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Short communication

Toxoplasma gondii in wild cervids and sheep in Finland: North-south gradient in seroprevalence

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ABSTRACT

A nationwide seroepidemiological study was conducted to estimate the prevalence of *Toxoplasma gondii* in selected wild and domestic ruminants in Finland. Serum samples from 1367 game cervids collected during the hunting season in 2008–2009 and 1940 sheep sera collected in 2008 were screened with a commercial direct agglutination test at a serum dilution of 1:40. *T. gondii*-specific IgG antibodies were detected in 116 (9.6%) of 1215 moose (European elk, *Alces alces*), 36 (26.7%) of 135 white-tailed deer (*Odocoileus virginianus*), 3 (17.6%) of 17 roe deer (*Capreolus capreolus*), and 477 (24.6%) of 1940 domestic sheep. Seropositive sheep were found in 74 (76.3%) of the 97 flocks examined. The odds of seropositivity in the adult moose was 2.9 times higher than the odds in calves; in white-tailed deer, the odds ratio was 3.2. The male moose had a significantly lower seroprevalence than the female, whereas the seroprevalence in the male white-tailed deer was higher than in the female; the odds ratios were 0.6 and 2.5, respectively. A clear geographical gradient in the seroprevalence was revealed in moose and sheep. The seroprevalences were lowest (1.6 and 8.6%, respectively) in the north and highest (24.6 and 36.4%, respectively) in the south-western regions, and ranged between these values in the other regions. In fact, the seroprevalence in moose from the south-west was not significantly different from the prevalence in white-tailed deer from the same area. Thus, the Finnish wild cervids and sheep are commonly exposed to *T. gondii*, especially in the southern part of the country.

1. Introduction

The protozoan parasite *Toxoplasma gondii* can infect and cause disease in a wide range of animal species, including humans (Hill and Dubey, 2002). The major risk factor for humans is consumption of undercooked meat containing infective *T. gondii* tissue cysts, accounting for up to 63% of human infections (Cook et al., 2000). Cultural differences partly explain the varying seroprevalences in humans reported from different parts of the world, but mutton and venison are generally regarded as important sources (Cook et al., 2000; Tenter et al., 2001; EFSA, 2007; Kijlstra and Jongert, 2008). Furthermore, not only consumption of meat but contact with the carcasses of wild game animals is among the discussed risk factors (Sacks et al., 1983; Ross et al., 2001; Vikoren et al., 2004).

Game cervids form a noteworthy source of meat in Finland. The annual game bag of roe deer, white-tailed deer and moose have been 3000, over 20,000 and up to 84,000, respectively; the latter forming 75% of the entire game meat yield of 10 million kilograms (Finnish Game and Fisheries Research Institute, 2009). Nevertheless, national estimates of the seroprevalence of *T. gondii* neither in these animals nor in domestic sheep have been tendered. The

