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Serological Survey of *Toxoplasma gondii* Infection in Free-ranging Eurasian Lynx (*Lynx lynx*) from Sweden

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ABSTRACT: To investigate the prevalence of *Toxoplasma gondii* infection in free-ranging Eurasian lynx (*Lynx lynx*) in Sweden, serosanguinous fluids and feces were collected from 207 carcasses of lynx killed or found dead from 1996 to 1998. Sera were tested for antibodies against *T. gondii* by the direct agglutination test, and 156 (75.4%) of the sera tested positive at antibody titers ≥40. Antibody prevalence was significantly lower in lynx originating from the northern parts of Sweden than in lynx from the more southern regions that are more densely populated by humans. Age-related differences also were found, with a significantly lower prevalence (55%) in juvenile (<1-yr-old) than in subadult and adult animals (82%). There was no significant difference in seroprevalence between males and females. Oocysts typical of *T. gondii* were not detected in any of the fecal samples.

Key words: *Lynx lynx*, parasite, serology, Sweden, *Toxoplasma gondii*, zoonosis.

Felids are the definitive hosts of the coccidian parasite *Toxoplasma gondii*, and a variety of warm-blooded animals, including man, are possible intermediate hosts (Dubey and Beattie, 1988; Tenter et al., 2000). Infection of either definitive or intermediate hosts usually results in mild clinical signs, and after a primary infection, most animal species will harbor the parasite in a latent stage in muscles and nervous tissues, probably for life. Such subclinically infected animals have circulating antibodies that can be demonstrated by different serological assays (Uggla and Buxton, 1990).

In Sweden, the infection is common in humans (Petersson et al., 2000), in meat-producing animals such as sheep (Lundén et al., 1992) and pigs (Lundén et al., 2002), and in game animals such as hares (*Lepus timidus* and *Lepus europaeus*; Gustafsson et al., 1988) and red foxes (*Vulpes vulpes*; Jakubek et al., 2001). Only two felids can serve as definitive hosts for *T. gondii* in Fennoscandia: domestic cats and Eurasian lynx (*Lynx lynx*; Nowell and Jackson, 1996). A seropositivity rate of 42% has been recorded in domestic cats in Sweden (Uggla et al., 1990). Lynx can be found in most parts of the country north of 60° latitude, and in 2001, the lynx population was estimated at about 1,500 animals (Swedish Hunter's Association, pers. comm.). Because the role of wild felids in the epidemiology of toxoplasmosis is potentially important in areas where they are abundant, the prevalence of *T. gondii* infection in Swedish lynx was investigated.

According to Swedish legislation, all carcasses of free-ranging lynx found dead or shot by hunters have to be submitted to the National Veterinary Institute (SVA), Uppsala, or the National Museum of Natural History, Stockholm, for postmortem examination. At SVA, all lynx are necropsied according to a standard protocol. From March 1996 to November 1998, serosanguinous fluids were collected from the heart or thoracic cavity of 207 carcasses submitted to SVA. Samples were immediately centrifuged, and the sera were stored at −20 C until analyzed. From each animal, fecal samples (3–5 g) were taken from the rectum and analyzed by a standard sodium chloride flotation method (Anonymous, 1986).

The lynx were collected from nine different counties in northern and central Sweden (Norrbotten, Västerbotten, Jämtland, Västernorrland, Gävleborg, Dalarna, Värmland, Örebro, and Västmanland).

FIGURE 1. Map of Sweden showing the origin of lynx serum samples (black dots) tested for antibodies against *T. gondii*. The lynx used in this study originated from two different areas including nine different counties: Area A (dark gray) includes mountainous regions and is sparsely populated (<5 inhabitants/km^2). Area B (light gray) consists of smaller, more densely populated counties (10–41 inhabitants/km^2) situated farther south and/or adjacent to the east coast.

This large study area was subdivided into two smaller areas, A and B (Fig. 1).

The sex and age of each animal were recorded. There were 127 males and 80 females. The age was determined in 183 animals by counting cementum annuli of a canine tooth (Matson's Laboratory, Milltown, Montana, USA). The youngest animal was approximately 5-mo-old, the oldest 13-yr-old, and mean age was 2.5-yr-old. The remaining 24 lynx were classified as <1-yr-old or ≥1-yr-old based on body size and tooth wear.

