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LEPTOSPIRAL ANTIBODIES IN WILD LIVING ANIMALS FROM NORTH TYROL

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Dedicated to Professor V. V. Kucheruk on the occasion of his 60th birthday

Abstract. The authors examined serologically 623 wild living animals (22 species) from North Tyrol for the incidence of leptospirosis. Positive reactions (MAL) in the titre 1 : 100 and more were found in 4.5 % of the animals examined; the serotypes concerned were these: *icterohaemorrhagiae*, *sorex-jalna*, *castellonis* or *arboreae*, *grippotyphosa*, *bratislava*, *pamona*, *sejroe*, *saxkoebing*. Positive reactions were obtained with the sera of *Sorex araneus*, *Erinaceus europaeus*, *Putorius putorius*, *Vulpes vulpes*, *Cervus claphus*, *Capreolus capreolus*, *Rupicapra rupicapra*, *Apodemus* sp., *Clethrionomys glareolus*. The serotypes forming natural foci of leptospirosis in North Tyrol are these: *sorex-jalna*, *grippotyphosa*, *bratislava*, *saxkoebing* and, apparently, *jalna*. Synanthropic foci are formed by *icterohaemorrhagiae* or *copenhageni* and *sejroe*.

Information on natural foci of leptospirosis in Austria is scarce and no data are available for large areas of this country. In Tyrol, an important area in the eastern part of Austria, and of interest from the zoogeographical point of view, no data are available on examinations of wild living animals except for a note on the finding of antibodies against *sorex-jalna* in the snow vole (*Microtus nivalis*) from the Grossglockner area (Šebek and Rosický 1973). Therefore, we welcomed the opportunity of a serological examination of a relatively large number of wild living mammals from North Tyrol in order to obtain information on the incidence of leptospirosis. Difficulties in catching small mammals particularly in high mountain areas impeded the collection of a representative number of specimens of several animal species. However, we hope that the results of our examination of a very variegated species material will offer valuable data on the incidence, distribution and serotype structure of natural foci of leptospirosis in North Tyrol.

MATERIALS AND METHODS

Table 1 contains data on the species and the number of animals examined in addition to data on positive reactions with the individual leptospiral serotypes. Blood for examination was obtained from the animals in two different ways: small mammals (shrew, mouse, vole) were caught in live-traps placed in all typical sites and inspected every 3—4 hr for a period of at least 24 hr. In this way we caught a total of 291 small, wild living mammals. The remaining material consisting of 332 blood samples from hedgehogs, carnivores, deer, squirrels and marmots was sent to us by our collaborators (hunters, prepara- tors, etc.).

Blood for serological examination was taken on strips of filter paper and examined by means of the microagglutination-lysis reaction in a basic dilution of 1 : 100 with 12 serotypes of live

Table 1. Results of serological examination of 623 wild living animals from North Tyrol for the incidence of leptospirosis

Species	No. examined	No. positive	%	Positive with *Leptospira*							
				ictero-haemor-rhagiae	*sorex -jalna*	*castel-lonis*	*grippo-typhosa*	*brati-slava*	*pomona*	*sejroe*	*saxkoe-bing*
Sorex araneus	12	1	—	0	1	0	0	0	0	0	0
Sorex minutus	4	0	—	0	0	0	0	0	0	0	0
Erinaceus europaeus	17	3	—	0	0	0	0	3	0	0	0
Mustela nivalis	8	0	—	0	0	0	0	0	0	0	0
Putorius putorius	1	1	—	0	1	0	0	0	0	0	0
Martes martes	4	0	—	0	0	0	0	0	0	0	0
Meles meles	4	0	—	0	0	0	0	0	0	0	0
Vulpes vulpes	9	5	—	1	5	0	0	0	0	0	1
Cervus elaphus	85	9	10.6	1	0	0	6	1	1	0	0
Capreolus capreolus	109	5	4.6	0	1	1	1	0	1	1	0
Rupicapra rupicapra	55	1	1.8	0	1	0	0	0	0	0	0
Capra ibex	4	0	—	0	0	0	0	0	0	0	0
Lepus europaeus	1	0	—	0	0	0	0	0	0	0	0
Rattus norvegicus	32	0	0.0	0	0	0	0	0	0	0	0
Mus musculus	10	0	—	0	0	0	0	0	0	0	0
Apodemus sp.	72	1	1.3	0	1	0	0	0	0	0	0
Clethrionomys glareolus	109	2	1.8	0	2	0	0	0	0	0	0
Microtus nivalis	27	0	—	0	0	0	0	0	0	0	0
Microtus arvalis	2	0	—	0	0	0	0	0	0	0	0
Microtus agrestis	23	0	—	0	0	0	0	0	0	0	0
Sciurus vulgaris	21	0	—	0	0	0	0	0	0	0	0
Marmota marmota	14	0	—	0	0	0	0	0	0	0	0
Total	623	28	4.5	2	12	1	7	4	2	1	1

