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## **PREVALENCE OF ANTIBODIES TO TOXOPLASMA GONDII AND *NEOSPORA CANINUM* IN WILDLIFE AND FARMED FOXES (*VULPES VULPES*)**

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### **ABSTRACT**

Serum samples collected from 105 adult farmed silver foxes and wildlife red foxes were tested for IgG antibodies to *T. gondii* and *N. caninum* by indirect fluorescent antibody test. The farmed foxes derived from 4 farms localized in south-west province of Poland. Red foxes were caught from forest area in the same province. Antibody titres to *T. gondii* equal or greater than 1:20 were found in 36.7% of 60 farm foxes and in 33.3% of 45 red foxes. Seroprevalence in farm foxes was related to the diet containing raw, non-frozen meat and was significantly higher in two farms (53.3% and 86.6%) than in the others using cooked feed (0.0% and 1.7%). Antibodies to *N. caninum* were detected in low titres in 2 (4.4%) of 45 red foxes and in 1 (1.7%) of 60 farmed silver foxes. It was the first report on *N. caninum* in canids from Poland.

**Key words:** *Toxoplasma gondii*, *Neospora caninum*, IFAT, red foxes, silver foxes.

### **INTRODUCTION**

*Toxoplasma gondii* and *Neospora caninum* are closely related obligate, intracellular protozoan parasites. Both parasites may infect a wide range of animals and have similar life cycle with the bradyzoites and tachyzoites stages [5]. Cats and some other members of a cat family are definitive hosts of *T. gondii*, excreting oocysts of

this parasite, while *Neospora* oocysts are produced in the feces of dogs [5,10]. *T. gondii* infection in immunocompetent individuals is usually asymptomatic infection. Nevertheless severe or fatal toxoplasmosis with various symptoms has been noted in humans and many species of animals and birds [7,9,14]. Furthermore the role of toxoplasmosis in immunocompromised patients and pregnant females has been generally known [6]. *N. caninum* can cause illness in several species but most frequently in cattle and dogs [8]. However this parasite has been diagnosed since 1984 and has not completely known yet [3]. *N. caninum* has particular significance as a cause of abortion in cattle, in which persistence of infection in the mother, recrudescence of the parasite during pregnancy, and the vulnerability of the placenta and fetus to invasion is important [5]. Nevertheless subclinical infection is widely spread [8]. The proportion of dogs that develop clinical versus of subclinical infection is very low, although dogs of any age can develop disease, which can be either generalized, with virtually all organs involved (including the skin) or localized. The most severe cases occur in young, congenitally infected pups, which show an initial hind limb paresis that progresses to paralysis [2].

To compare the distribution of *T. gondii* and *N. caninum* in farming and natural environment sera from farmed and wildlife foxes were examined for evidence of antibodies to both parasites.

### MATERIAL AND METHODS

Sera from 105 adult wildlife red foxes and farmed silver foxes (one species *Vulpes vulpes*) were collected over a 4-month period from November 2002 to February 2003. 45 sera derived from red foxes caught in forest area of the Lower Silesia (the province of south-west Poland) and 60 samples were taken from breeding silver foxes of 4 fur farms localized in the same province. 15 sera were collected from each farm. All tested foxes were clinically healthy. Cats were sporadically present in those farms. In 2 farms (farm A and B) foxes were fed cooked feed with poultry as a predominant component. The others (farm C and D) used raw, non-frozen slaughter offal.

Sera were examined for evidence of antibodies to *T. gondii* and *N. caninum* with a commercially available indirect fluorescent antibody test – IFAT (VMRD, Pullmann, USA). Sera in two-fold dilutions, from 1:20 were applied to individual wells of slides coated with *T. gondii* or *N. caninum* tachyzoites. After incubation at 37°C for 30 minutes, the slides were rinsed with a rinse buffer (pH 7.0) before goat anti-canine IgG antibodies labeled with fluorescein isothiocyanate (FITC) were applied. After incubation and rinsing slides were viewed under Nikon E-400 fluorescence microscope, fitted with U B2A filter cube, with a X 40 objective. In [Figures 1](#) and [2](#) positive and negative *Toxoplasma* results are shown.

