

Prevalence of *Toxoplasma gondii* Infection Diagnosed by PCR in Farmed Red Foxes, Arctic Foxes and Raccoon Dogs

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The aim of this study was to compare *Toxoplasma gondii* infection in three canid species: red fox *Vulpes vulpes*, arctic fox *Vulpes lagopus* and raccoon dog *Nyctereutes procyonoides* kept at the same farm. Anal swabs were taken from 24 adult and 10 juvenile red foxes, 12 adult arctic foxes, three adult and seven juvenile raccoon dogs. Additionally, muscle samples were taken from 10 juvenile red foxes. PCR was used to detect *T. gondii* DNA. *T. gondii* infection was not detected in any of the arctic foxes; 60% of raccoon dogs were infected; the prevalence of the parasite in material from red fox swabs was intermediate between the prevalence observed in arctic foxes and raccoon dogs. It is possible that susceptibility and immune response to the parasite differ between the three investigated canid species. *T. gondii* DNA was detected in muscle tissue of five young foxes. The results of this study suggest that *T. gondii* infection is not rare in farmed canids.

Key words: toxoplasmosis, Carnivora, fur animals, canids, *Alopex lagopus*.

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Toxoplasma gondii is an obligatory protozoan parasite of world-wide distribution. Definitive hosts of the parasite are only felids, but any mammal (and also other vertebrates) can serve as an intermediate host (TENTER *et al.* 2000). In humans *T. gondii* infection can be clinically asymptomatic, but can also evoke serious symptoms and even death, especially in pregnant women, fetuses and HIV carriers (DUBEY 1996; GUNDLACH & SĄDZIKOWSKI 2004). Similarly, in immunocompetent canids infection of the parasite is usually asymptomatic, but can also cause problems with reproduction (miscarriages, deliveries of stillborn puppies and neonate mortality) (ŚMIELEWSKA-ŁOŚ *et al.* 2001, 2003) and death of older individuals (KOPCZEWSKI *et al.* 2001, SØRENSEN *et al.* 2005). As reproduction is crucial for profitability of fur animal production, toxoplasmosis can impair income in fur animal farms. Moreover, toxoplasmosis in farmed canids is also a potential source of infection for people working with pelting

(JUOZAPAITIENE 1987). Only a few papers on *T. gondii* infection in farmed foxes (ŚMIELEWSKA-ŁOŚ *et al.* 1999; KOPCZEWSKI *et al.* 2001) and raccoon dogs (ŚMIELEWSKA-ŁOŚ *et al.* 2003) have been published. However, none of these studies compared prevalence of infection in different species kept on the same farm. Moreover, according to our knowledge only a few papers about *T. gondii* infection in raccoon dog (MURASUGI *et al.* 1996) were published at all.

The aim of this study was to compare *Toxoplasma gondii* infection in three canid species: red fox *Vulpes vulpes*, arctic fox *Vulpes lagopus* and raccoon dog *Nyctereutes procyonoides* originating from a single farm.

Material and Methods

Anal swabs from 24 adult (>one year old) red foxes, 12 adult arctic foxes, three adult raccoon

dogs and seven juvenile (<one year old) raccoon dogs were taken in October. In December anal swabs were taken from 10 juvenile red foxes as well as hind leg muscle samples from 10 juvenile red foxes.

Adult red foxes were chosen on the basis of results of a behavioural test carried out before taking swabs. The modified pencil test, i.e. the Nowicki and Przysiecki test described by GRONEK *et al.* (2008), segregates animals into four behavioural types: aggressive, curious, indifferent and fearful. Six females from every behavioural type were chosen for swab sampling.

All investigated animals were kept on a single farm in Wielkopolska, Poland. Row meat (offals from slaughterhouse) without hot processing was used for animal feeding. Rodents and cats have access to the farm area. The hygienic standard of the farm is not high.

Samples stored in microcentrifuge tubes at -70°C were thawed once before DNA extraction. DNA for amplification was isolated by the CTAB method or total genomic DNA was extracted with the QIAamp DNA Mini Kit (Qiagen). PCR analysis was carried out with primers Tg1 (5'-AAAAATGTGGGAATGAAAGAG-3') and Tg2 (5'-ACGAATCAACGGAAGCTG TAAT-3') designed and tested for specificity in the BLASTN data base (<http://www.ncbi.nlm.nih.gov/BLAST>) and predicted to be 100% specific for the B1 gene of *T. gondii* (JALAL *et al.* 2004). PCR with these primers amplified a 469-bp DNA fragment of the B1 gene with the following regime of amplification (25 l total volume): 10 min. at 95°C , followed by 35 cycles at 94°C , 52°C for 30 s and 72°C for 1 min., with a final extension step at 72°C for 7 min. Samples (15 l) of each reaction were analyzed on agarose 1.8% w/v mini-gels by electrophoresis for 25 min at 120 V, followed by staining with ethidium bromide and visualization under UV light.

Fisher exact tests were used to evaluate intra- and interspecies differences in prevalence of *T. gondii* infection.

Results

The presence of a 469 bp long DNA fragment unique for *T. gondii* was detected in some samples. Also other nonspecific bands were present on the gel.