cultures of Leptospirae. A detailed description of the methods employed has been given in an earlier paper (Šebek et al. 1972, 1976). Evident coagglutinations due to a similarity in the antigenic structure such as lower titres with *L. sorex-jalna* in the presence of high titres with *L. icterohaemorrhagiae* and vice versa have not been recorded.

RESULTS

Of the 623 animals examined, 28 gave positive reactions (4.5 %). These positive reactions were given with the serotypes *sorex-jalna* (2 %), *grippotyphosa* (1.1 %), *bratislava* (0.7 %), *icterohaemorrhagiae* (0.3 %), *pomona* (0.3 %), *castellonis* or *arboreae* (0.2 %), *sejroe* (0.2 %), *saxkoebing* (0.2 %). In two foxes, we found the concomitant incidence of antibodies against two serotypes: in one *sorex-jalna* and *icterohaemorrhagiae*, in the other *sorex-jalna* and *saxkoebing*. Unfortunately, in view of the small number of specimens available for the examination of several animal species, we were unable to evaluate the percentage of serological positivity. Neither could we make any conclusions as regards the importance of these species as reservoirs of leptospirosis. Our work was impeded also by technical difficulties and, therefore, our serological examination could not be completed by an examination of the cultures. Among the species for which the number of specimens available was low, were the brown rat — 32 specimens, and the house mouse — 10 specimens. Although both synanthropic rodent species are the principal reservoirs of three leptospiral serotypes in central Europe (*icterohaemorrhagiae, copenhageni, sejroe*) (Gsell 1952, Kmety 1956, Šebek 1965, Šebek and Rosický 1973, 1974), we failed to demonstrate antibodies against these serotypes in the two rodent species from North Tyrol. Evidently, the small number of animals available for examination was responsible for this failure because we found in domestic animals significant titres with these serotypes (Šebek et al. 1976).

Leptospiral antibodies were found in 9 of the 22 species of wild living animals examined. One of the 13 common shrews was positive with *sorex-jalna* in a titre of 1 : 400; 3 of the 17 hedgehogs examined were positive with *L. bratislava* in titres of 1 : 800 (once) and 1 : 1,600 (twice). The single polecat examined gave a positive reaction with *L. sorex-jalna* in a titre of 1 : 200. Of the 9 foxes examined 5 gave positive reactions: 3 with the serotype *sorex-jalna* in titres of 1 : 400 (once) and 1 : 1,600 (twice); one with *L. sorex-jalna* and *L. icterohaemorrhagiae* in the titre of 1 : 1,600 each; one reacted simultaneously with *L. saxkoebing* (titre 1 : 3,200) and *L. sorex-jalna* (titre 1 : 200). Of the 85 red deer specimens examined 9 (10.6 %) gave positive reactions: 6 with *L. grippotyphosa* (5 times in titres of 1 : 200, once in the titre 1 : 800); one with *L. icterohaemorrhagiae* (titre 1 : 200), one with *L. bratislava* (titre 1 : 400), one with *L. pomona* (titre 1 : 400). Of the 109 roe deer specimens, 5 (4.6 %) gave positive reactions: one with *L. sorex-jalna* (titre 1 : 400), one with *L. castellonis* (titre 1 : 1,600) or *L. arboreae* (titre 1 : 800), one with *L. sejroe* (titre 1 : 800), one with *L. grippotyphosa* (titre 1 : 12,800), one with *L. pomona* (titre 1 : 800). Of the 55 chamois, one gave a positive reaction with *L. sorex-jalna* (titre 1 : 400). Of the species of mice and voles, two species only gave positive reactions: one *Apodemus* sp.*) and two bank voles (two specimens). Both were positive with *L. sorex-jalna* in titres of 1 : 6,400 for *Apodemus* sp. and 1 : 400 and 1 : 1,600 for the two bank voles. Negative reactions were obtained from the remaining species examined, i.e., from the lesser shrew, the weasel, the pine marten, the badger, the ibex, hare, brown rat, house mouse, snow vole, common vole, field vole, red squirrel, marmot.

