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## SYLVATIC *TRICHINELLA* SPP. INFECTION IN FINLAND

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**ABSTRACT:** Although human infections caused by *Trichinella* sp. have not been reported in Finland for several decades and *Trichinella* sp. infection in pork has become virtually extinct in the last decade, sylvatic *Trichinella* spp. infection is still highly prevalent in Finland. Muscle digestion of 2,483 carnivorous wild animals from 9 host species during 1999–2005 showed 617 positive animals (24.8%). Molecular identification from 328 larval isolates revealed 4 different endemic *Trichinella* species, i.e., *T. nativa*, *T. spiralis*, *T. britovi*, and *T. pseudospiralis*. Seven percent of the infected animals carried mixed infections. *Trichinella nativa* was the most common species (74%), but *T. spiralis* was identified in 12%, *T. britovi* in 6%, and *T. pseudospiralis* in 1% of the animals. Host species showed different sample prevalence and *Trichinella* species distribution. Geographical distribution also varied, with the southern part of the country having significantly higher percentages than the northern part. Infection density was dependent on both the infecting *Trichinella* species and the host species. *Trichinella spiralis* was discovered in areas with no known domestic infection cases, indicating that it can also occur in the sylvatic cycle. Raccoon dogs and red foxes are the most important reservoir animals for *T. spiralis*, as well as for the sylvatic *Trichinella* species in Finland.

*Trichinella* spp. are ubiquitous nematode parasites with a broad host spectrum. In humans, there is trichinellosis, a foodborne zoonosis affecting a massive number of people worldwide (Dupouy-Camet, 2000; Pozio, 2007). Modern taxonomic studies indicate that *Trichinella* includes 8 valid species and 4 genotypes (Pozio, Hoberg et al., 2009). The genus has also been proposed to form 2 clades, i.e., encapsulated and non-encapsulated (Pozio and Murrell, 2006). All species of *Trichinella* can infect humans (Dupouy-Camet, 2000). *Trichinella spiralis* has typically been associated with pork in a domestic (=synanthropic) cycle, while other species are more often linked with wildlife in a sylvatic cycle (Kozar and Kozar, 1965; Chadee and Dick, 1982; Kjos-Hanssen, 1984; Kapel et al., 1998; Webster et al., 1999; Murrell and Pozio, 2000). The red fox and wild boar have been identified as typical reservoir animals for *Trichinella* spp. in the European Union (EU) (Murrell and Pozio, 2000; Nöckler et al., 2006; Pozio, Rinaldi et al., 2009). Although human infection with *Trichinella* spp. has been increasing in some EU countries, such as Romania and Bulgaria (Cuperlovic et al., 2005), in many countries no autochthonous human cases have been diagnosed for decades; moreover, no meat inspection findings from industrialized pork production have been made (Anonymous, 2005a). Therefore, new EU regimens allow countries to request derogations for mandatory *Trichinella* sp. testing in “areas with negligible *Trichinella* risk” (Anonymous, 2005a). This has intensified the need for regular surveys and a deeper understanding of the epidemiology of sylvatic *Trichinella* spp. infections in Europe (Anonymous, 2005b).

In Finland, meat inspection has revealed a small to worrisome number of swine infections starting in the early 1980s and peaking in 1996; more than 200 swine were found to be infected at the

maximum point, after which the number of cases decreased until 2004, when the last infected swine was diagnosed. The reduction was probably associated with the swine industry’s modernization process (Oivanen and Oksanen, 2009). As a human disease, trichinellosis is very rare in Finland, with only 8 human infections reported since the late 1800s and the last one more than 3 decades ago (Oivanen, 2005). Nonetheless, several wildlife surveys have indicated a high prevalence in lynx (Oksanen et al., 1998; Oivanen et al., 2002), foxes, raccoon dogs, and other carnivores (Freeman, 1964; Hirvelä-Koski et al., 1985; Oivanen et al., 2002). In older studies, no molecular methods for species-specific identification of *Trichinella* spp. were available. Moreover, previous surveys have had representative sample sizes from southern parts of the country, but only a limited number of samples from the northernmost part, i.e., Finnish Lapland. In the present survey, distribution of *Trichinella* spp. in host species was analyzed, with an emphasis given to the northern part of the country.

The aims of this study were: (1) to describe the distribution of *Trichinella* spp. infection in Finnish carnivorous wild mammals; (2) to analyze how infection intensity varies among animal hosts and *Trichinella* species; and (3) to determine if, and if so, how the infection probabilities depend on the population density of animal species.

### MATERIALS AND METHODS

Finland is located between the latitudes of 60° and 70°. It is bordered by Russia in the east, Sweden in the west, and Norway in the north. Approximately half of the western border to Sweden is across the Baltic Sea. In the south lies the Gulf of Finland, across which Estonia is located. To manage game animals, Finland is divided into 15 administrative units, i.e., game management districts, or GMDs, <http://www.riista.fi> (Fig. 1). The southern part of the country has a northern temperate climate, whereas the northern part has sub-Arctic conditions. During a normal winter, the whole country is snow-covered, with the mean monthly temperature in winter (December–February 1971–2000) varying from –2 C to –14 C for the south and north, respectively (<http://www.fmi.fi/saal/>), (Fig. 2).