Most lynx (179/207) had been killed by hunters during the winter season (December to March). The remaining animals had been found dead or euthanized due to disease or weakness. In 17 of these, the cause of death was noninfectious (mostly traumatic), 10 deaths were due to an infectious disease (in most cases sarcoptic mange), and in one case the cause of death could not be determined.

Serum samples were tested for *T. gondii*–specific antibodies by the direct agglutination test (DAT; Dubey and Thulliez, 1989) according to the instructions from the manufacturer (BioMérieux, Charbonnière-les-Bains, France). All sera were initially screened in dilutions 1:40 and 1:4000. Positive samples were then titrated in fourfold serial dilutions from 1:80 to 1:20,480. Serum from a domestic cat experimentally infected with *T. gondii* was used as positive control, and a preinoculation serum from the same cat as negative control. Titers ≥40 were considered indicative of previous natural exposure to *T. gondii* and regarded as positive. All others will be referred to as negative.

Data were analyzed using NCSS 2001 Statistical Software (J. L. Hintze, Kaysville, Utah, USA). Statistical significance of differences in seroprevalence between Areas A and B and between animals of different age and sex were analyzed using chi-square tests.

Antibodies to *T. gondii* were detected in sera from 156 (75.4%) of the 207 lynx tested. The prevalence ranged from 50% in Västmanland to 100% in Värmland and Västernorrland (Table 1) and was significantly higher ($P<0.001$) in Area B (91%) than in Area A (68%, Fig. 1). The seroprevalences in different age groups and distribution of antibody titers by age group are shown in Table 2. Positive titers were found in 55% of the lynx <1-yr-old,

TABLE 1. Prevalence of *T. gondii* antibodies in free-ranging Eurasian lynx from different counties of Sweden.

Area	County	No. examined	No. positive	% positive
Area A	Norrbotten	13	7	54
	Västerbotten	17	13	77
	Jämtland	112	77	70
Total Area A		142	97	69
Area B	Västernorrland	18	18	100
	Gävleborg	11	10	91
	Dalarna	18	17	94
	Värmland	8	8	100
	Örebro	6	4	67
	Västmanland	4	2	50
Total Area B		65	59	91
Total A and B		207	156	75

which was significantly less ($P<0.001$) than among animals ≥1-yr-old (82%). No difference was detected among the older age classes. There was no significant difference ($P=0.669$) in antibody prevalence between males (76%) and females (74%). Oocysts typical of *T. gondii* were not detected in any of the fecal samples.

To our knowledge, the present study is the first to describe the prevalence of *T. gondii* infection in lynx from Scandinavia. The observed seroprevalence in Swedish lynx (75.4%) is very similar to the 70% reported in lynx from Finland, but higher than that reported in other *Lynx* spp. from some areas of North America (Table 3). Other studies of *Lynx* spp. (Oertley and Walls, 1980; Oksanen and Lindgren, 1995; Labelle et al., 2001; Zarnke et al., 2001) had found no statistically significant difference in seroprevalence between females and males, but a highly significant difference between juvenile (<1-yr-old) and subadult or adult (≥1-yr-old) lynx. Zarnke et al. (2001) reported a gradual increase of seroprevalence with increasing age of the animals. In the present study, the prevalence was >50% in lynx <1-yr-old. This suggests that most Swedish lynx become infected during their first year of life. There was a significant increase of prevalence from the first to the second year of life, but no differences were observed between the older age classes.

Toxoplasma gondii can be transmitted in three principal ways: transplacentally, via carnivorism, and fecal-orally. In felids, the main sources of infection are infected prey, or raw meat and organs given as feed (Dubey, 1986). The prey spectrum of Eurasian lynx in Fennoscandia includes mainly hares, roe deer, and reindeer, as

TABLE 2. Distribution of *T. gondii* antibody titers (measured by the direct agglutination test) among Eurasian lynx from Sweden by age group.

	Titers and number of lynx							
Age[a] (yr)	<40	40	80	320	1280	5120	≥20,480	Total[b](%)
<1	23	8	3	5	5	6	1	28/51 (55)
1	12	4	6	16	10	8	0	44/56 (79)
2–4	8	6	9	5	7	7	2	36/44 (82)
5–7	6	5	4	1	4	5	1	20/26 (77)
≥8	2	6	1	2	2	1	0	12/14 (86)

[a] Sixteen lynx ≥1-yr-old could not be aged exactly and are not included in this table.