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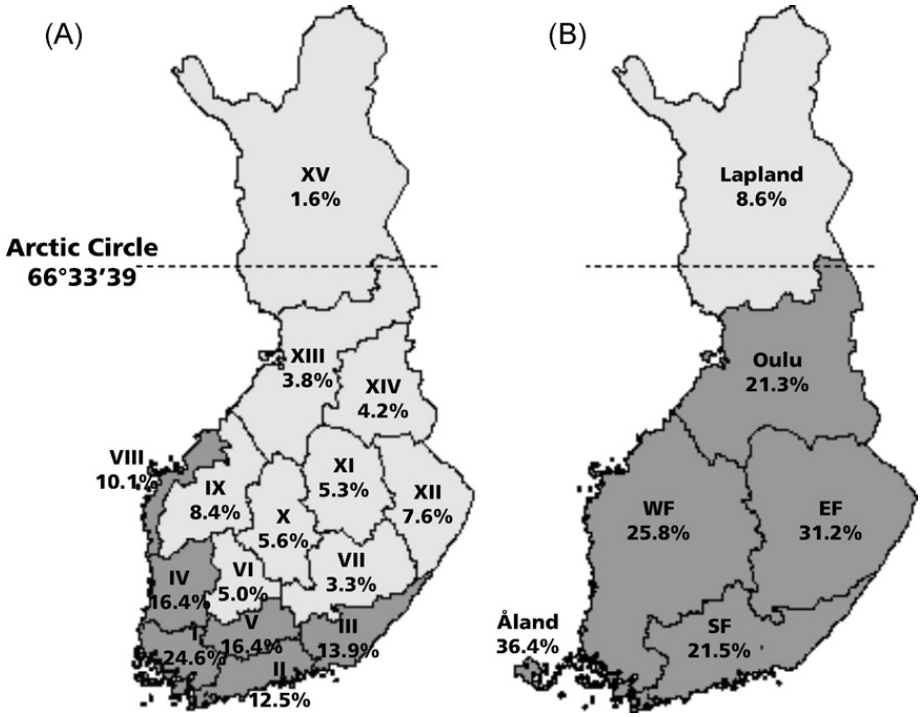

Fig. 1. Maps of Finland showing seroprevalence in moose by game management districts (I–XV) (A) and in sheep by provinces (B); the ones with a seroprevalence higher than 10% are hatched. The game management district was unknown for 27 moose. SF = Southern Finland; WF = Western Finland; EF = Eastern Finland.

main aims of this study were to estimate these seroprevalences and to investigate geographical variation within the seroprevalences in moose and sheep.

2. Materials and methods

2.1. Sample sizes

Sample sizes were calculated with the expected seroprevalences of 10–20% for the moose and 30–40% for the deer, and 10–45% for the sheep. The latter was revised with an intra-cluster correlation coefficient of 0.2 and sampling of 20 sheep per flock. Thus, the aim was to screen samples from 700 moose, 350 deer, and 1920 sheep.

2.2. Samples from wild cervids

For the 2008–2009 hunting season, altogether 2917 sampling packages were sent to the 15 game management districts (Fig. 1) to be distributed to hunters. Each package included two plastic blood sample tubes and a short questionnaire covering the game management district and the species, age group (less than 1-year-old calf, or adult), and sex of the animal. Samples arrived at the laboratory within 4 days after the kill, and sera were separated and stored at −20 °C until analyzed.

All of the animals sampled were hunted for human consumption. The samples from 1215 moose (European elk, *Alces alces*) originated from all 15 districts (Fig. 1, Table 1). The deer samples, from 135 white-tailed deer (*Odocoileus virginianus*) and 17 roe deer (*Capreolus capreolus*), were collected only from the south-western districts, where these cervids are most numerous.

2.3. Samples from sheep

Sera of 1940 domestic sheep were included in the study. These samples were taken in 2008 by local veterinarians originally for another disease surveillance program, which restricted the sampling frame to adult animals (over 1 year of age) and flocks with 20 or more ewes. The samples originated from all 6 provinces (Fig. 1), altogether from 97 separate flocks (Table 1).

2.4. Serology

The sera were screened for *T. gondii*-specific IgG antibodies with a direct agglutination test (Toxo-Screen DA; bioMérieux SA, Marcy-l'Etoile, France), according to the manufacturer's instructions. The negative and positive controls provided in the kit were included in all plates. The sera were diluted 1:40, and samples positive at this dilution were defined as seropositive.

2.5. Statistical analysis

Cross-tabulations and test statistics (Chi square or Fisher exact) were used to evaluate unconditional associations prior to the multivariable logistic regression analysis with Stata 9.2 (StataCorp, College Station, Texas, USA). The age group was a dichotomous variable (calf–adult), as was sex (female–male). The game management districts and provinces were included in the models as dummy variables.

