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TWO-YEAR STUDY OF EXAMINATION OF BLOOD FROM WILD RODENTS FOR THE PRESENCE OF ANTIBORRELIAN ANTIBODIES

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Abstract: The aim of our work was to find the positivity rate of antibodrrelian antibodies (IgG) in wild-living rodents in a locality situated in north Moravia, Czech Republic. Results of a survey for heart rinses (172) and sera (2) antibodies to *Borrelia burgdorferi* sensu lato (s.l.) from 6 species of 174 wild rodents from the northern part of the Czech Republic are presented. Samples were obtained in 2001–2002 at one locality (Studénka, 49º44', 18º05'). Host samples included yellow-necked mouse (*Apodemus flavicollis*, n = 106), wood mouse (*A. sylvaticus*, n = 170) and striped field mouse (*A. agrarius*, n = 13) from *Muridae* and bank vole (*Clethrionomys glareolus*, n = 3) and common/field vole (*Microtus* sp., n = 5) from *Microtidae* families. An indirect enzyme linked immunosorbent assay (ELISA) antibody test was used for testing heart rinses. Goat immunoglobulins against mouse were used as a conjugate. Antibodies to *B. burgdorferi* s.l. were found in all species. The highest positivity rate (58.8%) was recorded for wood mouse (58.8%), bank vole (45.5%), and yellow-necked mouse (44.3%). Mean positivity rate for both years of collection was 43.7%, mean annual positivity rate was 60.6% in 2001, and the value of 21.3% in 2002 appeared significantly different. Three times as many of the *Muridae* as of the family *Microtidae* were caught and the actual number of seropositive *Muridae* was not significantly higher than *Microtidae*. Positivity of compared males and females was not significantly different. Results indicate that spirochaetes were widely represented in the northern part of the Czech Republic.

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INTRODUCTION

Lyme borreliosis, also known as Lyme disease, is a wide-spread zoonosis caused by pathogenous spirochetes involved in a group called *Borrelia burgdorferi* sensu lato (s.l.). These spirochetes are common worldwide. In Europe, 5 genospecies of *B. burgdorferi* s.l. have been documented: *B. burgdorferi* sensu stricto (s.s.), *B. afzelii*, *B. garinii*, *B. valaisiana* and *B. lusitaniae* [10, 22]. *B. afzelii*, *B. garinii* and *B. burgdorferi* s.s. cause the Lyme borreliosis disease; of these, *B. afzelii* and *B. garinii* are the most frequent European genospecies, which also prevail in the area of the Czech Republic. There exist also reports about isolated strains of *B. burgdorferi* s.s. in patients [14] and from ticks [23].

Lyme disease is associated with blood-sucking arthropods, especially ticks, from which species of genus *Ixodes* are the most important. European species transferring *B. burgdorferi* are the following: *Ixodes ricinus*, feeding on at least 317 animal species, *Ixodes hexagonus*, parasiting

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in carnivors and hedgehogs, and *Ixodes uriae*, involved in transmission of borreliae in seabird colonies [6].

About 35 vertebrate species were identified as reservoir hosts of *B. burgdorferi* (animals participating in the circulation of *B. burgdorferi* in nature). Among them, small mammals and birds are the best known host species. Additional vertebrate species, such as hedgehogs and rabbits function as reservoirs [6]. Deer, domestic animals (cats, dogs) and reptiles (lizards) can also serve as reservoirs [3]. Rodent species, for example, *Apodemus* mice and *Clethrionomys* voles, have been studied as typical reservoir hosts of *B. burgdorferi* in various enzootic areas in Europe [17, 18].

This study attempted to find the species of reservoir hosts for *B. burgdorferi* s.l. living in the Czech Republic for which a locality rich in the wild-living rodents in north Moravia was chosen.

