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Prevalence of antibodies against *Neospora caninum* and *Toxoplasma gondii* in dogs and foxes in Austria

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Abstract

Sera from 1770 dogs and 94 red foxes from Austria were examined for antibodies against *Neospora caninum* using the indirect immunofluorescent antibody test (IFAT). 3.6% of the dogs were seropositive with titres ranging from 1:50 to 1:6400. Dogs from rural areas were significantly more often seropositive for *N. caninum* than those from the urban area of Vienna (5.3% versus 2.1%). There were no significant differences in sex or breed, but a slight increase in seropositivity with age was apparent, indicating postnatal infection. None of the foxes had antibodies against *N. caninum*. Additionally, sera from 242 dogs and 94 foxes were examined for antibodies against *Toxoplasma gondii* using the IFAT. Thirty-five percent foxes and 26% of the dogs were positive; 1.7% of the dogs were positive for both parasites. This is the first report of the prevalence of *N. caninum* infections in dogs and foxes in Austria.
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Keywords: *Neospora caninum*; *Toxoplasma gondii*; Dog; Fox; Indirect immunofluorescent antibody test

1. Introduction

Neospora caninum is an apicomplexa, which can infect a variety of host species (Dubey, 2003). Dogs (McAllister et al., 1998) and recently also coyotes (Gondim et al., 2004) have been described as definitive hosts, excreting oocysts, but dogs (and maybe other carnivores) are also intermediate hosts.

Serological studies of European dogs indicate prevalence rates of up to 29% (Dubey, 2003; Dubey and Lindsay, 1990; Lindsay and Dubey, 2000). Anti-*N. caninum* antibodies were also found in coyotes (Lindsay et al., 1996), dingoes (Barber et al., 1997a), gray and red foxes (Barber et al., 1997a; Buxton et al., 1997; Lindsay et al., 2001; Simpson et al., 1997) and wolves (Vitaliano et al., 2004), suggesting a role of wild canids in the epidemiology of neosporosis and the existence of a sylvatic cycle for *N. caninum*

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(Dubey et al., 1999). Oocysts are infectious for intermediate hosts (Dubey and Lindsay, 1990), mainly cattle, where infection can lead to neonatal mortality and abortion. In several hosts, the parasite can also be transmitted vertically, resulting in abortion or birth of congenitally infected progeny (Dubey and Lindsay, 1996). Consequently, the parasite can persist over several generations in cattle breeding units (Paré et al., 1996; Schares et al., 1998), serving as a reservoir for *Neospora*-infections of canids (Wouda et al., 1999).

It has been suggested that *Toxoplasma gondii* infection is common in wild animals, especially canids (Lindsay et al., 1996, 2001; Wolfe et al., 2001), due to their carnivorous feeding habits. Thus, wild canids may be included in a sylvatic cycle of these parasites, and may be important in the epidemiology of these coccidioses.

The aim of this study was to determine the prevalence of antibodies to *N. caninum* and the related parasite *T. gondii* in dogs and foxes from Austria.

2. Materials and methods

Sera from 1770 dogs were collected at various clinics or laboratories for small animals in Vienna, Lower Austria and Styria. Blood samples were obtained between 1996 and 2000 from dogs of both sexes and various ages by cephalic venopuncture, centrifuged and the collected sera stored at −20 °C until serologic testing. Wherever possible information about the origin, sex, age and breed of the tested animals were obtained. The origin was known for 814 dogs (381 urban and 433 rural). Six hundred and thirty-five female and 716 male dogs were tested, the gender of the remaining animals was unknown. The age of the dogs in different categories was not significally different. Ages were recorded for 1283 dogs, and they were 1-month to 25-year-old. Of 1368 dogs, 1071 were of pure and 297 of mixed breed.

Blood samples of 94 foxes were collected in the hunting season of 1999 in Burgenland/Austria, for an *Echinococcus multilocularis* prevalence study, sera were tested for the presence of IgG antibodies against *N. caninum* by the indirect fluorescent antibody test (IFAT) as described by Trees et al. (1993), using cell culture-derived tachyzoites of the NC-1 isolate (Dubey et al., 1988) as antigen.

In order to find cross reactions between *N. caninum* and *T. gondii*, sera from all 94 foxes and 242 of the 1770 dogs were examined for antibodies against *T. gondii* by IFAT [antigen: whole trophozoites of *T. gondii* (strain T—a laboratory strain from the Clinical Institute of Hygiene and Medical Microbiology, Department of Medical Parasitology; Medical University Vienna, propagated in vitro)] from cell culture-derived tachyzoites.

Anti-dog IgG (heavy and light chains) fluorescein-conjugated IgG-fraction from sheep (The Binding Site, Birmingham, UK; PBS dilution 1:100) was used as conjugate. All sera were screened at a dilution of 1:50 (cut-off value) in phosphate-buffered saline (PBS, pH 7.2), which was the positive threshold titre for *N. caninum* and *T. gondii*. Positive samples were then titrated to an endpoint using double dilutions. Sera from dogs naturally infected with *N. caninum* (Löschenberger et al., 2000) and *T. gondii* were used as respective positive controls.

The data were statistically analysed using the chi-square test to determine differences in prevalence between groups ($P \leq 0.05$).

3. Results

Antibodies to *N. caninum* were detected in sera of 63 (3.6%; confidence intervals (CI) 95%: 2.74–4.53%) of the 1770 dogs, but not in foxes. Eight of 381 urban dogs (2.1%; 95%CI: 0.88–4.15%) and 23 of 433 (5.3%; 95%CI: 3.38–7.89%) rural dogs were seropositive for *N. caninum*, which was a significant ($P = 0.017$) difference. *N. caninum* antibodies were found in dogs 6-month to 16-year-old. The prevalence of *N. caninum* antibodies slightly increased with age (Fig. 1). The seroprevalence in female dogs (2.8%) was not significantly different from that of male dogs (4.2%).

The highest antibody titre was 1:64,000. Most of the positive animals had titres of 1:200 and 1:400. The number of infected dogs at the respective serum dilutions tested is shown in Fig. 2. The 1368 dogs of known breed were divided into three groups according to their size (Table 1). There were no significant differences between these groups.

Sixty-three (26%, 95%CI: 20.6–32.0%) of the 242 dog sera and of 34 (35%, 95%CI: 26.5–46.7%) of the

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