### Study design

This was a cross-sectional survey performed in 1999–2005 in which volunteer hunters were asked to collect and send carcasses of carnivorous animals for investigation to the National Veterinary and Food Research Institute (currently Evira, Finnish Food Safety Authority) or to the Department of Basic Veterinary Sciences (DBVS), Faculty of Veterinary

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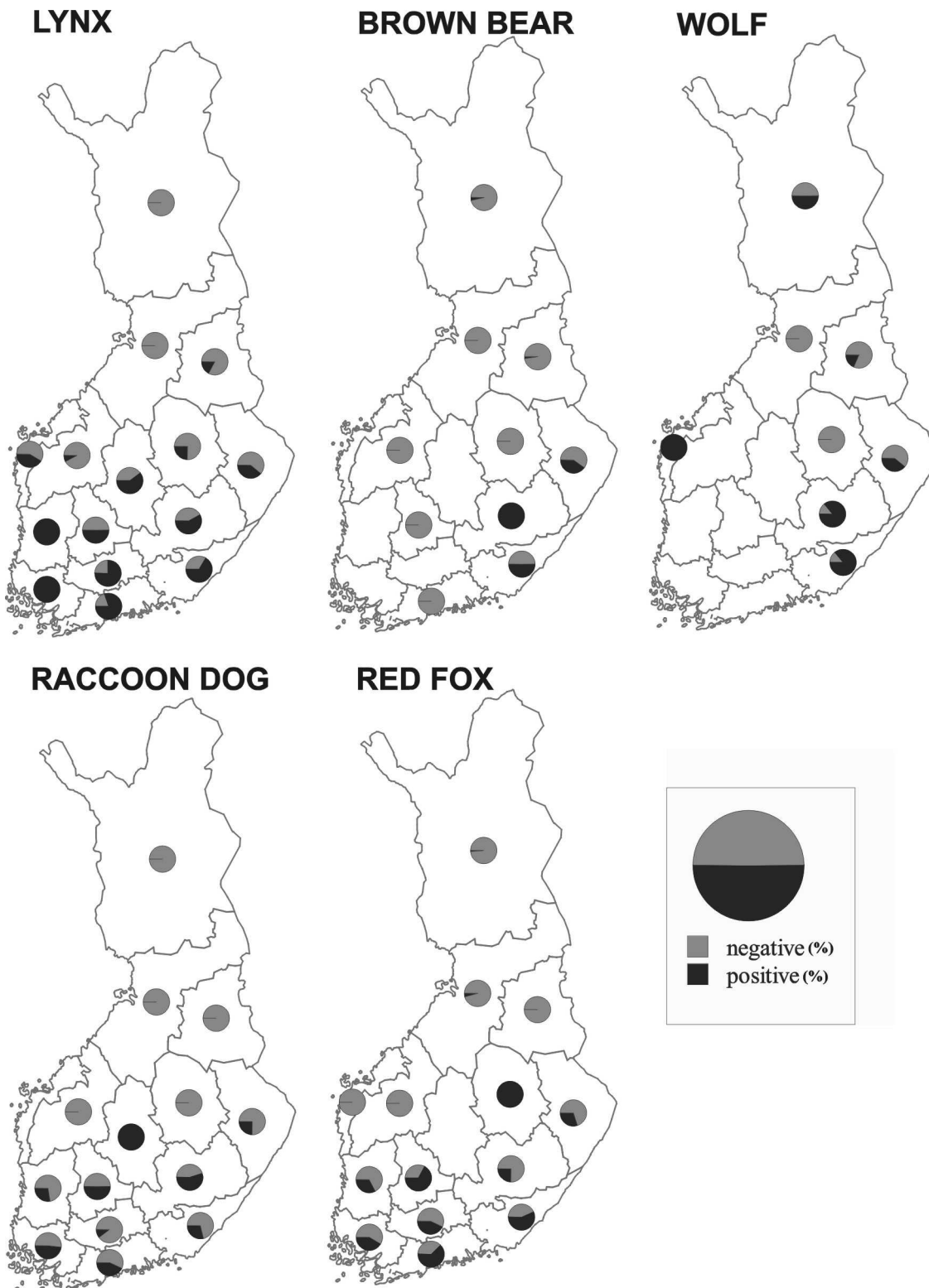


FIGURE 1. Sample prevalence of *Trichinella* spp. in 15 Finnish game management districts. All 5 host species with sample sizes of over 100 individuals are shown, i.e., lynx (*Felis lynx*), brown bear (*Ursus arctos*), wolf (*Canis lupus*), raccoon dog (*Nyctereutes procyonoides*), and red fox (*Vulpes vulpes*).

Medicine, University of Helsinki, Helsinki, Finland. Lynx samples were collected by the Finnish Game and Fisheries Research Institute. The collected samples have also been used for different purposes, such as rabies antibody monitoring and ecology studies, by both government and academic institutions.

Sampling covered all GMDs (excluding the archipelago between Sweden and Finland). Hunting site, sex, and age (juvenile/adult) of necropsied animals were recorded if the information was provided by hunters. However, since sex and age data were not recorded systematically, the information was not used in this study. The annual dynamics of