*) We used this name for the species *Apodemus flavicollis* and *A. sylvaticus* because at the time of blood collection we were unable to identify the species concerned.

DISCUSSION

Of the 8 leptospiral serotypes with which wild living animals from Tyrol gave positive reactions, 4 serotypes form natural foci in the area under consideration: *sorex-jalna, grippotyphosa, bratislava, saxkoebing*. Although antibodies against *L. jalna* could not be demonstrated in the wild living animals examined, it seems possible that this serotype forms natural foci in North Tyrol judging from our experience in other parts of Austria (Šebek et al. 1973), in Switzerland and several other European countries (Šebek and Rosický 1973). In support of this supposition is the finding of significant titres against this serotype in domestic animals from Tyrol (Šebek et al. 1976).

We found antibodies against *L. sorex-jalna* in the common shrew known to be the principal reservoir of this serotype in central Europe (Kmety 1954, 1956, Halaša et al. 1969, Šebek and Rosický 1974). The first finding of antibodies against this serotype in Austria was reported by Šebek (1971) for the alpine shrew from the Burgenland. In 1973, Šebek et al. found antibodies against *L. sorex-jalna* in 12 species of wild living animals; in the shrew, the percentage of positivity was high (16.8). Since natural foci of *L. sorex-jalna* are distributed with considerable frequency throughout Austria, they evidently are present also in the alpine zone judging from the finding of a high titre (1 : 50,000) in the snow vole from the Grossglockner area (Šebek and Rosický 1973). Our finding of antibodies against *L. sorex-jalna* in 7 species of wild living animals indicates that this serotype is frequent in North Tyrol, as this has been suggested also by the results of our serological examination of domestic animals from Tyrol (Šebek et al. 1976).

In our opinion, the incidence of natural foci of the serotype *grippotyphosa* seems to be less frequent and less widely distributed as it is in Czechoslovakia, with a dominant position mainly in Bohemia and Moravia (Šebek and Rosický 1974). Rosický and Šebek (1974) reported that the percentage of serological positivity was 18.7 for *L. grippotyphosa* in these areas (this is an average from numerous localities over a number of years). The authors drew attention to the fact that the rate of infestation of the principal reservoir, the bank vole, with the serotype *grippotyphosa* decreased in direction to the East and South of Czechoslovakia. This is in agreement with the results of our investigation of wild living animals from North Tyrol: we found antibodies against *L. grippotyphosa* in two species only, i.e., in the red and the roe deer: 5 times in the titre 1 : 200 for the first species and once in the titre 1 : 800; in the second species we found it once in the significant titre of 1 : 12,800. In Austria, Šebek et al. (1973) confirmed the presence of antibodies against *L. grippotyphosa* in 11 species of wild living animals. In North Tyrol, natural foci of the serotype, *grippotyphosa* appear to be distributed in areas of lower altitudes, i.e., in localities inhabited by the common vole. In view of the fact that we examined two common voles only, we have been unable to estimate the rate of infestation in the populations of this animal species in North Tyrol. The same applies to the field vole known to be an important reservoir of *L. grippotyphosa* in central Europe (Kmety et al. 1955, Pokorný et al. 1958, Šebek and Rosický 1974). Negative results obtained on the incidence of *L. grippotyphosa* from our examination of 275 specimens of the families Microtidae and Muridae, and the low percentage of positivity to this serotype in domestic animals (0.3 %) (Šebek et al. 1976) suggest that the incidence and distribution of *L. grippotyphosa* is scarce in Tyrol.

