leptospiroses represent a group of infectious diseases of humans, domestic and free-living animals and as such are worldwide zoonoses (Levett, 2001; Vinetz, 2001). The agents – leptospires – are typical parasites of animals, free-living rodents and insectivores, in particular. Every serotype of leptospires has its own predilection host considered to be the main reservoir (Sebek, 1985; Vinetz, 2001). These reservoir animals are responsible for the formation and persistence of endemic foci of leptospiroses of individual types in specific areas. Primary infections of reservoir animals are often accompanied by passing leptospires to the environment, the fact being responsible for their circulation and survival in water sources, in particular. Other species of small mammals as well as larger ones including humans may become infected under these circumstances and then serve as potential hosts of leptospires. Most reliable and precise information on the occurrence of leptospires and serotypes present in natural and synanthropic foci can be obtained by aimed studies of natural reservoirs (Sebek and Rosicky, 1974; Sebek *et al.*, 1983; Prokopcakova *et al.*, 1994). Ecological factors such as population density and geographic distribution of reservoir hosts play an important role in leptospiroses as well as other diseases occurring in natural foci (Pikula, 1996, 1998a; 1998b; Zapletal *et al.*, 1999, 2000).

The distribution of natural foci and the biotic and spatial structure of leptospiroses have been paid great attention to in the former Czechoslovakia. Research activities proved the existence of such foci of diseases in our country. Sebek and Rosicky (1974) and Sebek (1985) described foci of leptospiroses in the former Czechoslovakia in detail. The above authors mentioned existing differences in the occurrence of natural foci of leptospiroses between the Slovak and Czech Republics in their paper. There is a greater diversity of serotypes of leptospiroses in Slovakia and the eastern part of

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Moravia than in Bohemia. It is also interesting that similar differences were found in anthropourgic foci, i.e., those ones occurring in domestic animals. Pomona and tarassovi serotypes were relatively frequently found in the domestic pig and slaughter-house workers in Slovakia (Bakoss *et al.*, 1996), while in the Czech Republic they were lacking. As far as natural foci in the Czech Republic are concerned, those ones of the grippotyphosa serotype were proved both by serology and culture. Natural foci of the pomona serotype in Bohemia were found only in northern Moravia, where its natural reservoir, i.e., the Striped Field Mouse (*Apodemus agrarius*), is distributed. Foci of the bratislava serotype occur throughout the territory of the Czech Republic in areas of distribution of their main natural reservoir hosts, i.e., the Hedgehogs (*Erinaceus europaeus* and *E. concolor*). Foci of the sorex jalna serotype were found only by serology in the European Shrew (*Sorex araneus*) from Doupov highlands and the Bohemian Forest. Besides the mentioned natural foci other ones of the synanthropic type (icterohaemorrhagiae and copenhageni, respectively, and sejroe) were found in all areas of distribution of their main reservoir hosts such as the Norwegian Rat (*Rattus norvegicus*) and the House Mouse (*Mus musculus*) (Sebek *et al.*, 1983). It is clear that individual serotypes of leptospires are in specific conditions bound to their main hosts, i.e., both exoanthropic and synanthropic small mammals.

This study engaged in the examination for the presence of antibodies against leptospires in small mammals from various parts of the Czech Republic. Its aim was to find whether there were changes in the occurrence of natural foci of leptospiroses in the Czech Republic.

MATERIAL AND METHODS

We trapped and examined small mammals from various parts of the Czech Republic (Klatovy, Cesky Krumlov, Dobruska and Breclav) during the year 2001. Small mammals were trapped in the wild as well as around agricultural buildings using commercial snap traps. A total of 429 specimens belonging to 9 species were examined. Species determination was followed by autopsy of specimens trapped. Serology was based on the use of eluates of the heart or blood impression smears on filter paper. It was performed by the reaction of agglutination lysis according to standard methods (Sebek, 1979). For the reaction we used following serotypes and strains of leptospires: 1. L. grippotyphosa – P 125, 2. L. icterohaemorrhagiae – Fryšava, 3. L. sejroe – M 84, 4. L. canicola – C 7, 5. L. pomona – Šimon, 6. L. bratislava – Jež Bratislava, 7. L. arboreae – M 7, 8. L. sorex jalna – Sorex Jalna, 9. L. bataviae – Moldava, 10. L. tarassovi – S 42, 11. L. bulgarica – Nikolaevo, 12. L. pyrogenes – Salinem. Sera reacting in the standard dilution of 1 : 100 were examined with the respective serotype up to the titre.

