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Authors: Hejlíček, Karel, Literák, Ivan, and Nezval, Jiří

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TOXOPLASMOSIS IN WILD MAMMALS FROM THE CZECH REPUBLIC

Karel Hejlíček,[1] Ivan Literák,[2,4] and Jiří Nezval[3]

[1] VEDIA, Private Veterinary Laboratory, 38601 Strakonice, Czech Republic
[2] Dept. of Infectious Diseases and Veterinary Epidemiology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences, Palackého 1-3, 612 42 Brno, Czech Republic
[3] DYNTEC, Pražská 328, 411 55 Terezin, Czech Republic
[4] Author to whom correspondence should be addressed

ABSTRACT: The prevalence of *Toxoplasma gondii* was determined in wild mammals in the Czech Republic from 1981 to 1990. The biological prevalence of *T. gondii* was <1% in insectivores (n = 578), 12% in carnivores (n = 112), 1% in rodents except muskrats (*Ondatra zibethicus*) (n = 5,163), 24% in muskrats (n = 437), 5% in lagomorphs (n = 293), 0% in ruminants (n = 456), and 2% in wild boars (*Sus scrofa*) (n = 136). The seroprevalence (Sabin-Feldman dye test, titre ≥ 1:4) of *T. gondii* was 15% in ruminants (n = 421), and 15% in wild boars (n = 124). Antibodies to *T. gondii* also were found in four of 10 carnivores. Toxoplasmosis is a common infection in wild mammals from the Czech Republic, but its prevalence varies considerable according to taxonomic groups and different localities where wild mammals live.

Key words: *Toxoplasma gondii*, wild mammals, isolation, antibodies, prevalence, toxoplasmosis.

INTRODUCTION

Toxoplasmosis of wild mammals has been studied in various parts of the world (Dubey and Beattie, 1988; Smith and Frenkel, 1995). In the Czech Republic, studies on toxoplasmosis in wild mammals mainly described the results of serological examinations. Sometimes isolation tests were used for the demonstration of *Toxoplasma gondii* but the numbers of animals examined were usually small (see Discussion). The main objective of our work was to study the biological prevalence of *T. gondii* in sufficiently large number of different species of wild mammals in the Czech Republic, and to characterise the importance of individual species and/or species assemblages of wild mammals as reservoirs for *T. gondii*.

MATERIAL AND METHODS

Three populations of wild mammals in the Czech Republic were investigated. Population one (P1) was an assemblage of mammals from the Strakonice district in south Bohemia, collected in 1981–1990. The wild mammals were hunted in and around villages or close to farm buildings used for cattle, pigs, sheep or domestic fowl breeding. All surveyed sites were situated within a 15 km radius of Strakonice (49°16′N, 13°54′E). Small mammals (insectivores and rodents) were captured in January, April, July and October. Game animals were collected during hunting seasons, mostly in autumn and winter. Population two (P2) consisted of mammals from military training areas located near Karlovy Vary (50°14′N, 12°52′E), Mimoň (50°39′N, 14°44′E), Libavá (49°44′N, 17°31′E), Jince (49°47′N, 13°59′E), Boletice (48°49′N, 14°13′E), Vyškov (49°17′N, 17°00′E) and Hartmanice (49°10′N, 13°28′E). In 1987–1989, small mammals were captured in May, September and November; game species in these areas were captured also during hunting seasons. Military training areas are large areas that have been used by the armed forces for many years. Some parts of those facilities have been extensively damaged by military activities while others are well-preserved because they have been inassessible to the general public. Farming and forest exploitation was restricted to a minimum. Population three (P3) was comprised of mammals from suburban area of Brno (49°09′N, 16°40′E). Mammals were captured or hunted in 1986–1989.

For the isolation of *T. gondii* from tissues, *T. gondii*-negative outbred Swiss mice (Velaz, Praha, Czech Republic) were used. Tissue samples (mostly brain, heart, liver, spleen and skeletal muscles) pooled from each animal were homogenised in a buffered physiological saline with antibiotics: 600,000 IU of H benzyl penicillinum procainicum (Prokain Penicilin G, Biotika, Slovenská Ľupča, Slovak Republic) and 1 g of streptomycin (Streptomycin Sulphate$^{S.P.}$, V/O Medexport, former USSR) per litre of saline. Mice were then intraperitoneally injected with

1 ml of the homogenate. Five wks later, the mice were killed, bled and their cerebrum removed. Their blood serum was examined for the presence of *T. gondii* antibodies using the modified Sabin-Feldman dye test (DT) (Kouba et al., 1974). The basic serum dilution was 1:4. A compressed preparation was made of the cerebrum, which was then microscopically examined for *T. gondii* tissue cysts. Some of the wild mammals classified as game species in P2 also were tested serologically. The method for antibody detection was the same as above.