Antibodies against *L. bratislava*, in significant titres, were found in three hedgehogs from Tyrol. This serotype and its main reservoir, the hedgehog, are distributed, practically, in all parts of Europe (Šebek and Rosický 1973, 1974). It has been

described by Kmety (1955, 1956) from Czechoslovakia. In Austria, Šebek et al. (1973) found antibodies against this serotype in three species of wild living animals; the positivity in the hedgehog was 44.8 %. In North Tyrol, natural foci of *L. bratislava* were found in all localities inhabited by the hedgehog, i.e., in places of a lower altitude.

The serotype *saxkoebing* with its main reservoir, the yellow-necked field mouse, and its important potential reservoir, the long-tailed field mouse, appears to be absent in several areas of central Europe. On the territory of Czechoslovakia, reports on its incidence are available for Slovakia only (Kmety 1956, 1967), and not for Bohemia and Moravia (Šebek and Rosický 1974) in spite of the fact that both *Apodemus* species are frequent in these areas. In Austria, Šebek et al. (1973) found antibodies against this serotype in 6 species of wild living animals which indicates the considerably frequent distribution of *L. saxkoebing* in this country. In North Tyrol, antibodies against *L. saxkoebing* were found by the present authors in one fox only, but were not disclosed in either the main or potential reservoir. This may have been due to the relatively small number of animals examined; in addition, we did not identify the individual *Apodemus* species and, hence, did not obtain knowledge on the number of yellow-necked field mice examined. Evidently, antibodies against *L. saxkoebing* should be present in this species. In addition to a significant titre in the fox, our assumption that natural foci of *L. saxkoebing* are present in North Tyrol is supported by our finding of significant titres in domestic animals (Šebek et al. 1976).

The serotypes *icterohaemorrhagiae*, *copenhageni* and *sejroe* do, evidently, form foci of the synanthropic type in North Tyrol. Although our results of serological examinations of the principal reservoirs — rats and mice — were negative, the number of these animals was relatively small. Our finding of antibodies against *L. icterohaemorrhagiae* in the fox and red deer could not be considered to be significant because the serotype *sorex-jalna* reacted concomitantly with *icterohaemorrhagiae* in the same titre in the fox (coagglutination?); in the red deer, the titre of 1 : 200 was insignificant for such a large animal. Antibodies against *L. sejroe* were demonstrated in the roe deer in one instance only and, again, its titre of 1 : 200 was insignificant. Basing on our experience from other parts of Austria (Šebek et al. 1973), and from other European countries (Šebek and Rosický 1973) our supposition that synanthropic foci of the three serotypes are present in North Tyrol appears to be justified.

A most interesting finding was that of antibodies against *L. castellonis* or *L. arboreae* in a titre of 1 : 1,600 or 1 : 800 in a roe deer from Fieberbrunn. Although the titre with *L. castellonis* was higher than that with *L. arboreae*, it has been suggested by Kmety's (1967) results from Czechoslovakia, that the second serotype is more likely to be present than the first. All strains of the serological group *Ballum* isolated from laboratory mice and also from the pig in Czechoslovakia (Kmety 1967) belonged to the serotype arboreae. We are convinced that natural foci of *L. arboreae* do not exist in North Tyrol, because their presence has not been confirmed from other parts of Austria, from Czechoslovakia, Switzerland, Yugoslavia and Bulgaria (Šebek and Rosický 1973). Theoretically, red deer might acquire infection through indirect contact with the pig. Of interest is the significant titre with *L. castellonis* or *L. arboreae* found in one cow from the Kitzbühel district (Šebek et al. 1976).