**Fig. 1. Diffuse staining is considered positive *Toxoplasma gondii***

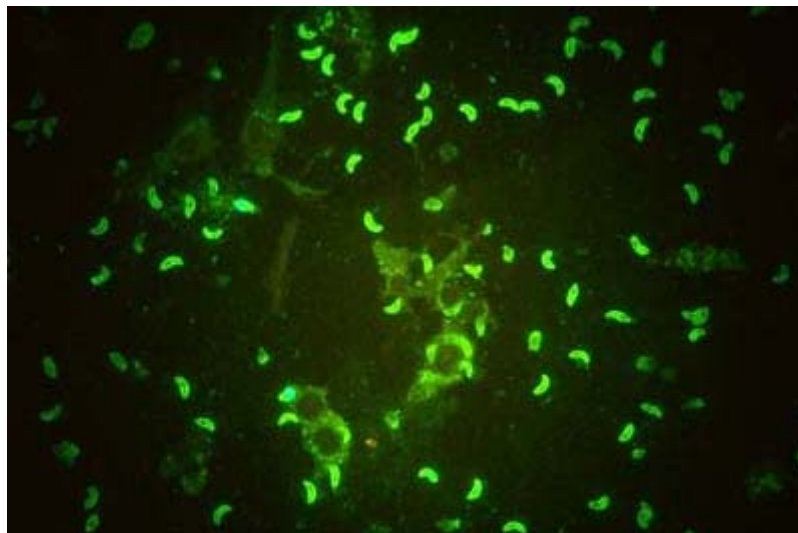
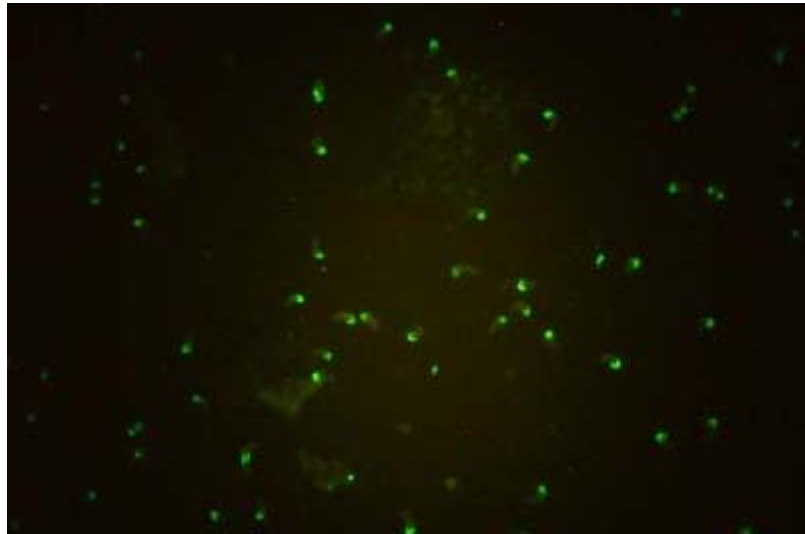


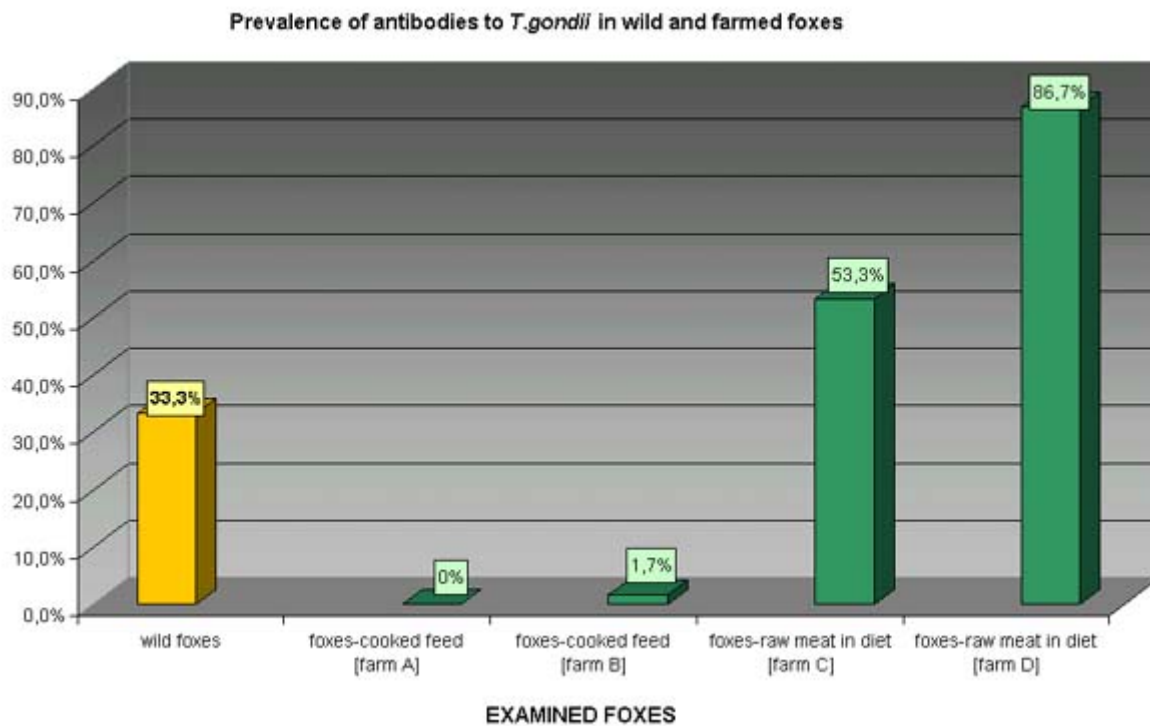
Fig. 2. Apical (polar) staining is not considered positive



