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ORIGINAL ARTICLE

Neospora caninum and *Toxoplasma gondii* antibodies in red foxes (*Vulpes vulpes*) in the Czech Republic

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Abstract

Introduction and objective. *Neospora caninum* and *Toxoplasma gondii* are worldwide spread parasites, causing serious illnesses in sensitive animals; toxoplasmosis is also important zoonosis. Although neosporosis is not considered as a zoonosis, it leads to aborted births in cattle, as well as paresis and paralysis in dogs.

Objective. The aim of this study was to discover the prevalence of *N. caninum* and *T. gondii* antibodies in red foxes (*Vulpes vulpes*) in the Czech Republic.

Materials and method. Sera of 80 foxes from 8 regions of the Czech Republic were tested for antibodies to *N. caninum* and *T. gondii* by competitive enzyme linked immunosorbent assay (cELISA) and indirect ELISA. All samples were simultaneously tested by indirect fluorescent antibody test (IFAT) to detect both *N. caninum* and *T. gondii* antibodies.

Results. Antibodies to *N. caninum* were found by IFAT in 3 (3.8%) red foxes with titre 50 and in 2 (2.5%) red foxes with inhibition 42.7% and 30.2 %. Antibodies to *T. gondii* were found in all tested animals in both IFAT (titres 50 – 6400) and in ELISA (S/P ranging from 34% – 133%).

Conclusion. This is the first prevalence study of *N. caninum* and *T. gondii* antibodies in red foxes in the Czech Republic. The results obtained show that red foxes are exposed at different levels to both protozoan infections, and thus could play an important role in the transmission cycle of *N. caninum* and *T. gondii* in sylvatic cycle.

Key words

neosporosis, serology, toxoplasmosis, *Vulpes vulpes*

INTRODUCTION

Neosporosis is a serious disease of cattle and dogs worldwide [1]. Definitive hosts of *N. caninum* are dogs and other canids such as dingo, coyote and grey wolf. *T. gondii* is a coccidian parasite with cats and other felids as the definitive host, and warm-blooded animals as the intermediate hosts. Experimentally-infected foxes remained asymptomatic irrespective of *T. gondii* strain [2].

Since foxes are at the top of food pyramid, their prevalence reflects the situation in the environment. Although foxes have not been proved as the definitive host of *N. caninum* based on experimental infection [3, 4], they play important role in maintaining *N. caninum* and *T. gondii* infection in the sylvatic cycle. In Europe, there are reports of *N. caninum* and *T. gondii* seroprevalence in red foxes (*Vulpes vuples*) in the range of 0% – 3.2% and 35% – 100%, respectively. There are no reports from the Czech Republic which is why the aim of this study was to examine the sera of red foxes from the Czech Republic for *N. caninum* and *T. gondii* antibodies.

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MATERIALS AND METHOD

Between February – March 2012, blood samples were collected from 80 red foxes in 29 districts of 8 regions of the Czech Republic as the part of survey for the detection of post- vaccination anti-rabies antibodies. The blood samples were centrifuged and sera stored at -20 °C until assayed. The area of the regions in km^2 and the number of samples taken in individual regions are summarized in Table 1. During 2012, 75,296 red foxes were hunted in the Czech Republic [5].

The sera were tested for *N. caninum* and *T. gondii* antibodies by the reference method of indirect fluorescent antibody test (IFAT) using a commercially available *N. caninum* and *T. gondii* substrate slide (VMRD Inc., Pullman, USA) and anti-dog IgG conjugate (Sigma Aldrich, St. Louis, Missouri, USA). The sera were diluted in a double dilution starting at 1:50 as the basic dilution; a titre of 50 was considered positive for both parasites. *Neospora caninum* positive and negative control canine sera (both VMRD) were used. Sera from dogs simultaneously positive in both latex agglutination test (LAT) and IFAT and IFAT negative sera from puppies of a laboratory Beagle served as *T. gondii* positive and negative controls, respectively. Both positive and negative control sera were included in each slide. In the case of positive samples, green antigens of *N. caninum* or *T. gondii* were observed under an immunofluorescence microscope.

The seroprevalence of antibodies to *N. caninum* were also investigated by competitive enzyme linked immunosorbent assay (cELISA, VMRD Inc.), according to the manufacturer´s

instruction. The plate was read by spectrophotometer using wavelength 630 nm. In the case of positive samples, other dilutions of sera were prepared. Inhibition (%) was calculated by following the formula: Inhibition (%) = 100 – [(OD sample × 100) – (average of negative controls OD)], where OD=optical density. Samples with inhibition ≥ 30 % were marked as positive.

The same samples were analyzed for Toxoplasma-specific IgG antibodies by indirect ELISA. Microtiter plates coated with *T. gondii* antigen (Test-line, Brno, Czech Republic) was used. Serum samples of 200 µl were diluted in 1:40 with sample dilution buffer (Test-line) and added per well. Microtiter plates were incubated for 1 h in a wet chamber at 37 °C. The wells were washed 3 times in wash solution (Test-line) and 100 µl of horseradish peroxidase Rabbit anti-Dog IgG conjugate, Fc fragment specific (Jackson Immunoresearch, West Grove, Pennsylvania, USA) diluted in 1:16,000 in PBS was added to each well. After incubation (30 min, 37 °C) and washing, 100 µl of TMB Complete substrate (Test-line) was added to each well. The reaction was stopped after 10 min of incubation by adding 100 µl of 1 M sulphuric acid (Test-line), and absorbance was read at 450 nm using the spectrometer. Positive and negative serum samples of dogs and blank wells were added onto the plate. ELISA was validated by panel of IFAT positive and negative canine sera. IFAT strong and weak positive canine sera and IFAT negative sera from puppies of the laboratory Beagle were used. Criteria of validity: absorbance of the blank have to be < 0.200, absorbance of negative control must be 3x smaller than absorbance of positive samples, minus absorbance of negative serum. The difference between absorbance of positive serum and negative serum must be in the range of 0.500–2.000. For each sample, S/P was calculated according to the formula: S/P (%) = (absorbance of sample/absorbance of positive control – absorbance of negative control) × 100. Samples with the S/P (%) ≥ 30% were considered positive.