Differences in prevalence occur localities and hosts were determined by the χ^2 and Fisher exact tests using an epidemiological statical software package (Epi Info 5.01 6-1991, Public Domain Software for Epidemiology and Disease Surveillance, Centers for Disease Control, Atlanta, Georgia). Significance was inferred at $P \leq 0.05$.

RESULTS

Toxoplasma gondii was isolated from 17 species of mammals. The differences in prevalence of *T. gondii* in different species were considerable (Table 1). The species we tested were divided into seven taxonomic groups. Insectivores had a prevalence based on isolation of 0.5% (n = 578), carnivores 12% (n = 112), rodents (except muskrats, which live in water) 1% (n = 5163), muskrats 24% (n = 437), lagomorphs 5% (n = 243), artiodactyles including ruminants 0% (n = 463), and wild boars (*Sus scrofa*) 2% (n = 136). The biological prevalences of individual groups reflected different environments, such as village rural areas of P1, military training areas of P2 and a suburban area of P3 (Table 2). The biological prevalence of carnivores from the suburban area was significantly higher than that from military training areas. The highest biological prevalence of ground rodents was ascertained in the suburban environment. The prevalence based on isolation in muskrats living in water was significantly higher in the suburban environment of Brno than in the rural area.

Antibodies to *T. gondii* were detected in four of 10 carnivores examined, 15% wild boars (n = 124) and 15% of 421 ruminants (Table 3). The seroprevalence was significantly higher in carnivores than in wild boars ($P < 0.05$) and ruminants ($P < 0.05$).

DISCUSSION

Our survey of toxoplasmosis in the Czech Republic revealed that the organism is common and that a number of mammalian species are natural reservoirs of *T. gondii*. Using the complement fixation test (CFT), antibody prevalences of 5 to 20% were regularly reported in small mammals (insectivores, rodents) from the mountains, lowland rural areas and the suburban environment in the Czech Republic (Rosický et al., 1969; Kadlčík et al., 1969; Jíra and Rosický, 1983). Using the DT, the seroprevalences of 5 and 7% were detected (Havlík and Hübner, 1958; 1960; Zástěra et al., 1966). Microprecipitation in agar gel (MPA) detected antibodies in 1% of small mammals (Hübner and Uhlíková, 1970a). Pokorný et al. (1961) isolated *T. gondii* from three domestic mice (*Mus musculus*). Zástěra et al. (1966) isolated it from a hedgehog (*Erinaceus* sp.) and five Norway rats (*Rattus norvegicus*). Hübner and Uhlíková (1970b) isolated *T. gondii* from a bank vole (*Clethrionomys glareolus*). Hejlíček et al. (1981) isolated *T. gondii* from five of 15 mice (*Mus musculus* and *Apodemus sylvaticus*) trapped near animal barns on farmsteads. Rašín (1971, 1973) first isolated *T. gondii* from three of 15 muskrats; on the basis of an examination of 100 muskrats, he subsequently reported a seroprevalence of 48% and biological prevalence of 22%. Of 253 muskrats from different sites tested, Nezval and Literák (1994) reported a *T. gondii* prevalence of 47% and 9% from a site where water was heavily polluted with municipal waste and three sites with water slightly polluted with waste, respectively. Antibodies to *T. gondii* were demonstrated in 28 to 31% in European hares (*Lepus europaeus*) using the DT (Havlík and Hübner, 1958, 1960; Zástěra et al., 1966), in 3% of these hosts using the CFT (Rašín, 1970a) and in 16% of hares using MPA (Vošta et

TABLE 1. Biological prevalence of *Toxoplasma gondii* in wild mammals from the Czech Republic.