T. gondii DNA was detected in samples from four of 24 adult red foxes (16.7%; confidence intervals (CI) 95%: 4.7-37.4%). Infection was not detected in any fearful and indifferent individuals (95% CI: 0-45.9%), but one of six aggressive (16.7%; 95%CI: 0.4-64.1%) and three of six curi-

ous animals (50%; 95% CI: 11.8-88.2%) were infected (Table 1). Differences between groups of different behaviour were statistically insignificant ($P>0.05$). *T. gondii* was detected in one of 10 (10%; 95% CI:0.2-44.5%) swabs from juvenile red foxes and five of 10 (50%; 95% CI:18.7-81.3%) muscle samples from juvenile red foxes. *T. gondii* DNA was not detected in any of 12 arctic foxes (95% CI: 0-26.5%). All three adult raccoon dogs were infected with *T. gondii* (95% CI: 29.2-100%), whereas three of seven (42.9%; 95% CI: 9.9-81.6%) juvenile individuals of this species were infected with the parasite. The difference in prevalence of infection between adult foxes of two species was insignificant ($P>0.05$), whereas interspecies differences were significant ($P<0.05$) when infection prevalence was compared in groups of three investigated canid species in October. These differences were statistically significant when only adult raccoon dogs were taken into account as well as when juvenile specimens of this species were included in the analysis.

Table 1

Prevalence of *Toxoplasma gondii* infection in adult red foxes (females) diagnosed by PCR of anal swabs

Type of behaviour	Number of infected foxes and prevalence of <i>T. gondii</i> infection
Aggressive (n=6)	1 (16.7%)
Curious (n=6)	3 (50%)
Indifferent (n=6)	0 (0%)
Fearful (n=6)	0 (0%)

Discussion

BUXTON *et al.* (1997) stated that antibodies against *Toxoplasma gondii* were present in 98% of studied wild red foxes in Belgium. Later studies carried out on wild red foxes in some European countries revealed that seroprevalences ranged from 20-68% (JAKUBEK *et al.* 2001, HAMILTON *et al.* 2005, WANHA *et al.* 2005, JAKUBEK *et al.* 2007, MURPHY *et al.* 2007).

PRESTRUD *et al.* (2005) stated that 43% of studied wild arctic foxes were seropositive to *T. gondii*.

ŚMIELEWSKA-ŁOŚ *et al.* (1999) stated that individuals with antibodies against *T. gondii* comprised 33.6% of farmed red and arctic foxes studied in Poland. Seroprevalences ranged from 0-80% in different farms.

MURASUGI *et al.* (1996) reported seroprevalence of *T. gondii* in 18.3% of raccoon dogs kept in a zoo in Japan.

However, authors using PCR as a method of parasite detection reported clearly lower prevalence. HŮRKOVÁ and MODRÝ (2006) observed *T. gondii* DNA in only 1.3% of investigated wild red foxes from the Czech Republic. MURPHY *et al.* (2007) detected antibodies against *T. gondii* in thoracic fluid of 56% of investigated wild red foxes from Ireland, but DNA of the parasite in only 3% of brains with histological lesions and pathological changes suggestive of parasite encephalitis.

The percentage of animals with *T. gondii* DNA in our study is relatively high, although size samples were small. This may be connected with the fact that all animals at the farm are fed with the same fodder, contrary to the situation of wild foxes.

We observed clear differences in percentage of infected animals of different species. None of the arctic foxes was infected whereas all adult raccoon dogs (and 60% of all studied raccoon dogs) were infected (prevalence of infection of red foxes was intermediate: higher than in arctic foxes and lower than in raccoon dogs). ŠMIELEWSKA-ŁOŚ *et al.* (1999) who studied *T. gondii* infection in red and arctic foxes compared farms, not species. Studies on ruminants provided information on the variation in prevalence of *T. gondii* infection in two species grazed on the same pasture (ESTEBAN-REDONDO & INNES 1997) and kept on one farm (GÓRECKI *et al.* 2005). Variability in prevalence, resistance and symptoms of *T. gondii* infection in different ruminant species have been described (DUBEY 1985; ESTEBAN-REDONDO & INNES 1997; INNES 1997; PITTA GONDIM *et al.* 1999). INNES (1997) reported that in other mammalian taxa susceptibility to *T. gondii* infection can be species-specific: e.g. humans are more resistant than monkeys. Hence, it is possible that the three canid species studied herein can differ in susceptibility and immune response to *T. gondii*. However, we emphasize caution in interpretation of these results due to the small number of investigated animals. Further investigations on larger samples should give more information on this topic.

Another interesting observation was that red fox females of different behavioural type differed in prevalence of infection: none of the fearful and indifferent foxes were infected, whereas three of six curious and one of six aggressive animals were infected. None of the arctic foxes was infected and all were fearful or indifferent vixens – the results of the behavioural test were not the basis for selection of animals for study. It is possible that existing differences in prevalence of *T. gondii* infection are connected with different levels of testosterone. Testosterone can influence both immunity and behaviour of animals. High concentrations of testosterone are known to have immunosuppressive

effects (ROBERTS *et al.* 2001). Results of some studies suggest that in humans of both sexes aggression (BENDERLIOGLU & NELSON 2004; BAILEY & HURD 2005) and susceptibility to *T. gondii* infection (FLEGR *et al.* 2005) can be related to exposition to high testosterone levels during prenatal life. BAKKEN (1992) stated that red fox cubs of either sex more active in an open-field tested at 30 days of age had also higher competition capacity at seven months of age. It is possible that curiosity and aggressiveness in red foxes may be traits of similar basis (testosterone). Testosterone influence on exploratory behaviour was observed for example in rats (TALAROVIČOVÁ *et al.* 2009). However, again we should be very careful in interpretation due to small number of studied foxes and the differences were statistically insignificant.

In conclusion, our results agree with the statement of ŠMIELEWSKA-ŁOŚ *et al.* (1999) that *Toxoplasma gondii* infection can be common in foxes kept on farms.

Moreover, to our best knowledge the first information of *T. gondii* infection in random farmed raccoon dogs is given here, as ŠMIELEWSKA-ŁOŚ *et al.* (2003) reported infection of the parasite not in random individuals, but in females with reproduction problems and dead neonates.

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