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RESEARCH NOTE

PREVALENCE OF *TOXOPLASMA GONDII* IN SMALL MAMMALS FROM THE ARDENNES REGION, FRANCE

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Abstract. Serum samples from 218 small mammals trapped in forest and grassland in the Ardennes region (North-eastern France) were tested for antibodies to *Toxoplasma gondii*. Using the modified agglutination test, positive results were found in 4/92 *Apodemus* sp., 3/64 *Clethrionomys glareolus*, 0/26 *Microtus agrestis*, 0/4 *Micromys minutus*, 3/5 *Sorex* sp., 2/9 *Arvicola terrestris*, and 7/18 *Talpa europaea*. *Toxoplasma gondii* was not isolated from the heart of seropositive individuals after bioassay in mice. Seroprevalence was significantly higher in large fossorial mammals living in grassland than in small forest mammals, probably related to ecological factors.

Even if felids are the only hosts that can excrete oocysts in the environment, infection by the protozoan *Toxoplasma gondii* (Nicolle et Manceaux, 1908) is widely prevalent in numerous species of warm-blooded animals (Dubey and Beattie 1988). Ecological and behavioural factors like feeding behaviour or use of space (De Thoisy et al. 2003) as well as the differences in soil contamination can influence the level of infection of intermediate hosts. Species of small mammals living under the surface of the ground and/or feeding from the ground are thus expected to be in contact with *T. gondii* oocysts. We predicted that the prevalence of *T. gondii* should differ from one species of small mammals to another, according to differences in their behaviour (fossorial *versus* non-fossorial species) and habitat (forest *versus* grassland) leading to differences in their potential exposure to the parasite. Our aim was thus to explore the prevalence of *T. gondii* in several small mammal species, which differ in their behaviour, in forest and grassland areas where felids were present.

Small mammals were trapped between October 2005 and October 2006 in the Ardennes region (North-eastern France) in two trapping sites: the 600-hectare Bel-Val private park (49°28'11"N, 5°02'13"E) composed by 80% of forest on one side, and the grassland surrounding the Boult-aux-Bois village (49°25'58"N, 4°50'57"E) on another side. Wildcats *Felis silvestris silvestris* (Schreber) and domestic cats *Felis silvestris catus* (L.) have been frequently observed in these two sites.

A standardized trapping method was used in forest. Lines of 34 INRA live-traps were placed every three meters during three nights, and checked once a day (Spitz et al. 1974). We used 12 (in October 2005) and 11 (in October 2006) trap lines located on different parcels that represent six main habitats of the Bel-Val park, considered as representative of forest cover surface in the Ardennes: open and closed forests, plot of regeneration, and their respective edges. Two lines of traps were placed in each habitat, except for the closed forest in October 2006, where only one line was placed. We then conducted 2,346 trap-nights. Fossorial small mammals of grassland were trapped with Topcat traps (TOPCAT, GmbH, Wintersingen, Switzerland). Twenty traps were placed in galleries between two hills from one to three nights, and checked every two to four hours. We then conducted 218 trap-nights.

All animals were dissected in the laboratory. Blood was obtained by overflowing the heart in a drop of distilled water and heart washings were tested for IgG antibodies to *T. gondii* using the modified agglutination test (MAT; Dubey and Desmonts 1987). The hearts of seropositive animals were stored in aqueous sterile sodium chloride (0.9%) solution (saline) and antibiotics from four to six days at 4°C. Hearts of seropositive animals were bioassayed individually in mice. For bioassay, heart was first pounded and mixed homogenized with a 0.25% trypsin suspension (90 min, 37°C). The mixture was filtered through gauze and centrifuged, and supernatant was suspended in saline solution containing penicillin G, streptomycin and amoxicillin and inoculated intraperitoneally in two mice. Hearts of all seronegative animals were mixed in pools of 10 to 16 hearts by species. The inoculation procedure was also proceeded in pools of hearts of the seronegative animals. Serum of inoculated mice was tested with MAT at 1:25 dilution four weeks after inoculation.

A total of 218 individuals was trapped (Table 1). Most of the 191 animals trapped in forest were wood mice *Apodemus* sp. and bank voles *Clethrionomys glareolus*. A few field voles *Microtus agrestis*, shrews *Sorex* sp. and harvest mice *Micromys minutus* were also trapped in this habitat. In grassland, 9 water voles *Arvicola terrestris* and 18 moles *Talpa europaea* were trapped. *Toxoplasma gondii* was not isolated from the heart of any animal.

The low seroprevalence in small forest rodents in the present study is similar to reports by others (Kapperud 1978, Hejlíček et al. 1997). By contrast, the high seroprevalence (22–39%) we found in fossorial mammals trapped in grassland is considerably higher than the 2% seroprevalence found previously in these animals in France (Doby et al. 1974, Kia et al. 2004). The seroprevalence was higher in grassland fossorial animals than in forest small mammals living on the surface of the ground (9/27 *versus* 10/191; Fisher's exact test, $P<0.001$). Although the sample size of grassland animals trapped in this study was low, it was sufficient to compare

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Table 1. Seroprevalence of *Toxoplasma gondii* in 218 small mammals trapped in the Ardennes (France), as determined by the modified agglutination test.

Species	Positive / Tested			Prevalence (%) [95 CI]
	2005	2006	Total	
Forest				
Apodemus sp.	3/83	1/9	4/92	4 [2 ; 11]
Clethrionomys glareolus (Schreber)	1/59	2/5	3/64	5 [2 ; 13]
Micromys minutus (Pallas)	0/4	–	0/4	0 [0 ; 49]
Microtus agrestis (L.)	0/10	0/16	0/26	0 [0 ; 13]
Sorex sp.	0/1	3/4	3/5	60 [23 ; 88]
Grassland				
Arvicola terrestris (L.)	No sample	2/9	2/9	22 [6 ; 55]
Talpa europaea L.	No sample	7/18	7/18	39 [20 ; 61]

seroprevalences between grassland and forest animals, due to the considerable difference between the two seroprevalences: the power of the test comparing the two groups was of 96% (i.e. the type II error was 4% with a type I error of 5%).

Several factors may explain this difference. First, the level of soil contamination may be different according to the habitat, because of their use by cats: wildcats and feral domestic cats frequently hunt in grassland while forests are most frequently only used by wildcats (Fitzgerald and Turner 2000). Additionally, water voles and moles are fossorial species that have extensive contact with soil (and oocysts), by digging soil for building galleries or searching food. Moles also eat earthworms, which have been identified as paratenic and transport hosts of the parasite (Ruiz and Frenkel 1980, Bettiol et al. 2000). Finally, water voles and moles have a large body mass compared to forest species. Large body mass is correlated to a higher prevalence for toxoplasmosis in small mammals (Afonso et al. 2007), probably because of a longer lifespan and higher energy requirements that can explain a higher exposure to *T. gondii*, or also a difference in susceptibility.

Animal species present in forest and grassland are exposed to *T. gondii* and may be a potential source of infection if they are consumed by cats, with insectivorous and grassland species being most often infected. In particular, the water vole (*Arvicola terrestris*) may represent an important host in the transmission of the parasite because it is generally the main prey of the cats when it is abundant (Fitzgerald and Turner 2000).

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