RESULTS

Antibodies to *N. caninum* were detected by IFAT in 3 (3.8%) of 80 foxes with titre 50. Two of these positive samples were collected from a district in South Bohemia and one from the Ústí nad Labem district. These positive samples were simultaneously positive for *T. gondii* antibodies by both methods (in IFAT with titres 3,200, 400 and 400). In cELISA, *N. caninum* antibodies were detected in 2 (2.5%) of 80 foxes with inhibition 42.7% and 30.2%. These samples were collected in the districts of South Bohemia and Ústí nad Labem and were simultaneously positive for *T. gondii* antibodies by both methods (in IFAT with titres 800 and 200, respectively).

Antibodies to *T. gondii* were detected by IFAT in all 80 foxes with titres in the range 50 – 6400 (Tab. 1). In ELISA, *T. gondii* antibodies were detected also in all 80 foxes with S/P ranging from 34% – 133%. This is the first prevalence study of *N. caninum* and *T. gondii* antibodies in foxes in the Czech Republic.

DISCUSSION

The results of this study indicate that *T. gondii* is fairly common (100%) in foxes in the Czech Republic in contrast with *N. caninum* that was detected only in 3.8% and 2.5% of foxes by IFAT and cELISA, respectively. Five samples had antibodies against both parasites and *Neospora caninum* titres were less than to the *Toxoplasma gondii* antibody titres. A cross- reactivity of coccidia might have had an impact on the results, but co-infection with both parasites cannot be ruled out either. It would be interesting to discover the risk factors, such as gender and age of animals, unfortunately, these data are not available.

In Spain, antibodies to *N. caninum* were demonstrated by cELISA and confirmed by IFAT in 3.2% of 95 red foxes [6], similar to the presented study; also similar to this study, a high *T. gondii* seroprevalence (100%) was found in 123 foxes in Belgium [7]. Antibodies to *T. gondii* were detected by immunoblot analysis in 74.5% of 204 and 84.7% of 176 red foxes in Germany [8], and by IFAT in 53.4% of 191 foxes in Italy [9]. Higher *T. gondii* prevalence compared to *N. caninum* was detected also in other countries. In Hungary, antibodies to *T. gondii* and *N. caninum* were found by direct agglutination test (DAT) in 68%, and by iscom-ELISA in 1.5% of 337 red foxes, respectively [10]. In Sweden, antibodies to *T. gondii* were found by DAT in 38% of 221 red foxes, but none of the foxes had antibodies to *N. caninum* by using iscom-ELISA [11]. In Austria, 35% of 84 red foxes tested by IFAT were positive for *T. gondii* antibodies, but negative for *N. caninum* antibodies [12]. The results of seroprevalence studies may differ according to the method, number of tested animals, location and year of sampling.

T. gondii antibodies were found in foxes in all districts of the Czech Republic, while *N. caninum* antibodies only in foxes from 2 districts (South Bohemia and Ústí nad Labem). It would be interesting to study possible risk factors, since there are differences in the size of the districts (Tab. 1), natural conditions and presence of potential sources of these parasites. In the Czech Republic, Hurkova and Modry [13] detected *T. gondii* and *N. caninum* by PCR directly in the tissues of 1.3% and 4.6% of 152 red foxes, respectively.

Foxes can be infected by drinking water contaminated with *T. gondii* or *N. caninum* oocysts or by hunting the animals (especially small mammals such as rodents and hares) that are infected with parasites. Kidawa and Kowalczyk [14] examined the stomachs of 224 foxes in Poland. They found that the most frequent prey were voles (46.9% of the diet by volume) and brown hares (10.7 % of diet by volume). In the Czech Republic, *T. gondii* and *N. caninum* antibodies were detected in 21% and 39% of 333 hares, respectively [15]; these animals could therefore serve as the source of infection in foxes.

Stray dogs and cats shed *N. caninum* and *T. gondii* oocysts, respectively, and contaminate the environment for a long time. Prey animals could be continuously infected and serve as the main source of infection for carnivorous. Therefore, it is important to continue monitoring *N. caninum* and *T. gondii* infection in foxes, small mammals and hares to assess the actual situation.

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Table 1. Distribution of antibody titres to *Toxoplasma gondii* in red foxes in 8 regions of the Czech Republic

District	Area (km^2)	Number of animals	Total positive	T. gondii – IFAT (titres)						
				50	200	400	800	1600	3200	6400
Jihočeský	10 056	15	15	1	3	5	5	-	1	-
Karlovarský	3 314	6	6	-	3	1	-	-	1	1
Královehradecký	4 758	9	9	2	1	5	-	1	-	-
Liberecký	3 163	21	21	3	3	9	3	-	2	1
Pardubický	4 519	4	4	1	-	1	-	1	1	-
Plzeňský	7 561	1	1	-	-	-	-	1	-	-
Středočeský	11 016	16	16	4	3	4	4	-	-	1
Ústecký	5 335	8	8	-	3	2	-	1	1	1
Total (%)	49 722	80	80	11 (14%)	16 (20%)	27 (34%)	12 (15%)	4 (5%)	6 (8%)	4 (5%)

IFAT – indirect fluorescent antibody test.

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