Species	Collection locality			Total	%
	Rural	Military	Suburban		
Insectivores					
Erinaceus spp.	0/3[a]	0/1	—	0/4	0
Sorex araneus	1/183	2/298	0/11	3/492	1
Sorex minutus	0/21	0/12	—	0/33	0
Neomys anomalus	0/1	—	—	0/1	0
Neomys fodiens	—	0/1	—	0/1	0
Crocidura leucodon	0/1	—	—	0/1	0
Crocidura suaveolens	0/42	0/3	—	0/45	0
Talpa europea	—	0/1	—	0/1	0
Carnivores					
Mustela erminea	—	—	0/2	0/2	0
Mustela nivalis	1/1	0/3	—	1/4	25
Putorius putorius	—	0/1	1/2	1/3	33
Martes foina	—	0/1	2/10	2/11	18
Martes martes	—	1/6	—	1/6	17
Meles meles	—	0/1	—	0/1	0
Vulpes vulpes	0/1	0/14	0/3	0/18	0
Canis familiaris (feral)	—	0/1	—	0/1	0
Felis catus (feral)	0/1	0/24	8/41	8/66	12
Rodents					
Sciurus vulgaris	0/2	0/1	—	0/3	0
Clethrionomys glareolus	2/83	1/221	—	3/304	1
Arvicola terestris	0/4	0/5	0/1	0/10	0
Ondatra zibethicus	21/155	0/1	84/281[b]	105/437	24
Microtus arvalis	8/706	4/723	2/175	14/1,604	1
Microtus agrestis	0/1	1/38	—	1/39	3
Micromys minutus	0/13	0/4	—	0/17	0
Apodemus flavicollis sylvaticus	9/915	8/1,134	0/20	17/2,069	1
Apodemus agrarius	—	7/96	—	7/96	7
Rattus norvegicus	0/38	0/44	1/2	1/84	1
Mus musculus	6/875	0/29	3/30	9/934	1
Muscardinus avellanarius	—	0/3	—	0/3	0
Lagomorphs					
Lepus europaeus	0/38	0/3	6/123	6/164	4
Oryctolagus cuniculus	0/6	—	6/73	6/79	8
Artiodactyles					
Sus scrofa	0/1	3/135	—	3/136	2
Cervus elaphus	—	0/309	—	0/309	0
Capreolus capreolus	0/4	0/110	0/3	0/117	0
Dama dama	—	0/8	—	0/8	0
Ovis musimon	—	0/29	—	0/29	0

[a] Number of positive isolations/number of animals tested.
[b] Data published in Nezval and Literák (1994).
[c] The two species *Apodemus flavicollis* and *A. sylvaticus* were not distinguished.

al., 1981). In a post-mortem examination, toxoplasmosis was diagnosed in 7 to 18% of European hares taken dead (Rašín, 1948; Pokorný, 1955; Ašmera et al., 1965). Šebek (1975) demonstrated *T. gondii* microscopically in the spleen of one of 15 European hares. Rašín (1970b) described 11 isolates of *T. gondii* from European

TABLE 2. Comparison of *Toxoplasma gondii* prevalences (%) of wild mammals from different localities in the Czech Republic according to the type of environment.

Group	Collection locality			Statistic evaluation of differences (χ^2 test)
	Rural	Military	Suburban	
Insectivores	<1	1	NE[a]	NS[b]
Carnivores	NE	2	19	$P < 0.01$
Rodents (except muskrats)	1	1	3	$P < 0.05$
Muskrats	14	NE	30	$P < 0.001$
Lagomorphs	0	NE	6	NS
Artiodactyles (except wild boars)	NE	0	NE	NT[c]
Wild boars	NE	2	NE	NT

[a] NE = not evaluated due to the small number (≤11) of animals examined.
[b] NS = not significant.
[c] NT = not tested statistically.

hares. A sample of carnivores showed the presence of antibodies (DT) in one of four and three of six red foxes (*Vulpes vulpes*), in two of 11 and eight of 23 weasels (*Mustela nivalis*) and in one Siberian polecat (*Putorius eversmanni*) (Havlík and Hübner, 1960; Zástěra et al., 1966). Pokorný et al. (1961) and Zástěra et al. (1966) isolated *T. gondii* in one and three weasels, respectively. Rašín (1971) isolated *T. gondii* in a beech marten (*Martes foina*). The DT of wild artiodactyles in the Czech Republic demonstrated antibodies in one of two and in two of three red deer (*Cervus elaphus*), and in 15 of 26 roe deer (*Capreolus capreolus*) (Havlík and Hübner, 1960; Zástěra et al., 1966). Our more comprehensive study allowed broader epidemiological and environmental conclusions about the prevalence of toxoplasmosis in wild mammals in the Czech Republic.

