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SMALL MAMMALS (*INSECTIVORA, RODENTIA*) AS A POTENTIAL SOURCE OF CHLAMYDIAL INFECTION IN EAST SLOVAKIA

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Abstract: The presence of antibodies against *Chlamydophila* (*Chlamydia*) *psittaci* in small mammals (*Insectivora, Rodentia*) in the region of East Slovakia are presented. The hosts were caught in several areas of Slovakia in habitats with different levels of anthropogenic disturbance. Research was carried out during 2000–2002. The authors examined 1,947 sera coming from 4 insectivore and 10 rodent species. Each serum was examined by micromethod of complement binding reactions using antigen *Chlamydophila* (*Chlamydia*) *psittaci*. Chlamydial infections were found in 251 individuals (prevalence 12.9%) of 8 mammal species. The antichlamydial antibodies were proved at levels ranging from 1:32–1:1024. The highest prevalence of antibodies was detected in the most abundant rodent species *Apodemus microps* (14.8%), *Apodemus agrarius* (13.9%), *Apodemus flavicolis* (12.4%), *Microtus arvalis* (12%), and *Clethrionomys glareolus* (10.9%). Positive hosts were registered in all studied localities. Testing of prevalence values in the individual research years confirmed significant changes. Our results showed that small mammals probably play an important role in the circulation of chlamydiae in nature.

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INTRODUCTION

Chlamydophila (*Chlamydia*) *psittaci* is the causative agent of psittacosis and a common pathogen in avian species but less common in mammals. The first naturally occurring infection with psittacosis agents of mammals other than man was reported in 1941 by Gőnnert [4], who found that these infectious organisms caused pneumonia and inapparent infections in laboratory mice. During the past two decades, spontaneous infections of many species of domestic and wild animals with this agent were identified etiologically as the cause of diseases, disguised in a variety of clinical symptoms.

Due to both the previously found positive antibodies against *Chlamydophila* (*Chlamydia*) *psittaci* in small mammals [1, 2, 3] and the unknown role they play in spreading and maintaining of chlamydial infection, we decided to serologically examine the extensive material of

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small mammals in Slovakia. The work summarises results obtained during a 3-year study of chlamydial infection in small mammals from East Slovakia.

MATERIALS AND METHODS

Study area. Small mammals were caught in several orographical areas of East Slovakia. The important part of examined small mammals came from 6 areas of East Slovakia (Tab. 1):

- Košice Basin (400 - numerical codes of orographical units of the fauna of Slovakia).
  Localities: Košice, Rozhanovce, Kechnec, Šebastovce, Grajciar.
  Habitats: alluvial habitats, windbreaks, acacia belts, fields, forest ecotones, gardens, deer parks, 190–280 m a.s.l.
  Research period: 2000–2002.
- Eastern Slovakia Plain (820).
  Localities: Zemplínske Hradište, Trebišov, Boťany.
  Habitats: fields, windbreaks, field forests, shrubs, gardens, lowland forests in protected areas, 100-120 m a.s.l.
  Research period: 2000–2002.
- Pieniny Mountains (600).
  Localities: Červený Kláštor, Lesnica, Veľký Lipník.
  Habitats: alluvial habitats in protected areas (national park), 430-460 m a.s.l.
  Research period: 2002.
- Beskydy Mountains (760).
  Locality: Nemcovce.
  Habitats: alluvial habitats in agricultural landscape, forest ecotones, 270–280 m a.s.l.
  Research period: 2002.
- Ondava Hills (740).
  Localities: Giraltovce, Mičakovce.
  Habitats: alluvial habitats in agricultural landscape, 190–220 m a.s.l.
  Research period: 2002.
- Other areas.
  A small part of the mammals (1.4%) was caught in other areas of the East Slovakia (Valaškovce and Úhorná).

All evaluated small mammals were trapped during 2000–2002. Small mammals were caught in standard snap traps set in lines. The traps were spaced 5 m apart, and each line was exposed usually during 2 nights. Trap lines were checked regularly each the morning. A wick soaked in oil and nut mixture was used as bait. Only a small part of examined hosts was collected by live traps.

The trapped mammals were examined by standard mammalogical and ectoparasitological methods in a field laboratory.

Blood samples taken from the heart of small mammals were examined after centrifugation with micromethod of complement binding reactions - CBR [6, 7]. Genus - specific antigen *Chlamydophila* (*Chlamydia*) *psittaci* was used for CBR method (product BIOVETA Ivanovice na Hané, Czech Republic).

Statistical significance of prevalence differences among individual samples were tested using asin transformation [8].

Table 1. The host number and occurrence of antibodies against *Chlamydophila* (*Chlamydia*) *psittaci* in small mammals in different areas of the East Slovakia (2000–2002).