Table 1. Results of trapping and examination of small mammals in various areas of the Czech Republic (numbers of examined/positives)

Species	Klatovy	Dobruska	Ceský Krumlov	Breclav	Total
Apodemus sp.	67/6	11/5	44/3	43/3	165/17
Apodemus microps	0/0	0/0	0/0	36/0	36/0
Clethrionomys glareolus	9/2	0/0	38/5	3/0	50/7
Microtus agrestis	0/0	0/0	1/0	0/0	1/0
Microtus arvalis	38/8	3/2	8/1	53/10	102/21
Mus musculus	0/0	54/4	0/0	0/0	54/4
Microtus subterraneus	0/0	0/0	4/1	0/0	4/1
Sorex araneus	5/0	1/0	7/0	1/0	14/0
Sorex minutus	3/0	0/0	0/0	0/0	3/0
Total	122/16	69/11	102/10	136/13	429/50

Table 2. Results of serology for the presence of antibodies against leptospires in small mammals

Species	Examined	Positives	% of the same species	% of examined	% of positives
Apodemus sp.	165	17	10.3	3.9	34.0
Apodemus microps	36	0	0	0	0
Clethrionomys glareolus	50	7	14	1.6	14.0
Microtus agrestis	1	0	0	0	0
Microtus arvalis	102	21	20.5	4.9	42.0
Mus musculus	54	4	7.4	0.9	8.0
Microtus subterraneus	4	1	25.0	0.3	2.0
Sorex araneus	14	0	0	0	0
Sorex minutus	3	0	0	0	0

RESULTS

Apodemus spp. mouses, including the Yellow-necked Mouse (*Apodemus flavicollis*) and the Wood Mouse (*Apodemus sylvaticus*), and the Common Vole (*Microtus arvalis*) were the most frequently examined species with the percentage of captured specimens amounting to 38.5% and 23.7%, respectively. The above-mentioned species were trapped in all the areas under study. We can thus conclude that these species are distributed throughout the territory of the Czech Republic. Other species occurred in lower numbers and were not present in all localities under study (Table 1).

Antibodies against leptospires were found in 50 cases, i.e., in 11.6%, in specimens belonging to 5 species. They were found in the specimens examined in all localities under study. The percentage of positives varied from 9.5% to 15.9% in localities of Breclav and Dobruska, respectively. Antibodies against leptospires were most often, i.e., in 42.0% of positives, found in the Common Vole (*Microtus ar-*

Table 3. Titres and serotypes of antibodies found in the small mammals examined

Species	V	+	Titres								Serotype
			100	200	400	800	1600	3200	6400	12800	
Apodemus sp.	165	17	8	1	6	2					L. grippotyphosa
Apodemus microps	36	0									
Clethrionomys glareolus	50	7	1	5			1				L. grippotyphosa
Microtus agrestis	1	0									
Microtus arvalis	102	21	1	2	4	5	3	2	2	2	L. grippotyphosa
Mus musculus	54	4	4								L. grippotyphosa
Microtus subterraneus	4	1	0	0	1						L. grippotyphosa
Sorex araneus	14	0									
Sorex minutus	3	0									
	429	50	14	8	11	7	4	2	2	2	

valis). In mouses of *Apodemus* species and the Bank Vole (*Clethrionomys glareolus*) it was in 34.0% and 14.0% of positives, respectively. In other species such as the House Mouse (*Mus musculus*) and the Common Pine Vole (*Microtus subteraneus*) antibodies were found only on occasion (Table 2).

Table 3 shows the serotypes present and the titres obtained. Even though we used 12 different strains of leptospires in the reaction, there were found antibodies against L. grippotyphosa only. Titres varied from 100 to 12 800 and most reactions in positive specimens were in the lower dilution, i.e., up to the titre of 800 (40 specimens representing 80% of positives). Higher titres against L. grippotyphossa were found only in the Common Vole (*Microtus arvalis*).

DISCUSSION

Small mammals, both synanthropic and exoanthropic ones, are the main reservoir of pathogenic leptospires. Under natural conditions the distribution of individual strains and serotypes of leptospires depends on specific hosts. From our results it is clear that small mammals are abundant throughout the territory of the Czech Republic. This study engaged in examination of 9 different species, most abundant of which were mouses of *Apodemus* spp. and the Common Vole (*Microtus arvalis*). Both species were regularly present in all localities under study and belong probably to the most common inhabitants of natural as well as agricultural habitats. From our results it is also clear that they are most important from the point of ecology of leptospiroses. Similar results concerning these two species have already been published (Treml and Nesnalova, 1993).