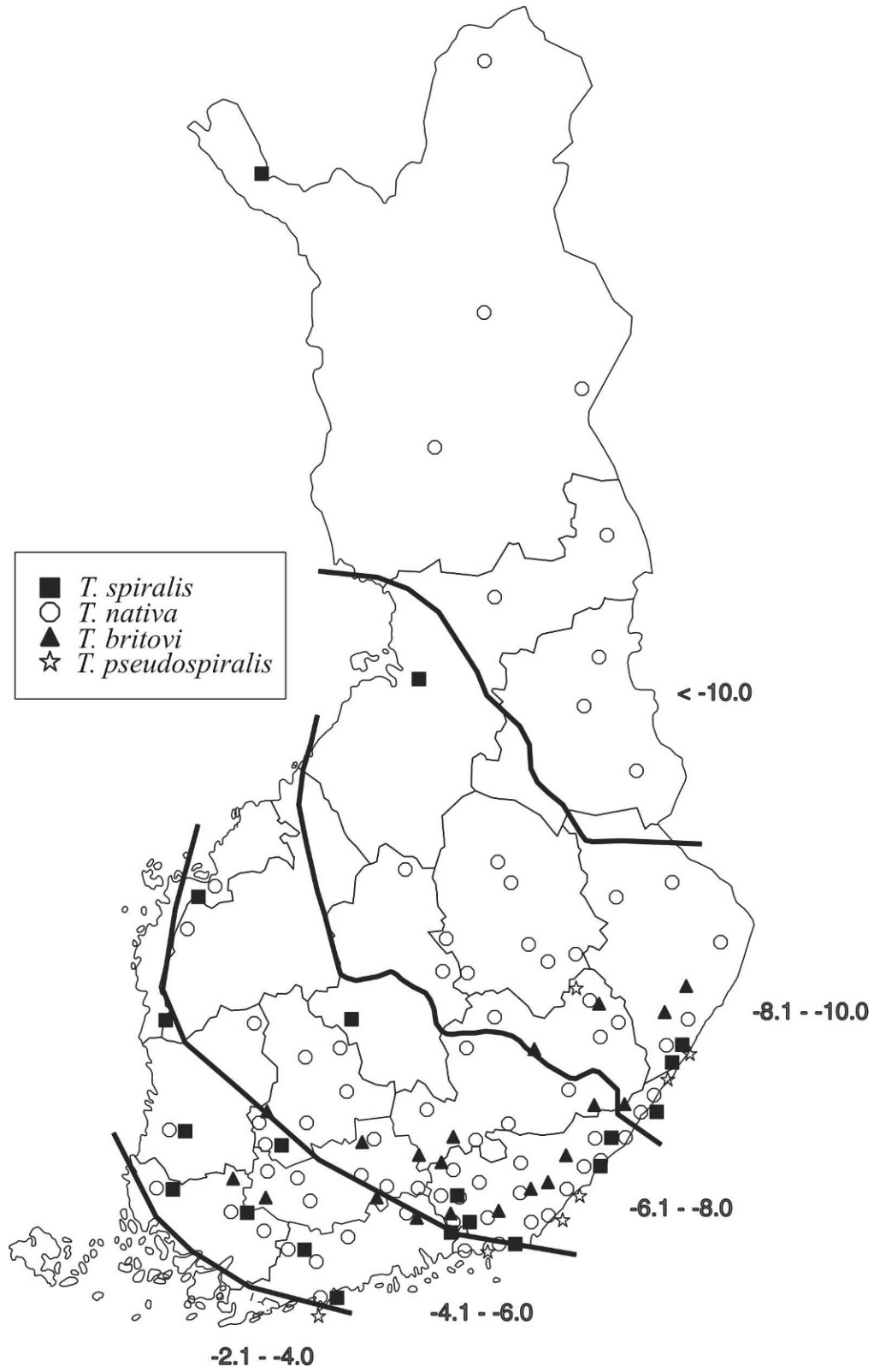


FIGURE 2. *Trichinella* spp. distribution by municipality with mean winter temperature isotherms in Finland (based on mean monthly temperature from December to February during 1971–2000). One symbol for each species in a given municipality is indicated irrespective of the number of positive samples observed.

TABLE I. Sampled carnivores in Finland during 1995–2005, discovered *Trichinella*-infected animals, sample prevalence with ranges between different game management districts (GMDs), number of observed carnivores, median infection densities with ranges, and estimated population sizes of the studied carnivores.

Host species	Positive (%; range*)	Number sampled	Median lpg† (min–max)	Population size
Red fox ( <i>Vulpes vulpes</i> )	189 (18.7; 0–62.2)	1,010	9.5 (0.02–342)	70,000–80,000 (over winter)‡ 150,000 (in autumn)‡
Raccoon dog ( <i>Nyctereutes procyonoides</i> )	186 (28.1; 0–54.8)	662	40 (0.05–760)	85,000 (over winter)‡ 230,000 (in autumn)‡
Lynx ( <i>Felis lynx</i> )	183 (45.5; 8.3–80.0)	402	1 (0.02–360)	1,200–1,250§
Brown bear ( <i>Ursus arctos</i> )	7 (5.6; 0–40.0)	125	0.2 (0.12–140)	800–850§
Wolf ( <i>Canis lupus</i> )	40 (39.2; 0–85.7)	102	3.6 (0.18–57.5)	250–260§
Pine marten ( <i>Martes martes</i> )	7 (9.3; 0–11.1)	75	6.8 (1.6–36.0)	No estimate available; (hunting bag about 20,800 in 2007)
Badger ( <i>Meles meles</i> )	4 (7.5; 0–33.3)	53	0.4 (0.16–27.5)	48,000 (over winter)‡ 50,000–70,000 (in autumn)‡
Otter ( <i>Lutra lutra</i> )	1 (3.2; 0–0)	31		2,000–2,550 (in 1995–1998)#
American mink ( <i>Mustela vison</i> )	0 (0; 0–0)	23		No estimate available; (hunting bag about 61,300 in 2007)
Total	617 (24.8; 0–85.7)	2,483		

\* Range showing difference between 15 GMDs with  $\geq 5$  individual animals of host species sampled.

† Larvae per gram of muscle.

‡ Kauhala, 2007.

§ Kojola et al., 2008.

|| Sulkava, 2006.

# RKTLL, 2008.

infection, as such, was not of interest in this study; the information regarding the date (year) of the observation has been used in linear models to normalize annual variation.

### Analyzed hosts

The carnivore sample consisted of 2,483 animals from 9 species (Table I), including 1,010 red foxes (*Vulpes vulpes*), 662 raccoon dogs (*Nyctereutes procyonoides*), 402 lynx (*Felis lynx*), 125 brown bears (*Ursus arctos*), 102 wolves (*Canis lupus*), 75 pine martens (*Martes martes*), 53 badgers (*Meles meles*), 31 otters (*Lutra lutra*), and 23 American minks (*Mustela vison*).

### Digestion

*Trichinella* spp. larvae were identified following artificial digestion of muscle tissues. Muscle digestion was performed at either Evira or DBVS. Larvae were isolated by artificial digestion of 10 g of muscle tissue from the diaphragm, mastigatory muscles, or forelimbs, which have been demonstrated to be predilection sites in carnivores (Hermansson, 1943; Kapel et al., 1994, 1995; Mikkonen et al., 2001). To evaluate the prevalence and distribution of different *Trichinella* species, larvae from positive samples ( $n = 617$ ) were collected and stored in 70% ethanol in distilled water at  $-20$  C or in 99% ethanol at 4 C until species identification.

### Molecular identification

Molecular analyses were carried out on larvae from 328 positive animals. Two samples were analyzed from each animal; the number of larvae per sample varied from 3 to 10.