MATERIALS AND METHODS

Rodent trapping. Trapping was practised in Bažantula locality, on a 5 ha study plot situated about 2 km north-east of the town of Studénka (North Moravia, Czech Republic; 49º44'N, 18º05'E). The floodplain Bažantula forest is oak forest - *Querco-Ulmetum alnetosum* association. The tree layer consists of 2 sub-layers: the highest tree is *Fraxinus excelsior*; at the edge there are *Alnus glutinosa* and *Tilia* sp. trees. The shrub stratum is poorly developed in the sampling area, and consists mainly of young trees. The herb layer forms small separate patches. In the damp relief depression, the herb cover is up to 100%, in drier elevated places herbs are almost absent; moss or fallen leaves and branches predominate. The southern part of the forest is regularly flooded. In some relief pits water stays throughout the year [2]. Rodents were obtained by trapping with snapping spring traps. In some cases rodents were trapped with "life-hunt" traps. The snapping traps were baited with a piece of wick fried with lard and "life-hunt" traps were baited with a piece of fish covered by cotton wadding. Traps were placed on solid ground in line at a distance of 7 m from each other. They were opened at least every 2 days and checked every 12 hours. Rodents were trapped from 2001 (July, August, November) until 2002 (April, July, September). Trapped mammals were collected and transferred to the laboratory for subsequent investigation early in the morning.

Heart rinses and sera as samples were prepared from 172 dead and 2 living rodents. Dead rodents caught by snapping traps were dissected. The heart of each individual was put into 0,85 % physiological solution for a period of 1 day at temperature of 4°C. After removing the heart the remaining solution was centrifuged and the drained supernatant stored at -18°C. In this way, 172 samples were collected. Blood taken from the neck artery of 2 anesthetized living individuals was stored at 4°C overnight. Next day, the serum was separated and also stored at -18°C.

Serology. The whole cell culture from *B. burgdorferi* s.l. was used as antigen: *B. afzelii* BRZ 16 - our own strain isolated from *I. ricinus* from the Pisárky Park area in Brno, southern Moravia [13]. Borreliae were cultivated at 33°C in BSK-H medium (Sigma) at 14-day intervals to the density of 10^6–10^7 cells The culture was washed three times in physiological solution and centrifuged at 10,000 rpm for 15 min. After resuspendation in physiological solution the supernatant was used as an antigen. The protein content was measured by the method of Wannemacher *et al.* [24].

Sera (2) and heart-rinses (172) were examined by a modified ELISA, available in commercial sets (TestLine, Prague) used for diagnosis of Lyme borreliosis in human medicine, in the following way: Microplates were parallelly filled with 100 µl of respective antigen diluted in carbonate buffer at pH 9.6 (2 µg/ml) and incubated overnight at 4°C. After washing 3 times with phosphate buffer (pH 7.4) containing 0.05% Tween 20, 100 µl portions of sera diluted at 1:100 in phosphate buffer with 0.05% Tween 20 and 0.3% cassein and heart-rinses (undiluted) and incubated at 37% for 1 hour. After a triple washing of the plates, 100 µl portions of anti-mouse IgG peroxidase conjugate (Sigma) were added per well, diluted at 1:2,000. After 1 hour of incubation and a subsequent washing, 100 µl per well of substrate solution (0.1 M citrate buffer pH 4.7–5.0 with 0.05% H_2O_2) with orthophenylene diamine were added. The reaction was stopped with 1 M H_2SO_4 after 10 min of incubation. The absorbance was measured at 492 nm. The serum from a wild immunized mouse was used as a positive control. As negative control the wild mouse sera negative to antigens ($OD_{492} < 0.300$) were used. Samples with $OD_{492} > 0.300$ were considered positive.