[b] Number positive/Number examined.

TABLE 3. Seroprevalence of *T. gondii* antibodies in different free-ranging lynx species originating from different geographic regions.

Lynx species	Geographic origin	No. samples	Seroprevalence	Source
Eurasian lynx (*L. lynx*)	Finland	70	73%	Oksanen and Lindgren, 1995
	Sweden	207	75.4%	Present study
Canada lynx (*L. canadensis*)	Alaska, USA	255	6–21%[a]	Zarnke et al., 2001
	Québec, Canada	106	44%	Labelle et al., 2001
Bobcat (*L. rufus*)	California, USA	86	69%	Franti et al., 1976
	California, USA	103	61%	Riemann et al., 1978
	Virginia and Georgia, USA	150	18%	Oertley and Walls, 1980
	Québec, Canada	10	40%	Labelle et al., 2001
	USA	52	50% (0–64.3%)[a]	Kikuchi et al., 2004

[a] Depending on the area.

well as red foxes, rodents, and occasionally domestic cats (Pulliainen et al., 1995; Nowicki, 1997). A high incidence of acute fatal toxoplasmosis has been reported in hares from Sweden (Gustafsson et al., 1988). Diseased hares may be easy prey for lynx but are unlikely to be a major source of infection because, during this acute stage of disease, it is mainly the tachyzoite stage of the parasite that is present in the host, and tachyzoites have a low probability of survival while passing through the stomach (Dubey et al., 1998). Vikøren et al. (2004) recently reported a seroprevalence of 33.9% in roe deer in Norway, suggesting that roe deer may be an important source of infection for lynx. In Scandinavia, *T. gondii* infection also has been demonstrated in other wild ungulate species and in rodents (Kapperud, 1978; Vikøren et al., 2004). A relatively high seroprevalence (38%) was recorded in Swedish red foxes (Jakubek et al., 2001). Thus, lynx also might be infected through consumption of these prey species.

Shedding of *T. gondii* oocysts by wild felids has been reported in bobcats (Miller et al., 1972). Aramini et al. (1998) observed oocyst shedding by free-ranging cougars on Vancouver Island and considered it as a possible source of human infections. In the present study, no oocysts could be detected in lynx feces. Oertley and Walls (1980) also reported a lack of oocysts in feces of investigated bobcats. When domestic cats become infected for the first time, they excrete oocysts for only 1–2 wk during the initial phase of infection. Thereafter they become immune against reinfection and will shed only reduced numbers of oocysts on rare occasions (Dubey, 1986). This is most likely also the case in wild felids.

The higher prevalence recorded in southern than in northern Swedish counties might be linked to climatic differences (i.e., to survival of *T. gondii* oocysts) and to the density of human settlements (and therefore the presence of domestic cats), as well as differences in prey availability. Although hares and reindeer are the most common prey of lynx in northern areas, roe deer are more commonly preyed on in the south (Nowicki, 1997). In contrast to roe deer, reindeer show a very low prevalence of *T. gondii* antibodies (1%; Vikøren et al., 2004).

The primary mode of infection for herbivores is probably through ingestion of the soil-borne oocysts while foraging (Riemann et al., 1978). Sporulated oocysts from cat feces have been shown to remain viable in soil for periods exceeding a year under favorable conditions, but are inactivated over a period of time by conditions of drying, direct sunlight, or repeated freezing and thawing (Yilmaz and Hopkins, 1972; Frenkel et al., 1975). Thus,

survival of the organism may be influenced by climatic factors in the different geographic regions of Sweden. However, the oocysts appear to survive in most parts of the country for a time period sufficient to infect enough animals to perpetuate the infection among wildlife.

The results of this survey indicate that the Eurasian lynx is a common host for *T. gondii* in Sweden. Lynx might be responsible for maintaining the parasite in the wild. Together with other studies on the prevalence of *T. gondii* in Fennoscandian wildlife (Kapperud, 1978; Oksanen and Lindgren, 1995; Gustafsson and Uggla, 1994; Jakubek et al., 2001; Vikøren et al., 2004), these findings suggest that *T. gondii* is widespread in the wild. Since hunting is very common in these countries, lynx and game animals may represent a potential source of infection for humans.

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