In our opinion, natural foci of the serotype *pomona* do not exist in North Tyrol. The main reason is the absence of the striped field mouse, the natural reservoir of this serotype, in this area. On the other hand, we are inclined to believe that there are foci of the anthropourgic type, but that these are scarce in occurrence as indicated by the results of our serological investigation of domestic animals (Šebek

et al. 1976). The titre of 1 : 400 in the red deer cannot be considered to be significant. More convincing is the titre of 1 : 800 in a roe deer. It is possible that this animal had previously been infected with *L. pomona*. Since both red- and roe deer come close to isolated dwellings particularly in the winter, there might be the possibility of indirect contact of these animals with the pig. The occurrence of natural foci of other serotypes not mentioned in this report appears to be unlikely in North Tyrol.

In view of the small number of specimens of several animal species, particularly small mammals, available for examination, we consider the presented results of this first serological investigation of wild living animals to be a general information only on the incidence of leptospirosis. However, we hope that our report will initiate further detailed studies on leptospirosis of wild living animals in North Tyrol.

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АНТИТЕЛА К ЛЕПТОСПИРАМ У ДИКИХ ЖИВОТНЫХ В СЕВЕРНОМ ТИРОЛЕ

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Резюме. Авторами серологически исследовано на лептоспироз 623 диких животных (22 видов) из северного Тироля (Австрия). Положительные реакции (MAL) в титрах 1 : 100 и выше наблюдались у 4,5 % обследованных животных, а именно с серотипами серологических групп *icterohaemorrhagiae*, *sorex-jalna*, *castellonis* или *arborcae*, *grippotyphosa*, *bratislava*, *pomona*, *sejroe*, *saxkoebing*. Положительные реакции давали сыворотки от *Sorex araneus*, *Erinaceus europaeus*, *Putorius putorius*, *Vulpes vulpes*, *Cervus elaphus*, *Capreolus capreolus*, *Rupicapra rupicapra*, *Apodemus* sp., *Clethrionomys glareolus*. Природные очаги в северном Тироле образуют серотипы *sorex-jalna*, *grippotyphosa*, *bratislava*, *saxkoebing* и по всей вероятности также *jalna*. Синантропные очаги образуют *icterohaemorrhagiae* или *copenhageni* и *sejroe*.

REFERENCES

GSELL O., Leptospirosen. Bern, 1952.

HALAŠA M., PLEŠKO I., HRÚZIK J., Leptospirózy zvierat a ludí. Bratislava, 1969.

KMETY E., Jež prírodný rezervoár leptospír typu australis? Čs. hyg. epidem. mikrob. imunol. 3: 41—43, 1954.

—, *Leptospira sorex-Jalná* — nový druh leptospír? Brat. lek. listy 35: 261—265, 1955.

—, Úloha našej leptospirológie pri výskume prírodných ohnísk nákaz. In: Prírodné ohniská nákaz. Bratislava, pp. 145—155, 1956.

—, Vorkommen und Verbreitung in Europa: 17. Tschechoslowakei. In: KATHE J., MOCHMANN H., Leptospiren und Leptospirosen. Teil II., Jena, pp. 881—892, 1967.

—, CHYLO E., KRATOCHVÍL J., *Microtus agrestis* rezervoár leptospír v prírode. Zool. ent. listy 4: 291—294, 1955.

POKORNÝ B., LEHKÝ F., ŠEBEK Z., VOŠTA J., Weitere neue Leptospirenträger in Böhmen. Zbl. Bakt. I. Orig. 172: 135—141, 1958.

ROSICKÝ B., ŠEBEK Z., To the evolution of natural foci of *L. grippotyphosa* in central Europe. Folia parasit. (Praha) 21: 11—20, 1974.

ŠEBEK Z., A characterization of leptospirosis foci on the territory of Czechoslovakia. In: Theoretical questions of natural foci of diseases. Proc. of a symp. held in Prague, 26—29, 11. 1963. Prague, pp. 69—82, 1965.