Prior to molecular identification, a sufficient number of larvae were rehydrated in a decreasing ethanol series (70%, 50%, 30%, 10%, 5%, 0% in MilliQ-water [Millipore, Billerica, Massachusetts]). Two pools consisting of 3–10 larvae were analyzed from each host animal. Identification of *Trichinella* species was performed with multiplex polymerase chain reaction (multiplex-PCR) according to a previously published protocol (Zarlenga et al., 1999), with slight modifications.

### Carnivore host abundance

The annual and nationwide monitoring scheme, i.e., wildlife triangle censuses, regarding small carnivore mammals has been used in Finland since 1989 (Lindén et al., 1996). This monitoring method is based on counting animal tracks crossing the census line forming equilateral triangles, i.e., wildlife triangles, with 4-km sides, resulting in an estimate of

animal tracks on snow/10 km/24 hr. There are more than 1,700 wildlife triangles in Finland, covering the whole country in a regionally representative way.

We used wildlife triangle census data for abundance approximation when estimating the effects of carnivore population sizes, i.e., potential host abundance, on the probability of individuals being infected by *Trichinella* spp.

For abundance estimates used in statistical analyses, we calculated normalized abundance indices. We calculated average abundances from 1998 to 2005 for each species and GMD. Then, we divided the resulting species-specific values with the corresponding national average values for each species, and finally converted the values to a logarithmic ( $\text{LOG}_2+1$ ) scale (for details of this procedure, see Pellikka et al., 2005). When describing the combined abundances of multiple carnivore species, we simply summed the log-transformed species-specific abundance indices and termed the resulting values wildlife richness indices (WRIs) for hosts.

The annual amount of *Trichinella* spp. infected tissue in red foxes and raccoon dogs at the national level was calculated using minimum and maximum hunting bag estimates between 2000 and 2005 at the GMD level and roughly assuming that the hunting mortality rates for the red fox and raccoon dog populations are 40% and 50%, respectively, of the animals alive at the beginning of the autumn hunting season in each GMD (Kauhala, 2007), the average body and muscle weights of the hosts (Siivonen and Sulkava, 1999; Kojola and Heikkinen, 2006), and the observed *Trichinella* spp. sample prevalence in a given host species and GMD. Muscle proportion of the body was estimated to be 50% (White, 1953). All of these parameters were first calculated at the GMD level to account for unequal distribution of hosts. If the number of samples in the GMD was less than 5 animals of a species, data were combined with neighboring GMD(s). The lower and upper 95% confidence bounds of the observed prevalences were used in the calculations.

### Statistical methods

Associations between the following variables were calculated using Spearman's rho correlation test: percentage of *Trichinella* infection-positive raccoon dogs and red foxes; abundance for each host species, i.e., raccoon dogs, red foxes, lynx, and wolves, and together, comprising a wildlife richness index for the host species; year and x- and y-coordinates (as 100 km) of sample locations; as well as their nearest distance to the southeast border of Russia (in 100 km), since this is the direction from which raccoon dogs originally invaded Finland. Associations between being *Trichinella* spp.-infected (yes/no) and the GMDs, as well as the role of the host species, were explored using a binary logistic regression procedure. Only information regarding the red fox and raccoon dog were

TABLE II. Correlating associations (Rs) between the regional abundance of *Trichinella* hosts, regional sample prevalence, and geographic gradient in Finland based on data from 1999 to 2005. Spearman's rho correlation matrix with correlation coefficient, and 2-tailed *P* value (in parentheses). Measurements are at game management district level (15 game management districts, GMDs, except 11 for proportion of *Trichinella* infection\*).

Variables	1.	2.	3.	4.	5.
1. Proportion of <i>Trichinella</i> -infected raccoon dogs and red foxes*	1.000				
2. Abundance index for raccoon dog	0.764 ( <i>P</i> = 0.006)	1.000			
3. Abundance index for red fox	0.473 ( <i>P</i> = 0.142)	0.686 ( <i>P</i> = 0.005)	1.000		
4. WRI for hosts†	0.591 ( <i>P</i> = 0.056)	0.750 ( <i>P</i> = 0.001)	0.386 ( <i>P</i> = 0.156)	1.000	
5. North-south coordinate	-0.618 ( <i>P</i> = 0.043)	-0.750 ( <i>P</i> = 0.001)	-0.832 ( <i>P</i> = 0.000)	-0.643 ( <i>P</i> = 0.010)	1.000
6. West-east coordinate	-0.409 ( <i>P</i> = 0.212)	-0.415 ( <i>P</i> = 0.124)	-0.642 ( <i>P</i> = 0.010)	0.082 ( <i>P</i> = 0.771)	0.322 ( <i>P</i> = 0.242)

GMDs, game management districts; WRI, wildlife richness indices.

\* *Trichinella* infection proportion was not calculated for GMDs with <5 animals sampled.

† WRI consists of the abundances of the raccoon dog, red fox, lynx, and wolf in GMDs.

used in these analyses since other animals had less than 5 observations in some categories. In Finland, the raccoon dog has been recognized in earlier studies as an important reservoir and potential vector animal (Oksanen et al., 1998; Oivanen et al., 2002) and was, therefore, the animal of main interest.

Logistic regression analyses were used to verify whether any *Trichinella* species promoted certain host animal species in single parasite species infection; only the fox, raccoon dog, and lynx were used in these analyses.

The role of different variables in the infection density of hosts was explored by using multivariable linear regression analysis (the GLM univariate procedure with Tukey's HSD post-hoc test for homogeneous subsets). The dependent variable for infection density was the e-base logarithm of number of larvae per gram of muscle. Independent variables were animal species, *Trichinella* sp., x- and y-coordinates (as 100 km), and year. Only information regarding the raccoon dog, red fox, wolf, and lynx with a *Trichinella* specification (in single or mixed infections) was used, since they had considerable numbers of observations for *Trichinella* species. In a single host species, 1-way analysis of variance (ANOVA) with Games-Howell / Dunnett T3 post-hoc tests were used to compare parasite burden (larvae per gram, lpg) of different *Trichinella* species.