3. Results

T. gondii-specific IgG antibodies were detected in 116 (9.6%) of 1215 moose, in 36 (26.7%) of 135 white-tailed

Table 1
Toxoplasma gondii—seroprevalence in moose, sheep, and sheep flocks from different geographical regions.

Province	GMD	Moose		Sheep		Sheep flocks	
		n, positive/total	Prevalence (%); 95% CI	*n*, positive/total	Prevalence (%); 95% CI	*n*, positive/total	Prevalence (%); 95% CI
Åland		–	–	160/440	36.4 (32.0–40.9)	19/22	86.4 (67.2–96.4)
Southern Finland		33/230	14.3 (10.3–19.3)	43/200	21.5 (16.2–27.6)	8/10	80.0 (48.1–96.5)
	II	7/56	12.5 (5.6–23.2)				
	III	14/101	13.9 (8.1–21.7)				
	V	12/73	16.4 (9.2–26.3)				
Western Finland		60/479	12.5 (9.8–15.7)	98/380	25.8 (21.6–30.4)	15/19	78.9 (56.7–92.9)
	I	28/114	24.6 (17.3–33.1)				
	IV	10/61	16.4 (8.6–27.3)				
	VI	4/80	5.0 (1.6–11.6)				
	VIII	7/69	10.1 (4.5–19.0)				
	IX	7/83	8.4 (3.8–16.0)				
	X	4/72	5.6 (1.8–12.9)				
Eastern Finland		14/264	5.3 (3.1–8.5)	81/260	31.2 (25.7–37.0)	13/13	100.0 (79.4–100)
	VII	3/91	3.3 (0.8–8.7)				
	XI	5/94	5.3 (2.0–11.4)				
	XII	6/79	7.6 (3.1–15.1)				
Oulu		6/151	4.0 (1.6–8.1)	64/300	21.3 (17.0–26.2)	10/15	66.7 (40.8–86.6)
	XIII	3/79	3.8 (1.0–10.0)				
	XIV	3/72	4.2 (1.1–10.9)				
Lapland		1/64	1.6 (0.1–7.5)	31/360	8.6 (6.0–11.9)	9/18	50.0 (27.8–72.2)
	XV	1/64	1.6 (0.1–7.5)				
Total		116/1215	9.6 (8.0–11.3)	477/1940	24.6 (22.7–26.5)	74/97	76.3 (67.1–84.0)

n = number of animals or flocks.
CI = confidence interval.
GMD = game management district; listed below the corresponding provinces; not known for 27 moose.

Table 2
T. gondii—seroprevalence in moose, white-tailed deer, and roe deer according to age group and sex.

Age group	Sex	Moose		White-tailed deer		Roe deer	
		n, positive/total	Prevalence (%); 95% CI	*n*, positive/total	Prevalence (%); 95% CI	*n*, positive/total	Prevalence (%); 95% CI
Calf		24/454	5.3 (3.5–7.6)	10/64	15.6 (8.2–26.1)	0/3	0.0 (0.0–63.2)
	Female	14/213	6.6 (3.8–10.5)	2/27	7.4 (1.3–22.4)	0/2	0.0 (0.0–77.6)
	Male	10/234	4.3 (2.2–7.5)	8/36	22.2 (10.9–37.9)	0/1	0.0 (0.0–95.0)
Adult		90/729	12.3 (10.1–14.9)	26/71	36.6 (26.1–48.3)	3/14	21.4 (5.8–48.0)
	Female	47/298	15.8 (12.0–20.3)	8/30	26.7 (13.2–44.4)	0/4	0.0 (0.0–52.7)
	Male	43/430	10.0 (7.4–13.1)	18/41	43.9 (29.4–59.3)	3/10	30.0 (8.3–62.0)
Total		116/1215	9.6 (8.0–11.3)	36/135	26.7 (19.7–34.6)	3/17	17.6 (4.7–40.9)
	Female	61/514	11.9 (9.3–14.9)	10/57	17.5 (9.3–29.1)	0/6	0.0 (0.0–39.3)
	Male	53/671	7.9 (6.0–10.1)	26/77	33.8 (23.9–44.9)	3/11	27.3 (7.5–57.8)

n = number of animals.
CI = confidence interval.
Age group unknown for 32 moose; sex unknown for 30 moose and 1 white-tailed deer.

deer, in 3 (17.6%) of 17 roe deer, and in 477 (24.6%) of 1940 domestic sheep (Tables 1 and 2). Seropositive sheep were found in 74 (76.3%) of the 97 flocks examined (Table 1).