Statistic analysis was performed using chi-square test.

## RESULTS

Antibodies to *T. gondii* at titres of 1:20 or more were detected in 15 (33.3%) of 45 wildlife red foxes and in 22 (36.7%) of 60 farm silver foxes. The difference was statistically non significant. All foxes from farm A were seronegative. Positive seroreagents were found in 1 (1.7%) of 15 foxes from farm B, 8 (53.3%) of 15 foxes from farm C, and 13 (86.6%) of 15 foxes from farm D. The seroprevalence in farm foxes fed raw meat (farms C and D) differed significantly from seroprevalence in all farms and in wild foxes ( $P < 0.01$ ).



The presence of *N. caninum* antibodies at titres equal or higher than 1:20 was found in 2 (4.4%) of 45 red foxes and in 1 (1.7%) of 60 silver foxes. In foxes seropositive for *Neospora*, antibodies to *T. gondii* were not detected. Antibody titres to *T. gondii* and *N. caninum* in tested fox sera are shown in [Table 1](#).

**Table 1. Reciprocal IgG IFAT titres to *T. gondii* and *N. caninum* in sera from wild red foxes and farmed silver foxes**

Reciprocal titre	Wild red foxes		Farmed silver foxes	
	<i>T. gondii</i> n (%)	<i>N. caninum</i> n (%)	<i>T. gondii</i> n (%)	<i>N. caninum</i> n (%)
< 20	30 (66.7)	43 (95.6)	38 (63.3)	59 (98.3)
20	6 (13.3)	1 (2.2)	4 (6.7)	0 (0.0)
40	3 (6.7)	1 (2.2)	5 (8.3)	1 (1.7)
80	2 (4.4)	0 (0.0)	10 (16.7)	0 (0.0)
160	1 (2.2)	0 (0.0)	0 (0.0)	0 (0.0)
320	1 (2.2)	0 (0.0)	1 (1.7)	0 (0.0)
640	2 (4.4)	0 (0.0)	1 (1.7)	0 (0.0)
1280	0 (0.0)	0 (0.0)	1 (1.7)	0 (0.0)

## DISCUSSION

Studies of fox and other wild animals sera for *T. gondii* and *N. caninum* could give an indication as to likelihood of this and other source of infection. Surveys of red fox sera in several countries showed that antibodies to *T. gondii* are very common in foxes. The proportion designated as positive depends on the method of examination. With a Sabin-Feldman due-test, Tizard and others (1976) selected titres of 1:20 in fox sera as indicating exposure to *T. gondii* found that 84% of 94 foxes in Ontario, Canada, were infected [21]. Kapperud (1978) found positive due-test titre of 1:8 or higher in 9 (31%) of 29 red foxes from Norway and Sweden [11]. That seroprevalence was relatively lower, however the same study showed also low prevalence of antibodies in cats (24% of 87) what might indicate generally lower *Toxoplasma* infection rate in those countries. In Belgium 121 (98.4%) of 123 red foxes had IFAT antibody titres to *T. gondii* at 1:128 or more [4]. In our studies only red foxes living in a forest area far-away from urban and rural areas were tested. It could be assumed that sporadic presence of cats in forest results in lower prevalence in small mammals preyed by foxes. The majority of the foxes will have become infected with *T. gondii* by ingesting small mammals and birds persistently infected with *Toxoplasma* tissue cysts [4]. In wildlife carnivores, high percentages seropositives (84% of foxes, 64% of coyotes, 65% of minks) were found. The order of infection seems to be related to the extent of the carnivorous element in the diet [21]. Nevertheless the prevalence of *Toxoplasma* infection among small rodents from Polish forests and lands has been noted low ranging from 2.9% to 6.5% [17], in contrary to domestic animals such as cattle (55.5%), sheep (up to 80%) or pigs (21.2%), in which the high level of infection could be related to the great number (52.5-70.6%) of infected house-less or semi-domestic cats [20,22,23]. Those facts may be the explanation why in our studies farmed silver foxes fed raw slaughter offal potentially containing cysts of the parasite (farm C and D), were much more frequently infected with *T. gondii* than forest red foxes. General seroprevalence in farmed silver foxes found in this study was close similar to previously reported both in silver and polar foxes from 14 Polish fur farms [18]. An outbreak of toxoplasmosis with a number of 3-4 month-old farmed foxes contracted a disease was noted lately in farmed polar foxes [12]. Congenital toxoplasmosis with abortions and cub losses was confirmed in farmed silver and polar foxes [19]. Our studies may indicate that losses caused by *T. gondii* infections are possible only in farms using raw, non-frozen feed (slaughter offal, carcass).