All statistical analyses were performed using the analytical software package SPSS 16.0 for Windows (SPSS Inc., Chicago, Illinois). For cross-tabulated data, Pearson chi-square tests were used, with Monte Carlo estimation if cell counts were low. The 95% confidence intervals for binomial distributions were calculated with Excel according to Casella and Berger (1990). Desktop mapping software package MapInfo Professional 8.5 (MapInfo Corporation, Troy, New York) was used in mapping the spatial distribution of observations.

## RESULTS

Of the 2,483 animals analyzed, *Trichinella* species were found in 617. Different host species showed varying sample prevalences (range 0–46%; Table I) Almost half (46%) of the lynx (Table I) harbored *Trichinella* spp., followed by wolves (39%), raccoon dogs (28%), and red foxes (19%). Prevalences of less than 10% were detected in pine martens, badgers, bears, and otters. No larvae were detected in American mink samples.

It is noteworthy that, in a given animal species, prevalence varied markedly between GMDs, e.g., the range for red fox was 0–62% and the range for lynx 8–80% (Table I). The overall *Trichinella* spp. prevalence from all sampled host species was also unequally distributed (Wald's *P* < 0.001), varying from 2.6% in northern Finland (Lapland) to 67% in the middle of the country. However, only 6 of the sampled animals were from the district of the highest prevalence. The 3 northernmost GMDs (Fig. 1) had the lowest sample prevalence (a total of only 6% for all *Trichinella* spp. infection cases, but 37% of all studied samples). Sample prevalence of all individual host species in the GMD showed a

clear tendency to be higher in the south (Fig. 1). Moreover, the prevalence showed variation within each GMD also in common host species, such as the raccoon dog (data not shown), indicating patchy distribution at the local level.

Variable autocorrelation caused fundamental obstacles when analyzing geographical and host species-specific differences in prevalence by multivariable logistic regression, i.e., most of the independent variables intended for exploring the effect showed high correlation (Table II). The most abundant and ubiquitous carnivores in the country are red foxes and raccoon dogs; to simplify the correlation matrix, these 2 species were combined. The combined *Trichinella* spp. prevalence in red foxes and raccoon dogs was positively correlated with the abundance of raccoon dogs and negatively correlated with the north-south coordinate. This indicates that the prevalence of positive raccoon dogs and red foxes decreases toward the north. In addition, the prevalence of *Trichinella* spp. infection was high when there were high numbers of raccoon dogs in the area. Red foxes, raccoon dogs, and the WRI for hosts, i.e., the abundance of raccoon dogs, red foxes, lynx, and wolves, also decreased toward the north (Table II).

*Trichinella* species were successfully identified in 303 animals by multiplex PCR; for 25 animals, amplification did not yield a specific reaction (7.6% of all 328 animals analyzed). Four species were identified, i.e., *T. spiralis*, *T. nativa*, *T. britovi*, and *T. pseudospiralis*. Single *Trichinella* species were found in 281 (93%) of the host animals successfully analyzed, with mixed infections in 22 (7%). *Trichinella nativa* was the most common single species (80.1%), followed by *T. spiralis* (12.8%), *T. britovi* (6.0%), and *T. pseudospiralis* (1.1%), the last of which was found as a single infection in only 3 animals, and in a mixed infection in 4 more individuals. From mixed infections, more than 2 different species were never found, but all possible 2 species combinations of the 4 species were observed. The most common combination was *T. nativa* with *T. britovi* (9/22), followed by *T. nativa* with *T. spiralis* (8/22), and *T. nativa* with *T. pseudospiralis* (2/22). Other possible combinations (*T. spiralis* with *T. britovi* or *T. pseudospiralis* and *T. britovi* with *T. pseudospiralis*) were seen only once.

All 4 *Trichinella* species were present in the southern part of the country (Fig. 2); in the northern part, only *T. nativa* and *T. spiralis* were found. Moreover, all 4 species were sympatric in areas where the mean winter temperature is from -2 C to -10 C (monthly average from December to February during 1971–2000; <http://www.fmi.fi/saal/>).

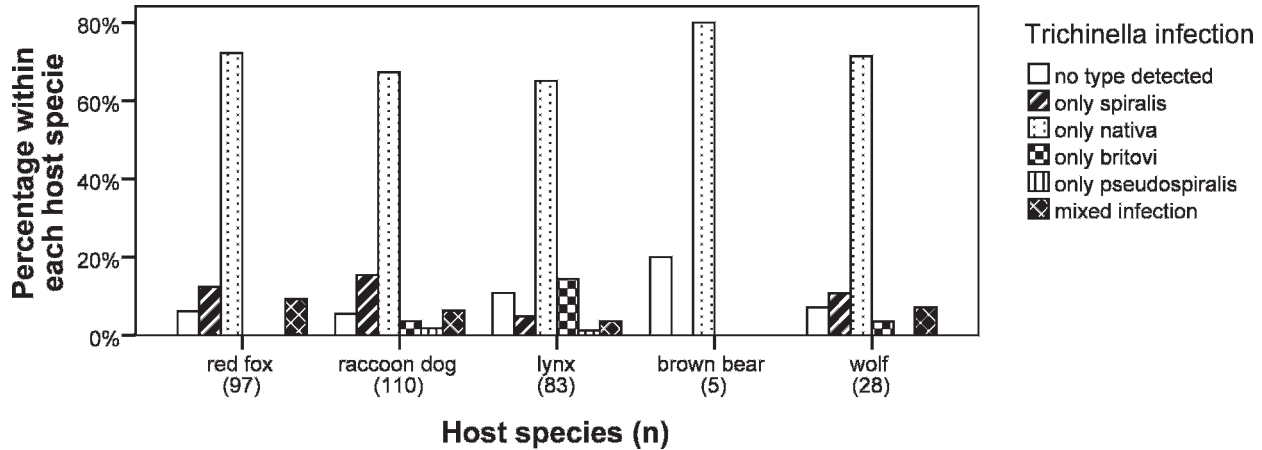


FIGURE 3. Relative distribution of the outcome of *Trichinella*-specific multiplex-PCR by host species with 5 or more larval isolates (n), red fox (*Vulpes vulpes*), raccoon dog (*Nyctereutes procyonoides*), lynx (*Felis lynx*), brown bear (*Ursus arctos*), and wolf (*Canis lupus*).