Statistical evaluation was carried out by the test for "comparison of population probabilities" [26]:

$$Z = \frac{|p_1 - p_2|}{\sqrt{p(1-p)}} \sqrt{\frac{n_1 n_2}{n_1 + n_2}} \qquad Z_{1-\frac{\alpha}{2}} = 2.576 \qquad (\alpha = 0.01)$$

p positivity (%)
n number of tested samples

RESULTS

In the years 2001 (99) and 2002 (75) a total of 174 wild rodents were trapped in Bažantula locality. Of these animals 38 belonged to *Microtidae* (*Clethrionomys glareous*, *Microtus arvalis*, *Microtus* sp.), 136 to *Muridae* (*Apodemus agrarius*, *Apodemus flavicollis*, *Apodemus sylvaticus*) families (Tab. 1). Generally, the rate between *Muridae* and *Microtidae* in the total amount of trapped animals was ca. 3.5:1. *Apodemus flavicollis* (yellow-necked mouse) (106) was the most frequently trapped species of *Muridae* and *Clethrionomys glareolus* (bank vole) (33) of *Microtidae*. Of all samples, 89 males and 85 females were trapped (Tab. 2).

Antiborrelian antibodies were detected in heart-rinses of 172 individuals and in sera of 2. In 2001–2002, mean

Table 1. Positivity of rodent species in both years.

Family	Species	Number of tested	Number of positive (%)
Muridae	*Apodemus agrarius*	13	2 (15.4)
	Apodemus flavicollis	106	47 (44.3)
	Apodemus sylvaticus	17	10 (58.8)
Total		136	59 (43.4)
Microtidae	*Clethrionomys glareolus*	33	15 (45.5)
	Microtus arvalis	4	1 (25.0)
	Microtus sp.	1	1 (100.0)
Total		38	17 (44.7)

No significant difference between *Muridae* and *Microtidae* family, $Z = 0.143$, $\Delta = 0.01$.

Table 2. Positivity of males (m) and females (f) in the years of 2001–2002.

	2001		2002		Total	
	m	f	m	f	m	f
Number of tested	54	45	35	40	89	85
Number of positive (%)	33 (61.1)	27 (60.0)	9 (25.7)	7 (17.5)	42 (47.2)	34 (40.0)

No significant difference between total numbers of males and females, $Z = 0.956$, $\Delta = 0.01$.

Table 3. Positivity of rodent species in 2001.

Family	Species	Number of tested	Number of positive (%)
Muridae	*Apodemus agrarius*	4	1 (25.0)
	Apodemus flavicollis	49	33 (67.3)
	Apodemus sylvaticus	16	10 (62.5)
Microtidae	*Clethrionomys glareolus*	25	14 (56.0)
	Microtus arvalis	4	1 (25.0)
	Microtus sp.	1	1 (100.0)
Total		99	60 (60.6)

Table 4. Positivity of rodent species in 2002.

Family	Species	Number of tested	Number of positive (%)
Muridae	*Apodemus agrarius*	9	1 (11.1)
	Apodemus flavicollis	57	14 (24.6)
	Apodemus sylvaticus	1	0 (0.0)
Microtidae	*Clethrionomys glareolus*	8	1 (12.5)
Total		75	16 (21.3)

positivity was 43.7%. Mean annual positivity in 2001 was 60.6% (Tab. 3) in 2002 - 21.3% (Tab. 4), which was significantly lower ($Z = 6.276$, $\alpha = 0.01$). Positivity of species belonging to *Muridae* was 43.4%, in *Microtidae* - 44.7%. Statistically, there was no difference between positivity of *Muridae* and *Microtidae*. The most positive species of *Muridae* was *A. sylvaticus* (wood mouse) (58.8%), in *Microtidae C. glareolus* (bank vole) (45.5%) (Tab. 1). Positivity of males was 47.2% and positivity of females – 40.0% which was not significantly higher (Tab. 2). Tables 5–6 present the positivity of genders in individual years.

DISCUSSION

The presence of IgG antibodies against *B. afzelii* was detected by indirect ELISA method. Genospecies *B. afzelii* seems to be suitable for using as an antigen because they were mostly isolated from the rodents or their ectoparasites [9, 11]. *B. afzelii* is described as being commonly found in rodents [7, 8, 12]. An anti-mouse IgG conjugate was used for the detection of antibodies to *B. afzelii* or to other genospecies of *B. burgdorferi* s.l. [5, 21]. Used dilution of conjugate and sera was determined on the basis of tests made during this study. Sera of immunized wild rodents kept alive in nature were used as a positive control. Positive samples from immunized wild rodents showed 0.300–1.500 OD, so that samples with a higher number were considered to be positive, and negative ones 0.050–0.200 OD. There were no cases of 0.200–0.300 OD value.