TABLE 3. Prevalence of *Toxoplasma gondii* antibodies in wild mammals from the Czech Republic (Sabin Feldman dye test, titre ≥ 1:4).

Species	Number tested	Number positive	%
Vulpes vulpes	4	3	25
Felis catus (feral)	6	1	17
Sus scrofa	124	19	15
Cervus elaphus	303	46	15
Capreolus capreolus	95	13	14
Dama dama	3	3	100
Ovis musimon	20	2	10

Domestic cats have been identified as the main source of toxoplasmosis in wild mammals. Their oocysts infect insectivores, rodents, lagomorphs and artiodactyles which then infect carnivores. The significantly highest prevalences in carnivores and rodents were found in suburban areas, where the density of domestic cat populations is the highest. In Brno, there was 0.9 cats/ha (Obrtel and Holišová, 1980). Alternatively, in large military training areas cats were less numerous and the probability of wild mammals coming into contact with *T. gondii* oocysts excreted in their feces was much smaller than in suburban areas. The few cats living in military training areas usually were linked to some specific locations (barns, residential houses, garbage dumps) where the *T. gondii* prevalence may be relatively high. In rural areas in the Czech Republic, the domestic cat is the only source of *T. gondii* oocysts. In the Czech Republic about 1% of cats excreted oocysts (Svobodová and Svoboda, 1986). The importance of the domestic cat in the epidemiology of toxoplasmosis in wild mammals also has been reported from Austria (Werner et al., 1973) and Norway (Kapperud, 1978).

Our results support Smith and Frenkel's (1995) theory that carnivores and omnivores are more likely to be infected with *T. gondii* then herbivores. Using results of standard isolation tests, the *T. gondii* prev-

alence in carnivores, whose diet consists mostly of small rodents, was 12%, the prevalence in omnivorous wild boars was 2%, and the herbivorous rodents (except muskrats) had a 1% prevalence. We failed to isolate *T. gondii* in the strictly herbivorous artiodactyles.

The prevalence based on isolation of *T. gondii* in insectivores was <1%. A small home range, short life-span and the diet consisting mostly of insects are limiting factors for toxoplasmosis in most insectivores. Rosický et al. (1969), and Jíra and Rosický (1983) noted that *T. gondii* frequently infected shrews (*Sorex* spp.). Our results do not support their conclusion.

The muskrat is exceptional for herbivores because of the high prevalence (24%) based on isolation of *T. gondii*. Muskrats, living permanently in the bank zone of streams, are probably infected by oocysts of *T. gondii* that survive for a long period in damp environments and reach streams via rain water runoff or sewage (Nezval and Literák, 1994). Spreading of oocysts via water was considered rather important, especially for water mammals including the coypu (*Myocastor coypus*) in Great Britain (Holmes et al., 1977), rabbits (*Oryctolagus cuniculus*) in Australia (Cox et al., 1981), American soldiers in Panama (Benenson et al., 1982) and wild birds in the Czech Republic (Literák et al., 1992).

The lagomorphs also were an exception to the concept that the prevalence of *T. gondii* is lower in herbivores. The prevalence of *T. gondii* in European hares and rabbits (*Oryctolagus cuniculus*) were 4% and 8% respectively. However, results of our study do not support the speculation (Gustafsson and Uggla, 1994) of a very low prevalence of latent (subclinical) infections in European hares.

The results of serological examinations also showed that the seroprevalence of antibodies to *T. gondii* was higher in carnivores (40%) than in omnivorous wild boars (15%) or herbivorous ruminants (15%). This also supports Smith and Frenkel's theory (1995).

Toxoplasmosis in wild mammals is not only frequent in the Czech Republic, but also in other European countries. Results indicated this in Italy (Berengo et al., 1969; Zardi et al., 1980), Poland (Dymon et al., 1971), Austria (Werner et al., 1973; Edelhofer et al., 1989), Slovakia (Čatár, 1974) and France (Doby et al., 1974).

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