The *Trichinella* species were not equally distributed in the different host species (Pearson chi-square  $P < 0.05$ ). All 4 *Trichinella* species were found as single or mixed infections in the lynx, red foxes, and raccoon dogs (Fig. 3). In all host species, the most common *Trichinella* species was *T. nativa*, which was found alone or in mixed infection in 80.5% of the 303 positive samples (Fig. 3). Of the 5 *Trichinella*-positive bears, 4 harbored *T. nativa* alone (1 could not be typed), as did the only positive otter in this material. *Trichinella spiralis* was recovered in single or mixed infections from 46 sylvatic animals of 303 samples (15%); the host species involved were the raccoon dog, red fox, lynx, and wolf (Fig. 3). *Trichinella pseudospiralis* was found in a single infection only 3 times, twice in the raccoon dog and once in the lynx, but in mixed infections twice in the lynx, once in a red fox, and once in a raccoon dog. Of all 28 animals with *T. britovi*, 46% were lynxes, 21% were raccoon dogs, and 21% were red foxes. Interestingly, lynxes were relatively more often infected with *T. britovi* than other species (Fig. 3; Wald's  $P < 0.05$ ), and when compared with the raccoon dog, the latter had 3 times higher odds of being infected with *T. spiralis*.

The parasite burden was unequally distributed. Different hosts showed variations in infection density, and different *Trichinella* species also had different burdens (Fig. 4). One raccoon dog had the highest burden, 760 lpg, in a mixed infection of *T. nativa* with *T. britovi*. Only 3 cases with *T. pseudospiralis* alone were observed, but it also exhibited the highest single species burden (median 488 lpg; 569 lpg in 2 raccoon dogs and 15 lpg in 1 lynx). The burden was second highest in mixed infections (median 19 lpg), followed by single infections of *T. spiralis*, *T. nativa*, and *T. britovi* (medians 9.75, 8.50, and 3.00 lpg, respectively). Linear regression analysis showed that interaction of host species and *Trichinella* species has a significant ( $P = 0.013$ ) effect on infection density (lpg) when the red fox, raccoon dog, or wolf is used as a host in the model. To balance variation of sampling host species at different times, year was used as a covariate in the model. The interaction between animal species and *Trichinella* species is reflected in, for instance, *T. spiralis* having a higher larval burden in the raccoon dog than in other animals. However, in the raccoon dog, infection intensities did not differ significantly between *Trichinella* species ( $F$ -test,  $P > 0.05$ ). In lynxes, *T. spiralis*

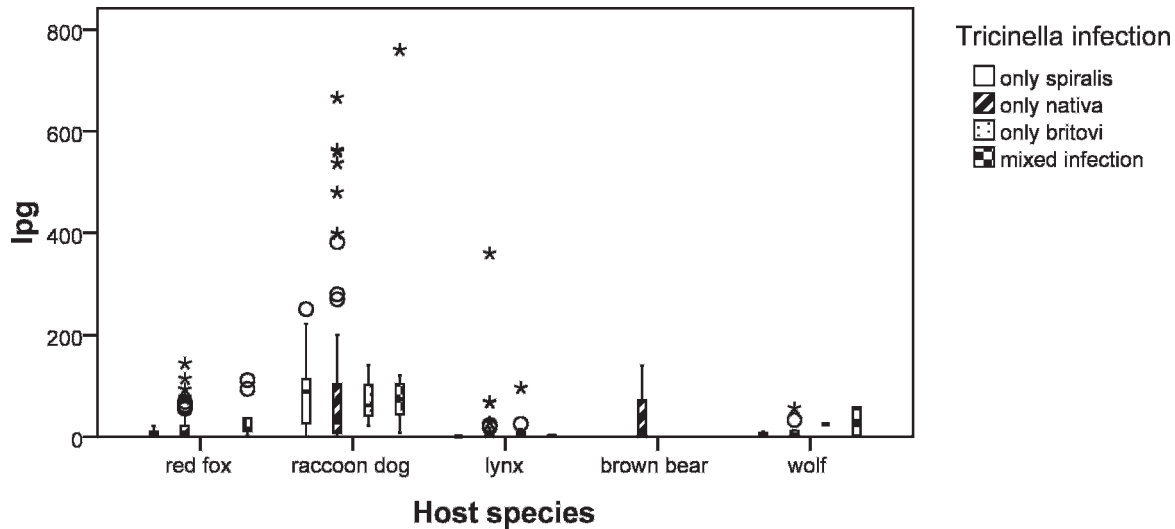


FIGURE 4. Box and leaf plot of infection density as larvae per gram (lpg) of muscle by different *Trichinella* species in host species with 5 or more isolates in Finland during 1999–2005. The few “only pseudospiralis” cases are omitted.

TABLE III. Estimated number of the population and infected individuals in Finnish carnivore hosts and *Trichinella* burden in the species calculated based on observed *Trichinella* prevalence in each GMD and population estimated based on annual bag in GMDs.

Host species	Annual national population size calculated based on estimated minimum and maximum population sizes at GMD level during 2002–2005	Estimated* annual (min–max) number of infected individuals in the country	Estimated (min–max) weight of infective muscle tissue (kg)†
Red fox ( <i>Vulpes vulpes</i> )	87,750–189,500	16,475–89,145	45,306–245,150
Raccoon dog ( <i>Nyctereutes procyonoides</i> )	138,000–277,800	21,509–134,866	64,528–404,598

GMD, game management district; min–max, minimum–maximum.

\* Based on estimated minimum and maximum annual population of 2002–2005 in GMDs and lower and upper binomial 95% confidence intervals for *Trichinella* infection prevalence at GMD level and then summing up to get the national data.