Studies aimed at finding occurrence of living *B. burgdorferi* s.l. or antibodies against it in its reservoir hosts have been made both in America and Europe, including the Czech Republic. Therefore, a certain comparison is possible, even if the method of detection was not always identical. In North America the higher positivity is in *Muridae* in contradiction to *Microtidae* [19]; in Europe the situation is more complicated. In Denmark, *Muridae* could be the most important reservoir hosts for *B. burgdorferi* s.l., although *Microtidae* also play an important role in this process [5]; in Switzerland, infected ticks attack mostly mice (*Muridae*) [12]. In Poland, high positivity was found both in *Microtidae* and *Muridae* [20]. In Russia, myomorph rodents (*Muridae*) are the main reservoir hosts for *B. burgdorferi* s.l. [16].

This study showed about 43.7% positivity on IgG antiborrelian antibodies of rodents trapped in Bažantula locality in the 2 following years. Positivity in individual years was significantly different probably because of different amounts of trapped rodents or various infestation rates in individual years. We also found that the positivity of males and females was similar. Positivity of *Muridae* was not statistically different from that of *Microtidae*. The most positive species of both familes were *A. sylvaticus* (58.8%), *A. flavicollis* (54.7%) and *C. glareolus* (44.1%); another study made in Central Bohemia reported the following results of antiborrelian antibodies detection by indirect haemagglutination assay (IHA) method: *C. glareolus* (8.7%), *A. sylvaticus* (5.9%), *A. flavicollis* (4.2%) [25]. Juřicová *et al*. [15] found antibodies to *B. burgdorferi* s.l. in *C. glareolus* (12.8%) and *A. flavicollis* (10.2%) in Bohemia and in the Břeclav region of southern Moravia by using indirect fluorescence assay (IFA) and IHA methods.

There are great variations of results in other European countries. In Poland, Pawełczyk & Siński [21] examined

3 rodent species for the presence of antiborrelian antibodies by enzyme-labelled protein G assay (ELGA). The prevalence of antibodies was as follows: 58% *C. glareolus*, 16.6% *A. flavicollis* and 10.5% *M. arvalis*.

In Denmark, Frandsen *et al.* [5] found *B. burgdorferi* antiborrelian antibodies in 100% *M. musculus*, 42.1% *A. sylvaticus*, 32.7% *M. agrestis*, 27.9% *A. flavicollis*, 17.4% *C. glareolus* and 4.9% *M. minutus* by the IFA method. The same method was used in Bulgaria where antibodies to *B. burgdorferi* s.l. were found in 55.0% *A. agrarius*, 55.3% *A. flavicollis* and 35.9% *A. sylvaticus* [1]. In France Doby *et al.* [4] found antibodies to *B. burgdorferi* s.l. in 28.0% *A. sylvaticus* and in 6.0% *C. glareolus* by IHA method.

There are quite large values of prevalence of positive rodents in our results compared to other studies made in the Czech Republic. This could be the result of using different methods, examining of rodents in various regions, or by different infection rates of rodents.

Our positivity rates correspond mostly with results from Bulgaria, but there a different method was used for detection. Data from Poland, obtained by a similar method, show lower positivity of *A. flavicollis* and *M. arvalis* but rather higher positivity of *C. glareolus*. A similar method was used and a similar region was observed; therefore the reason for their different rates could be the variation in numbers of wild-living rodents of each species.

We can conclude, that mice and voles are both reservoir hosts for *B. burgdorferi* s.l. in Studénka. Rates of antibodies could reflect a high number of infected ticks attacking wild rodents. Our results confirm the great importance of *Apodemus flavicollis*, *Apodemus sylvaticus* and *Clethrionomys glareolus* (as the most positive species) in the circulation of pathogenic borreliae in nature.

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