Host examined	Košice Basin	Eastern Slovakia Plain	Pieniny Mountains	Beskydy Mountains	Ondava Hills	Other areas	Total
Sorex araneus	19/0	21/2	2/0	-	2/0	-	44/2
Sorex minutus	-	1/0	-	-	-	-	1/0
Crocidura suaveolens	1/0	-	-	-	-	-	1/0
Neomys fodiens	-	-	-	1/0	-	-	1/0
Sciurus vulgaris	1/1	-	-	-	-	-	1/1
Clethrionomys glareolus	93/13	68/8	16/0	4/0	8/0	4/0	193/21
Microtus arvalis	14/0	240/31	-	-	4/0	-	258/31
Microtus subterraneus	4/0	-	-	-	-	1/0	5/0
Apodemus flavicollis	279/34	168/24	16/0	-	-	22/2	485/60
Apodemus sylvaticus	5/2	1/1	-	-	-	-	6/3
Apodemus microps	24/2	128/23	29/2	1/0	-	-	182/27
Apodemus agrarius	271/33	386/52	13/0	74/16	16/5	-	760/106
Mus musculus	2/0	-	-	-	-	-	2/0
Mus spicilegus	8/0	-	-	-	-	-	8/0
Total	721/85	1,013/141	76/2	80/16	30/5	27/2	1,947/251
Prevalence	11.8	13.9	2.6	20.0	16.7	7.4	12.9

* number of examined hosts/number of positive hosts

Examined material consisted of the following species: *Sorex araneus* (Linnaeus, 1758), *Sorex minutus* (Linnaeus, 1758), *Crocidura suaveolens* (Pallas, 1811), *Neomys fodiens* (Pennant, 1771), *Sciurus vulgaris* (Linnaeus, 1758), *Clethrionomys glareolus* (Schreber, 1780), *Microtus arvalis* (Pallas, 1778), *Microtus subterraneus* (de Sélys-Longchaps), *Apodemus flavicollis* (Melchior, 1834), *Apodemus sylvaticus* (Linnaeus, 1758), *Apodemus microps* (Kratochvíl et Rosický, 1952), *Apodemus agrarius* (Pallas, 1711), *Mus musculus* (Linnaeus, 1758), *Mus spicilegus* (Petényi, 1882).

RESULTS AND DISCUSSION

During the 3-years research (2000–2002), 1,947 individual small mammals belonging to 14 species in several areas of the East Slovakia were examined serologically (Tab. 1).

The antibodies against *Chlamydophila* (*Chlamydia*) *psittaci* were detected in 8 small mammal species. The antibodies were found in 1 specimen of insectivore (*S. araneus*) and 7 rodent species (Tab. 1). The values of antichlamydial antibodies prevalence in the 2 most abundant rodent families - *Arvicolidae* (N = 456 examined individuals, P = 11.4%) and *Muridae* (N = 1443, P = 13.6%) were compared. Prevalence differences were insignificant (t = 1.239, ns). On the other hand, only a small amount of insectivores (*Soricidae*) were caught (47 individuals, Tab. 1), therefore it was not possible to make any further comparison.

Table 2. Testing differences in antichlamydial antibody prevalence in study areas.

	Košice Basin	Eastern Slovakia Plain	Pieniny Mountains	Beskydy Mountains
Ondava Hills	0.752 ns	0.412 ns	2.389 p < 0.05	0.403 ns
Beskydy Mountains	1.921 ns	1.400 ns	3.755 p < 0.01	
Pieniny Mountains	3.110 p < 0.01	3.689 p < 0.01		
Eastern Slovakia Plain	1.307 ns			

The highest prevalence of antibodies was found in the most abundant rodent species - *Apodemus microps* (14.8%), *A. agrarius* (13.9%), *A. flavicollis* (12.4%), *Microtus arvalis* (12%), and *Clethrionomys glareolus* (10.9%). The tests for differences in values of prevalence among 5 dominant rodent species were statistically insignificant.

There was only a small number of examined animals of species *Sciurus vulgaris* and *Apodemus sylvaticus* available, consequently the antibody values were biased significantly (Tab. 1, 3). Examination of *Mus spicilegus* which is probably new mammal species of the East Slovakia [5] was very interesting from the epidemiological standpoint. To date, there is an absence of data about the serological examination of this species. A small number of *M. spicilegus* caught in Kechnec locality (Košice Basin) did not have any antibodies against chlamydiae (Tab. 1).

Table 3. View of host material examined on antichlamydial antibodies in the single research years.

Host examined	Research year			Total		
	2000	2001	2002	N	Positive	
					N	%
Sorex araneus	22/2*	8/0	14/0	44	2	4.5
Sorex minutus	-	1/0	-	1	0	
Crocidura suaveolens	-	-	1/0	1	0	
Neomys fodiens	-	-	1/0	1	0	
Sciurus vulgaris	-	1/1	-	1	1	(100)
Clethrionomys glareolus	41/1	65/8	87/12	193	21	10.9
Microtus arvalis	157/19	54/6	47/6	258	31	12.0
Microtus subterraneus	1/0	2/0	2/0	5	0	
Apodemus flavicollis	92/7	230/29	163/24	485	60	12.4
Apodemus sylvaticus	-	6/3	-	6	3	(50)
Apodemus microps	34/4	68/8	80/15	182	27	14.8
Apodemus agrarius	240/27	291/44	229/35	760	106	13.9
Mus musculus	2/0	-	-	2	0	
Mus spicilegus	-	-	8/0	8	0	
Total	589/60	726/99	632/92	1,947	251	12.9
Prevalence	10.2	13.6	14.6	12.9		

* number of examined hosts/number of positive hosts

Table 4. Values of antichlamydial antibodies in small mammals in all material and in host material from single areas.