† Assumptions for weight (Siivonen and Sulkava, 1999): red fox 3–8 kg (5.5 kg used in calculations); raccoon dog 3–9 kg (6 kg used in calculations).

was found less often than *T. britovi* or *T. nativa* (Fig. 3), and the intensity also differed from that of other *Trichinella* species, although both *T. britovi* and *T. nativa* had an equal burdens (median 0.40 lpg with *T. spiralis* vs. 2.0 and 1.4 lpg in *T. britovi* and *T. nativa*, respectively; log-transformed lpg, 1-way ANOVA without *T. pseudospiralis*,  $P = 0.04$ ; Fig. 4).

A clear north-south gradient in both prevalence and animal population densities led us to compare reservoir animals at the regional level (Table I). The most abundant and ubiquitous host species were red foxes and raccoon dogs. These 2 host species were compared at the national level as reservoir species based on summarized data from the GMD level (Table III). At the national level, comparison of species-specific prevalence (Fig. 1) showed that the red fox was more often infected than the raccoon dog. When calculated as a proportion of estimated infected host animals of the estimated total population size at the GMD level (Table III), the range of the national prevalence was 19–47% for the red fox and 16–49% for the raccoon dog (Table III). When the numbers of estimated infected individuals were compared, there were 1.3–1.5 times more infected raccoon dogs than red foxes in the country. When infected tissue mass was compared, there were 1.4–1.7 times more *Trichinella* spp.–infected raccoon dog muscle. Raccoon dogs had 3.8 times higher average infection intensity than red fox (100.3 lpg vs. 26.4 lpg, respectively). Therefore, one can estimate that, at the national level, the raccoon dog as a host species carried a 5.4–6.3 times heavier *Trichinella* spp. burden than the red fox.

## DISCUSSION

Sylvatic animals acquire *Trichinella* spp. infection by consumption of prey, by scavenging, or by cannibalism. The red fox has been identified as an important reservoir host, and by being ubiquitous in its distribution, it is a good indicator species for continent-wide comparisons. Davidson et al. (2006) have reviewed the literature and summarized European studies on parasite prevalence in red fox. Data from northern Europe show *Trichinella* spp. prevalence to vary from 0% in Denmark to 4.5% in Sweden and 4.8% in Norway. In the Baltic countries, prevalence in red fox is 28.9–40.6% (Malakauskas et al., 2007), 4.4% in northwestern Poland (Balicka-Ramisz et al., 2007), 16% in Slovakia (Hurníková et al., 2006), and 1.8% in Hungary (Széll et al., 2008). Earlier studies from Finland have reported varying prevalence, i.e., 16% (6/38) (Risilakki, 1956), 4% (4/105) (Freeman, 1964), 33% (5/15) (Hirvelä-Koski et al., 1985), and 37%

(58/158) (Oivanen et al., 2002). In our investigation, the red fox prevalence varied in different districts from 0% to over 60% (Table I, Fig. 1). Thus, in Finland, conclusions based on infected individuals and the total number of analyzed animals from various parts of the country are problematic. This indicates that the uneven distribution of host species in the country must be considered before any national-level conclusions regarding the national prevalence of host species can be made. Although sylvatic *Trichinella* spp. infection is very common, large geographical differences in prevalence were found with all host species studied (Fig. 1). The southern part of the country exhibited very high sample prevalence, but in Lapland only moderate prevalence occurred, e.g., for the red fox, 53% in the south versus 1.4% in Lapland. Oivanen et al. (2002) made similar observations in the red fox, with limited numbers of positive animals from the northern part of the country and a prevalence of 4% (2/54) in Lapland, 52% (35/68) in the southwest, and 62% (21/34) in the southeast. Of reports from Europe, only the prevalence in Baltic countries is similarly intense to the southern part of Finland (Malakauskas et al., 2007). Interestingly, data from the Baltic countries indicate that wildlife prevalence has been increasing during the last few decades (Malakauskas et al., 2007). In other parts of the world, intranational geographical differences have also been identified (Prestrud et al., 1993; Széll et al., 2008). Therefore, representativeness of the sampling schemes should be considered when comparing different studies of *Trichinella* spp. prevalence intranationally or between countries. In Norway, Davidson et al. (2006) reported, based on red fox data, that in the southeastern part of the country, the prevalence was higher than in other parts, decreasing toward the north, similar to the pattern observed here.

Interestingly, combined *Trichinella* spp. prevalence in the red fox and raccoon dog was strongly and positively correlated with the population density of raccoon dogs in the GMD (Table II). This finding supports the earlier observation of a high raccoon dog population in an area being a risk factor for *Trichinella* spp. infection in Finnish lynxes (Oksanen et al., 1998).

When evaluating the importance of host species by estimating *Trichinella* spp. burden in the host species, the raccoon dog was shown to have a higher burden than the red fox, although the prevalence was higher in red foxes in most districts (Fig. 1). The raccoon dog population is relatively more concentrated in the southern part of the country, where the overall prevalence of *Trichinella* spp. is higher and the raccoon dog had a higher infection density (Fig. 4). Therefore, the *Trichinella* spp. burden is



5–6 times higher in raccoon dogs than in red foxes (Table III). The total mass of infective material from red foxes and raccoon dogs in the country is remarkably high, being 109,800–649,700 kg yearly (Table III); one may estimate (Kauhala, 2007) that half of this amount will be circulating in nature. This should be considered when informing hunters on how to deal with small carnivorous carrion. Hunting practices in Russia, where skinned wolves and other carnivore carcasses are commonly used as bait, are associated with an exceptionally high local prevalence of 97.5% (Poizio et al., 2001). Similar behavior may partially explain the patchy prevalence distribution in Finland, with “hot spots” in some GMDs. However, even without hunters, raccoon dog carcasses are commonly available for scavenging animals in the Finnish countryside. Raccoon dogs reproduce in good numbers, but many young animals die during the winter (compare autumn and spring data, Table I). Road kill, e.g., raccoon dogs and foxes, is commonly found along the sides of the motorways.