The seroprevalence in the adult moose, 12.3%, and adult white-tailed deer, 36.6%, were significantly higher than those of the calves: 5.3% and 15.6% ($P < 0.001$ and $P < 0.05$), respectively. In addition, this difference between the age groups was significant in the subgroups of female and male moose ($P < 0.005$ and $P < 0.05$, respectively). The female moose had a significantly higher seroprevalence, 11.9%, than the male, 7.9% ($P < 0.05$), and this difference between the sexes was also significant in the subgroup of adult moose ($P < 0.05$). Conversely, the seroprevalence in the female white-tailed deer was lower, 17.5%, than the 33.8% in the male ($P < 0.05$). The three seropositive roe deer were adult bucks.

A geographical gradient in the seroprevalence was detected in moose and sheep, the species with a sampling covering the whole country (Fig. 1, Table 1). The proportion of positive samples in adult moose was low, 2.1%, in the northernmost game management district compared with 30.9% in the south-western one ($P < 0.001$), and a similar tendency was seen in calves, with seroprevalences of 0 and 15.6%, respectively. The overall seroprevalences in the other districts varied between the estimates in these two, 1.6 and 24.6% ($P < 0.001$), respectively, with higher prevalences in the south. A similar gradient was observed in sheep, with the lowest seroprevalence of 8.6% in the northern province of Lapland and the highest, 36.4%, in the south-western archipelago ($P < 0.001$). Seropositive animals were found in 50.0 and 86.4% of the flocks, respectively ($P < 0.05$).

Age group, sex, and game management district were the variables included in the final logistic regression model for *T. gondii* seropositivity in the moose (Table 3), age group and sex in the white-tailed deer, and province in the sheep. Due to the small sample size, no further analysis was performed on the roe deer data. In the moose, age group was a confounder. No interaction existed between age group and sex. Age group had a significant effect on seroprevalence in the moose and in the white-tailed deer, with odds ratios of 2.9 and 3.2, respectively. The effect of sex had odds ratios of 0.6 and 2.5, respectively. In the moose, 10 of 14 game management districts, and in the sheep, 4 of 5 provinces were affirmed as significant variables ($P < 0.05$) in the models. With the south-western one as the reference region, the odds ratios for the northernmost regions were 0.04 and 0.16, respectively.

4. Discussion

This study proves that wild game cervids and sheep, species used for human consumption, are commonly exposed to *T. gondii* in Finland. The seroprevalence in sheep, 24.6%, is higher than the ones reported from Sweden (19%), and Norway (16%, in lambs) but within the worldwide range of 3–96% (Lunden et al., 1992; Skjerve et al., 1998; Dubey, 2009). The seroprevalence in moose, 9.6%, is close to the ones reported from Alaska (1–23%), Canada (15%) and Norway (13%) (Kocan et al., 1986; Siepierski et al., 1990; Zarnke et al., 2000; Vikoren et al., 2004). The 95% confidence interval of the seroprevalence of 17.6% in roe deer encom-

Table 3
Final logistic regression model for *T. gondii*—seropositivity in 1168 moose.