Antibodies against L. grippotyphosa serotype were only found in the specimens examined. Antibodies were found in 5 species and prevailed in the Common Vole (*Microtus arvalis*) in 42.0% of positives. The dominance of natural foci of the grippotyphosa type in various areas of the Czech Republic was confirmed. Considering this point of view, there were no marked changes in the natural focality of leptospiroses in our country during last years. Recently, this fact has been noted by Sebek and Rosicky (1974), Sebek *et al*. (1983), Sebek and Vlcek (1990), Asmera (1991) as well as Treml and Nesnalova (1993) finding marked prevalence of antibodies against the grippotyphosa serotype in small mammals in various areas of the Czech Republic. Similar studies have been published by a number of authors from abroad, although there were differences in the prevalence of antibodies as well as the serotypes. Kocianova *et al*. (1993) examining small mammals from southern Bavarian forest found antibodies in 8 species in 7.9% with antibodies against L. grippotyphosa prevailing. Likewise Prokopcakova *et al*. (1994) in Slovakia examined 1 106 small mammals and found antibodies against leptospires in 4.5% of cases in *Apodemus flavicolis* and *Apodemus sylvaticus*, in particular. Stanko *et al*. (1997) examined 2 493 specimens of 22 species and found antibodies in 5% of cases and 6 species. It was also in their examinations, that the antibodies against leptospires of the grippotyphosa serotype prevailed (63.2% of positives). The above-mentioned authors obtained the results using the same method as that of our study. Considering this, the paper by Adler *et al*. (2002) is interesting because these authors used a new and modern method of PCR assay to detect leptospires. Employing this method they found DNA of leptospires in kidneys of small mammals in the area of Zurich in 12.6% of cases.

According to Sebek and Rosicky (1974), main reservoir hosts of leptospires of the grippotyphosa serotype under conditions of Central Europe are both the Field Vole (*Microtus agrestis*) maintaining archaic foci (marsh ones) and the Common Vole (*Microtus arvalis*) prevailing in the agrobiocoenosis and migrating to other non-typical habitats during periods of overcrowding, the fact responsible for more numerous contacts with other small mammalian species. The isolation of leptospires of the L. grippotyphosa serotype from the Common Vole (*Microtus arvalis*) was successful in various areas of the Czech Republic. The percentage of positive individuals, however, varied and amounted to the highest numbers during episodes of overcrowding (Asmera, 1991; Jarekova *et al*., 1993). Our results verify the previous ones and as it is clear it was especially the Common Vole (*Microtus arvalis*) that was most often positive (42.0% of all positives). The percentage of positives in the Common Vole amounted to 20.5% of examined specimens and the titres found witness for the considerable importance of the species for the maintenance of natural foci of leptospiroses of the grippotyphosa type in agrobiocoenoses of the Czech Republic.

Antibodies against L. grippotyphosa serotype were found also in 4 other small mammalian species. They, however, were found in lower percentage of specimens and in lower titres. We can thus con-

sider these species only potential hosts of leptospires of the grippotyphosa serotype. There are different opinions regarding the importance of potential hosts. Some authors mean that such species are important for the maintenance of the infection in the foci during periods of minimum population density of the main reservoir host, i.e., the Common Vole (*Microtus arvalis*) (Ananin, 1971). Other ones state that the importance of the so-called potential hosts is small and that during periods of latency of the foci they are free from infection and the infection persists only in the main reservoir host (Sebek and Chmela, 1972; Jarekova *et al.*, 1993). Results of our one-year study cannot confirm or exclude the above opinions because a longer study would be necessary. It is, however, clear that potential hosts get infected due to contacts with the Common Vole (*Microtus arvalis*) as the main reservoir host of these leptospires. As it has already been mentioned, this species migrates during episodes of overcrowding to non-traditional habitats (Zapletal *et al.*, 2001), where it can contaminate the environment increasing thus the risk of infection of other potential host species. Regarding the fact that the Common Vole (*Microtus arvalis*) occurs throughout the Czech Republic in areas employed for agricultural activities as well as other habitats we have to bear in mind that natural foci of leptospiroses of the grippotyphosa type can occur anywhere in our country. It is important both from the epizootiological and epidemiological point of view because such environment can result in infections both of humans and domestic animals. Our results witness the already known facts on the occurrence and structure of foci of leptospiroses in the territory of the Czech Republic. Even though we verified unambiguously the existence and dominance of foci of the grippotyphosa type, the possibility of occurrence of other types of leptospiroses such as L. sejroe and L. icterohaemorrhagiae resp. copenhageni bound to synanthropic small mammalian species cannot be neglected. In areas of abundant synanthropic species such as the House Mouse (*Mus musculus*) and the Norwegian Rat (*Rattus norvegicus*) we have to expect the existence of the above mentioned serotypes of leptospires. The same concerns natural foci of the pomona type bound to the occurrence of the Striped Field Mouse (*Apodemus agrarius*). We cannot confirm or exclude the presence of such natural foci of leptospiroses because the above mentioned small mammalian species were absent from our collection of specimens examined.

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