—, Zur Verbreitung der Alpenspitzmaus (*Sorex alpinus* Schinz, 1837, Soricidae, Insectivora) in der Tschechischen Sozialistischen Republik. Zool. listy 20: 319—329, 1971.

—, HENEBERG D., HENEBERG N., Natural and anthropurgic foci of leptospirosis in the Socialist republic of Macedonia. Folia parasit. (Praha) 19: 53—60, 1972.

—, ROSICKÝ B., Vergleichungsstudie der Leptospirose-Naturherde in einigen europäischen Ländern. In: 1. Internationales Arbeitskolloquium über Naturherde von Infektions-

krankheiten in Zentraleuropa. 17.—19. April 1973, Illmitz und Graz. Sonderheft 1 der "Wissenschaftlichen Arbeiten aus dem Burgenland", Eisenstadt, pp. 90—97, 1973.
—, —, K rozšíření, charakteristice a struktuře ohnisek leptospirózy v ČSSR. Čs. epidem. mikrob. imunol. 23: 10—21, 1974.
—, SIXL W., STÜNZNER D., SIXL-VOIGT B., VALOVÁ M., WITHALM H., Zur Leptospirose in Österreich: I. Untersuchungen bei Wildtieren. In: 1. Internationales Arbeitskolloquium über Naturherde von Infektionskrankheiten in Zentraleuropa. 17.—19. April 1973, Illmitz und Graz. Sonderheft 1 der "Wissenschaftlichen Arbeiten aus dem Burgenland", Eisenstadt, pp. 98—104, 1973.
—, WALLNER H., SIXL W., KAASERER G., VALOVÁ M., Leptospiral antibodies in domestic animals in Tyrol. Folia parasit. (Praha) 23: 000—000, 1976.

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In memoriam Prof. A. S. Monchadsky

Professor Alexandr Samoilovich Monchadsky, Doctor of Biological Sciences, leading scientist of the Zoological Institute of the USSR Academy of Sciences in Leningrad and one of the outstanding world medical entomologists, died after a long and serious illness, against which he struggled persistently. The day of 31st December 1974 was his last.

A native from Petersburg (born on March 19, 1897), after the demobilization of the Red Army in 1921 he continued his biological studies interrupted by the World War I. Under the guidance of Prof. V. A. Dogel, the prominent zoologist and parasitologist, he studied the respiratory system of water insect larvae. He finished his postgradual studies by an excellent thesis entitled "Stigmal plates of Culicidae larvae". He continued his scientific and pedagogic activities at the Department of Invertebrates of the Leningrad University and at the Petergof Instituto of Natural Sciences. In 1935 he became one of the first workers at the Department of Parasitology organized by E. N. Pavlovsky at the Zoological Institute of the USSR Academy of Sciences and since 1942 till 1972 he headed this Department, later reorganized to a Laboratory, in a close cooperation with Academicians E. N. Pavlovsky and B. E. Bykhovsky.

The main scientific papers of Prof. Monchadsky are concerned with three subjects of medical entomology. The first one is the modern taxonomy of mosquitoes based on a detailed knowledge of their larvae. Both his knowledge of this group and his extraordinary organisational abilities predestinated Prof. Monchadsky for the post of the secretary of the All-Union commission for the study of malaria mosquitoes, which played an important role in the development of the Soviet medical entomology and malariology. Of the basic works of A. S. Monchadsky, the following dealt with this subject: "Larvae of Mosquitoes in the USSR and Neighbouring Countries (Subfamily Culicinae)" (in Russian), (Seria "Opredeliteli po faune SSSR", 1951), "Blood-sucking Diptera" (in Russian), Izd. AN SSSR, Moskva—Leningrad.

Another line of Prof. Monchadsky's studies were the questions of ecology of blood-sucking insects and the general ecological conclusions deduced from them. His papers on the laws of infestation of man and other hosts with blood-sucking insects, on the types of reaction of poikilothermic animals to the changes in the environmental temperatures, as well as the papers dealing with the concept of factors in ecology and their classification belong now to the classic works in the medical ecological entomology.