	Titre of antibodies					
Species	1:32	1:64	1:128	1:256	1:512	1:1024
Sorex araneus	-	-	1	1	-	-
Sciurus vulgaris	-	1	-	-	-	-
Clethrionomys glareolus	1	13	4	2	-	1
Microtus arvalis	3	10	9	7	1	1
Apodemus flavicollis	3	25	22	4	4	2
Apodemus sylvaticus	-	3	-	-	-	-
Apodemus microps	6	11	6	2	1	1
Apodemus agrarius	16	46	27	13	3	1
Studied areas						
Košice Basin	8	45	22	5	2	3
Eastern Slovakia Plain	21	56	37	19	5	3
Pieniny Mountains	-	1	1	-	-	-
Beskydy Mountains	-	4	5	5	2	-
Ondava Hills	-	2	3	-	-	-
Other areas	-	1	1	-	-	-
Total	29	109	69	29	9	6

The examined material of mammals came from 6 orographical areas of East Slovakia. The prevalence of antichlamydial antibodies ranged from 2.6% (C - Pieniny Mountains) to 20.0% (D - Beskydy Mountains). In both areas, similar material of mammals (76 and 80 respectively) were examined. The large differences in values of prevalence among Pieniny Mountains. (C area) and other areas were statistically significantly high, while the differences among rest areas (A, B, D, E) were insignificant (Tab. 2).

During the 3 years of our research, the value prevalence slowly increased (Tab. 3). The differences in prevalence values of antichlamydial antibodies were significant only between the years 2000–2002 ($t = 2.326$, $p < 0.05$).

The antichlamydial antibodies were proved at levels ranging from 1:32–1:1024 titres in small mammals (Tab. 4). Of the total number of 251 positive samples, 55% of samples had lower titres (1:32 and 1:64). Relative frequently - 39% of samples with higher titres (1:128 and 1:256). The highest titres (1:512 and 1:1024) had only 6% of positive hosts (Tab. 4). Similarly, the titres in all 5 dominant rodent species proved a great range of antichlamydial antibodies, but without distinctive differences in values between prevalence in *Arvicolidae* rodents or *Muridae* rodents.

During of our previous research in 1991–2000, 1,004 individuals of small mammals belonging to 17 species in 7 areas of East Slovakia were examined serologically [3]. The antibodies against *Chlamydophila* (*Chlamydia*) *psittaci* were detected in 11 species of the small mammals. The values of antichlamydial antibodies prevalence in the 3 most abundant families (*Soricidae*, *Arvicolidae* and *Muridae*) were compared. The difference between prevalence values in both rodent families were statistically insignificant, similar to the present research. On the other hand, the prevalence of antichlamydial antibodies in 5 dominant species of small mammals evaluated in a previous paper [3] - *A. agrarius* (24%), *M. arvalis* (20.9%), *A. flavicollis* (20.8%), *C. glareolus* (19.7%), and *A. microps* (= *A. uralensis*, 13.8%), were higher by comparison with recent data. The comparison of prevalence antibodies among older and actual material of dominant rodent species confirmed a significant decrease in 4 species - *A. agrarius* ($t = 3.306$, $p < 0.01$), *A. flavicollis* ($t = 3.118$, $p < 0.01$), *M. arvalis* ($t = 2.126$, $p < 0.05$), *C. glareolus* ($t = 1.969$, $p < 0.05$). The difference was insignificant ($t = 0.219$, ns) only in the material of *A. microps* from East Slovakia.

Item higher prevalence of antichlamydial antibodies in both of the long-term monitored areas (Košice Basin and Eastern Slovakia Plain, respectively) previously were observed [3]. The testing of differences showed significant decreasing of values in Košice Basin ($t = 3.558$, $p < 0.01$), as well as in the East Slovakia Plain ($t = 5.581$, $p < 0.01$). The differences could probably be influenced by the larger number of examined animals (more than double) in recent research, and some changes in species by composition in the individual years. The tendency of value decreasing in recently examined material was also confirmed by the proportion of antibody titres. While the majority of the positive samples in research in 1991–2000 showed higher titres - 1:128 and 1:256 (altogether 54%

the samples) [3], in research in 2000–2002 these titres comprised only 39% of the samples. The highest titres - 1:512 and 1:1,024 in 1991–2000, comprised 20.3% of examined animals [3], and in 2000–2002, only 6%. The reasons for this phenomenon are not clear, but the presence of antichlamydial antibodies during long-term researches (1991–2002) point out the role of small mammals in spreading and maintaining chlamydial infection.

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