In opposite to *T. gondii*, much fewer reports have been focused on seroconversion to *N. caninum* in wildlife and domestic animals. IFAT antibodies to *N. caninum* were detected in Australia (9%), South America (20%) and Africa (22%) [1]. The current incidence of neosporosis in dogs is not known, and seroprevalence rates range from 0% in Kenya to 29% in Italy [13]. Sawada and others (1998) found *N. caninum* specific antibodies in 14 (7.1%) of 198 urban dogs, 15 (31.3%) of 48 farm dogs, and 17 (85%) of 20 sheep dogs [15]. Higher prevalence of *N. caninum* in farm and breeding dogs compared with urban dogs suggest that horizontal transmission of the parasite from cattle to dogs and from dog to dog may occur. Antibodies to the parasite have been found in red foxes (*Vulpes vulpes*), grey foxes (*Urocyon cinereoargenteus*), American coyotes (*Canis latrans*), and Australian dingos (*Canis dingo*) [8]. In Belgium 21 (17%) of 123 red foxes had IFAT antibody titres to *N. caninum* greater than 1:64 [4]. In contrary to dogs there was no evidence for a relationship between *N. caninum* infection in wild foxes and cattle. Simpson and others (1997) examined foxes which had been defecating on the silage in farms, where *N. caninum* infection has been diagnosed in aborted cows. Only one of 16 foxes was weakly positive (IFAT titre of 1:50) [16]. Ingestion of bovine placenta infected with *N. caninum* may also be a potential source of infection. The experimental oral infection of cats and dogs with *Neospora* tachyzoites has induced seroconversion [8]. In our studies both wild and farm foxes were seropositive for *N. caninum* sporadically, what could suggest the minimal role of foxes in epidemiology of neosporosis, nevertheless it may be the confirmation of the presence of this parasite in Poland.

IFAT antibody titres found in our studies were generally low. *T. gondii* antibody titres of 1:80 and higher were determined only in 13.3% of red foxes and 21.7% of silver foxes. *N. caninum* titres ranging 1:20 – 1:40 were found in *Toxoplasma* seronegative foxes, what eliminates the possibility of cross-reaction. This fact is in agreement with results obtained by Buxton and others (1997), where a large number of foxes positive for *T. gondii* were negative for *N. caninum* [4]. In canine sera usually IFAT titres of 1:50 or more indicate exposure to both parasites, although for *N. caninum* titres more than or equal to 1:80 or 1:200 were assumed positive [2,8]. To maximize sensitivity and minimize non-specificity it is considered that IFAT titre of 1:128 or more is likely to indicate specific seroconversion to either parasite, although it must be accepted that a proportion of foxes with titres below this may also have been exposed to infection [4].

The role of foxes in epidemiology of toxoplasmosis is not so important as in domestic animals, although it can be potential source of infection for people work in the fur farms. Higher percentage of fur farm workers reacted positively for *Toxoplasma* compared with control individuals were confirmed [24]. The infection rate in foxes could be one of the indicators of *Toxoplasma* prevalence and circulation in those environments. In our study higher seroprevalence in farm foxes eating raw meat in comparison with wild foxes was found. It could be concluded that *T. gondii* prevalence in farming animals is higher than in forest what is closely related to the more frequent presence of cats transmitting oocyst to environment. This fact may confirm that limiting of house-less cat population should be the first step in eradication of toxoplasmosis.

Circulation of *N. caninum* has been not well known. Obtaining of single seropositives in our study may suggest that further epidemiological studies of both wild and domestic animals are needed for indication of most important intermediate hosts and routes of transmission of the parasite.

## CONCLUSIONS

1. Seroprevalence to *Toxoplasma gondii* in foxes relates to the diet
2. In epidemiology of neosporosis (*Neospora caninum*) foxes have a small role

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