*Trichinella nativa* was the most common species identified among all sampled host species. This is congruent with other wildlife studies, indicating that *T. nativa* dominates in the Palearctic and Arctic, while *T. britovi* does so in more temperate regions (Handeland et al., 1995; Poizio and Murrell, 2006). The southern part of Finland, up to a latitude of 62°, is an area in which both *T. nativa* and *T. britovi* co-exist. In Estonia, the –6 C January isotherm has been speculated to be the thermal limit of *T. britovi* and –4 C is the limit for dispersal of *T. nativa* in more temperate areas (Poizio et al., 1998). Nevertheless, in the southern part of Finland, both *T. nativa* and *T. britovi* existed in an area where the average temperature from December to February drops to –8 C to –10 C. We found all 4 *Trichinella* species in the southern part of the country (latitude 62°; Fig. 2). All 4 *Trichinella* species have also been reported in sympatric Baltic areas (Malakauskas et al., 2007).

The domestic species, *T. spiralis*, was remarkably identified in 15% of the sylvatic isolations and was recovered from all parts of Finland. Intriguingly, *T. spiralis* was found in a fox (Fig. 2) from an area in Lapland with no previous reports of any domestic infection outbreaks. To our knowledge, this is the northernmost isolation of *T. spiralis* (see the map published in the review by Poizio and Murrell, 2006). In Lithuania and Latvia, *T. spiralis* was reported in wildlife only in areas with pig infections (Malakauskas et al., 2007). The latter authors concluded that a domestic source is needed to contaminate wildlife with disposed offal from an infected pig. Our finding of *T. spiralis* in Lapland contradicts their conclusion, indicating that *T. spiralis* may exist in a sylvatic cycle without external sources from synanthropic animals or swine offal. A similar pattern was seen in an epidemiological survey in Germany, which revealed *T. spiralis* larvae in 0.07% of sampled foxes (Wacker et al., 1999), although the German pork industry has been deemed virtually free of *Trichinella* spp. infection and human outbreaks have been associated with imported meat products (Jansen et al., 2008).

Host-parasite interaction was a significant factor in parasite intensity, indicating that certain species may reproduce better in particular hosts. The raccoon dog exhibited the highest parasite intensity for all *Trichinella* species, but burdens caused by different species of *Trichinella* did not differ in raccoon dogs, although the few detected *T. pseudospiralis* cases exhibited the highest burden. This is in accordance with earlier experimental studies where both *T. nativa* and *T. spiralis* were found to be

equally well adapted to this host species (Näreaho et al., 2000; Mikkonen et al., 2001). It is noteworthy that *T. spiralis* was both relatively and absolutely most often found in the raccoon dog and, moreover, that the parasite reproduces with higher intensities in raccoon dogs than in other host species. The raccoon dog has a high prevalence both in Baltic countries (Malakauskas et al., 2007) and in Finland, where it is an invasive alien species. However, in areas where it is native, reports do not show such a high prevalence, e.g., only 1.6% in Japan (Kobayashi et al., 2007). Data from Lithuania indicate that foxes are more often *T. spiralis* carriers than are raccoon dogs (8.2% of all isolates vs. 4.3% in raccoon dogs; Malakauskas et al., 2007). Although found less frequently than in raccoon dogs in the present study, *T. spiralis* was also commonly detected in the red fox; both animal species are closely associated with farm houses and barns. In the Finnish epidemiologic situation, they more likely act as donors of the infection to the domestic cycle rather than as recipients.

Interestingly, lynxes were relatively more frequently infected with *T. britovi* than with other species. *Trichinella* spp. prevalence in lynxes varied in different GMDs, from 8% to 80% (Table I). A study of Swiss lynxes has shown a prevalence of 27% (15/55), with variation between cantons (Frey et al., 2009). The same study reported a prevalence of 1.6% in foxes, with obvious geographical differences. The study reported only *T. britovi* in both foxes and lynxes, but the investigators did not provide infection intensities. In our data, both *T. nativa* and *T. britovi* had similar infection intensities in lynxes, but *T. spiralis* exhibited lower infection intensities and infected lynxes relatively less often. This may indicate a host-parasite adaptation that does not favor *T. spiralis* in lynxes.

*Trichinella pseudospiralis* was identified in only 7 animals, 4 times in different combinations of mixed infections and 3 times as single infections, twice in a raccoon dog and once in a lynx. Therefore, the low numbers of identification do not allow any conclusions to be drawn about host adaptations. However, the highest burden of *T. pseudospiralis* within the host species in both raccoon dogs and lynxes is in accordance with earlier studies showing a high reproduction index of this species in sylvatic hosts, but a low reproduction index in domestic animals (Poizio and Murrell, 2006).

We found 1 otter to harbor *T. nativa*. Reports of *Trichinella* spp. in otters are very rare. Data summarizing 20 yr of discoveries in the International *Trichinella* Reference Centre report only 2 otters infected by *T. britovi* (Poizio et al., 2009). The otter is mentioned, however, as a host species in Campbell's (1983) comprehensive review.

The high parasite burden with all *Trichinella* species identified in the raccoon dog shows that the raccoon dog is an adaptive host for *Trichinella* spp. High *Trichinella* spp. reproduction in naturally infected raccoon dogs has also been reported in other studies (Oivanen et al., 2002; Malakauskas et al., 2007). In Finland, the risk of the red fox or raccoon dog being infected with *Trichinella* spp. is strongly dependent on the sampling area. Their combined prevalence showed a significant association with the north-south coordinate and with raccoon dog abundance (Table II).

Although sylvatic infection is common in Finland and *T. spiralis*, a typically domestic species, is commonly associated with the sylvatic cycle, there are very few reports of human trichinellosis in Finland (see references in Oivanen, 2005). This

reflects effective transmission barriers. Pig production has been industrialized and meat inspection is efficient. Backyard pork production and consumption are uncommon. Hunters know the risk of sylvatic *Trichinella* infection and, in Finnish cuisine, foods are well cooked.

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