	OR	95% CI	*P*-value
Age group	2.85	1.75–4.62	0.000
Sex	0.61	0.41–0.92	0.019
GMD I			
GMD II	0.47	0.18–1.16	0.102
GMD III	0.50	0.24–1.03	0.061
GMD IV	0.53	0.23–1.21	0.133
GMD V	0.62	0.29–1.34	0.227
GMD VI	0.18	0.06–0.55	0.003
GMD VII	0.10	0.03–0.36	0.000
GMD VIII	0.31	0.12–0.76	0.010
GMD IX	0.29	0.12–0.72	0.007
GMD X	0.18	0.06–0.55	0.003
GMD XI	0.19	0.07–0.51	0.001
GMD XII	0.24	0.09–0.63	0.003
GMD XIII	0.11	0.03–0.38	0.000
GMD XIV	0.12	0.03–0.40	0.001
GMD XV	0.04	0.01–0.32	0.002

P (likelihood ratio) < 0.0001.
OR = odds ratio.
CI = confidence interval.
GMD = game management district.
The most south-western district (GMD I) was used as the reference region.
Adults and males were coded as 1, calves and females as 0.

passes the higher prevalences (34 and 39%) reported from other European countries (Vikoren et al., 2004; Gamarra et al., 2008). Among the species included in the study, we found the highest overall seroprevalence in white-tailed deer: 26.7%, which is lower than the 30 and 44% reported from two locations within the United States (Lindsay et al., 1991; Vanek et al., 1996). Although this seroprevalence is markedly higher than the overall one in moose, the deer samples originated only from the south-western part of the country and the difference becomes non-significant when compared with the one in moose from the same region.

In moose, the difference between the lowest seroprevalence (1.6%) in the northernmost district and the highest (24.6%) in the south-western one was striking. Furthermore, this is not exaggerated by the age group or sex of the animals sampled from these regions. On the contrary, among the samples from the northern region, the proportion taken from adults was 74.6%; of the samples from the south-west, 60.2% were from adults. Of the samples from the north, 41.3% were from female moose, and from the south-west, 51.3%, but the only positive moose from the north was a bull.

Intriguingly, a fairly similar north-south gradient was found in the seroprevalence in sheep. Thus, it is present in a wild and a domestic ruminant, and a corresponding gradient has been seen in semi-domesticated reindeer (herded in Finland only in the north). Seroprevalence was highest in the southernmost reindeer herding districts, a finding explained by the higher degree of domestication: corral feeding during winter providing contact with cats (Oksanen et al., 1997). Geographical differences in *T. gondii* seroprevalence have also been found in several other studies (e.g. Vikoren et al., 2004; Gamarra et al., 2008), and recent findings have suggested different infection kinetics in different areas (Halos et al., 2010). Revealing this variation is important for further use of the results, especially regarding wild game: while mutton is often consumed in a different region than where it is produced, the majority of meat from game animals is consumed locally.

Finland is located between the 60th and 70th parallels of latitude, with a northern temperate climate in the southern part of the country and a subarctic climate in the north, which could partly explain the gradients. However, for the survival of sporulated oocysts the temperatures are not too extreme (Hill and Dubey, 2002; EFSA, 2007), and as herbivorous animals, moose and sheep most likely encounter *T. gondii* via feed or water contaminated with this infective material shed by felids (Skjerve et al., 1998; Tenter et al., 2001; Kijlstra and Jongert, 2008). While the contribution of the small population of wild lynx (*Lynx lynx*) is unknown, domestic cats probably form the major source of infection. As cats are more numerous near human settlements, the concentration of the human population in the southern part of Finland could explain the gradient.

The Finnish wild cervids and sheep should be considered potential sources of human *Toxoplasma* infections. Currently, *T. gondii* is not among the pathogens routinely monitored at slaughterhouses, and the meat from game animals consumed in the households of hunters is not subjected to meat inspection. It is common to freeze some of this meat for later consumption, and, as this practice is effective in killing the parasite, it is an advisable preventive measure (Kijlstra and Jongert, 2008).

Conflict of interest statement

None of the authors had any conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.vetpar.2010.04.008.

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