Yersinia spp. in wild rodents and shrews in Finland

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Introduction

Wild small mammals are considered an important reservoir of several different zoonotic agents and the incidence of zoonoses associated with wildlife reservoirs are increasing (Jones et al. 2008). Several foodborne pathogens, including *Salmonella* and *Escherichia coli* O157, have been isolated from wild small mammals (Davies and Wray 1995**,** Cizek 1999, Garber 2003**,** Nielsen 2004**,** Meerburg 2006**,** Zaytseva 2007). Also for enteropathogenic *Yersinia* spp. small mammals are suspected to be an important reservoir but evidence is still insufficient.

Enteropathogenic *Yersinia* spp. including *Y. enterocolitica* and *Y. pseudotuberculosis* are important foodborne zoonotic bacteria causing enteric yersiniosis, which is commonly reported among humans in Europe (EFSA 2015). The mechanisms of pathogenicity in enteropathogenic *Yersinia* spp. are complex, including several chromosomally and plasmid-located genes (Revell and Miller 2001). One of the most important chromosomal virulence genes is the *ail* gene coding for the Ail (attachment-invasion-locus) outer-membrane protein responsible for the colonization and invasion of *Y. enterocolitica* to epithelial cells in the ileum during the initial stages of infection (Revell and Miller 2001, Mikula et al. 2013). *Y. enterocolitica* strains can be biochemically divided into six biotypes (1A, 1B and 2 to 5) whose pathogenicity varies widely: biotype 1A strains are considered non-pathogenic due to the lack of most common chromosomal virulence genes and the approximately 70-kb virulence plasmid (pYV), which are found in both the weakly pathogenic strains of biotypes 2-5 and in the highly pathogenic biotype 1B strains (Bhagat and Virdi 2011). All *Y. pseudotuberculosis* strains are considered pathogenic. Most environmental *Yersinia* spp. strains are non-pathogenic. The *ail* gene is typically found only in pathogenic *Y. enterocolitica* strains and it is the most commonly used target for polymerase chain reaction (PCR) detection of pathogenic *Y. enterocolitica* (Nakajima et al. 1992, Fredriksson-Ahomaa and Korkeala 2003, Lambertz et al.

2008). All *Y. enterocolitica* and *Y. pseudotuberculosis* strains causing yersiniosis in humans and animals carry the plasmid pYV, which is essential for these bacteria to survive and multiply in the lymphatic tissue (Cornelis et al. 1998).

The role of wild small mammals in the epidemiology of enteropathogenic *Yersinia* spp. is still obscure, even though several findings suggest the importance of small mammals. For instance, grated carrots and lettuce, possibly contaminated by wild small mammals or other wildlife, have been vehicles for *Y. pseudotuberculosis* epidemics in Finland (Nuorti et al. 2004, Jalava et al. 2006, Kangas et al. 2008, Rimhanen-Finne et al. 2009). In addition, *Yersinia* spp. including human pathogenic biotypes of *Y. enterocolitica* and *Y. pseudotuberculosis*, have been isolated from wild rodents in Japan, where rodents also seem to play an important role in the epidemiology of *Y. enterocolitica* bioserotype 1B/O:8 (Fukushima et al. 1990, Iinuma et al. 1992, Hayashidani 1995). Moreover, similar pathogenic *Y. enterocolitica* 4/O:3 strains were isolated from pigs and rodents on Swedish pig farms but not from rodents outside the farms (Backhans et al. 2011). Laukkanen et al. (2008) suggested that the higher prevalence of *Y. pseudotuberculosis* on organic pig farms may be due to the higher number of pest and pet contacts with organic pigs than with pigs in in-house conventional farming. In order to predict human epidemics, the role of wild small mammals needs to be quantified.

The aim of our study was to clarify the role of small mammalian wildlife hosts as a reservoir for *Yersinia* spp.

Materials and Methods

Study area

The study was conducted in an area of Finland (60-64^{o}, 22-29^{o}, Figure 1). Wild small mammals were trapped from 37 trapping locations (Tables 1 and 2). Most of trapping locations (30) were selected without earlier knowledge about the presence of *Yersinia* spp. and made two transects across Southern and Central Finland. Additional five locations (locations 31-35 in the Table 2 and Figure 1) were source farms of *Y. pseudotuberculosis* epidemics a year before trapping started. The remaining two locations (locations 36-37) were specifically chosen for Borna virus research. All trapping locations are shown in Figure 1. Habitats in trapping areas covered typical boreal (taiga) forest (Norway spruce, birch and Scots pine) that covers most of the landscapes, wild meadows (abandoned oldfields) and field edges.

Rodents and shrews

Small mammals were trapped after the reproductive season from September to November in 2001-2007. Voles undergo strong population cycles in Finland; generally, 2001 was an increase year, 2002 a peak year, 2003 was a crash, 2004 an increase and 2006 a peak. The 30 trapping sites in the two transects across Finland remained the same through the study period with the exception that in the crash year of the rodent cycle in 2003 only every second locality was trapped. At each trapping locality, altogether 150 traps were set at five different sites, 30 traps in each, within 1 km^{2}. Traps were set in the afternoon of day 1, checked and animals collected on day 2, and animals collected and traps removed on day 3. Most of the animals were trapped in the first night. At source farms of *Y. pseudotuberculosis* epidemics 200-300 snap traps were used for 1-2 days. Locations for Borna virus research were trapped once with 200 traps. Snap traps, which killed the animals immediately, were used. During the trapping months, nighttime temperatures were already low, from -2^{o}C to +10^{o}C and animals were frozen in dry ice immediately after trap checking. Oral cavity samples including the tongue and larynx, and/or the intestine including the end of the colon and rectum were later dissected in the rodent laboratory of Finnish Forest Research Institute (now Natural Resources

Institute Finland), and were subsequently investigated at the Department of Food Hygiene and Environmental Health, University of Helsinki. The animals were stored at -25ºC before dissection. Intestinal and tongue samples were removed from partially thawed animals and immediately refrozen at -20ºC before culturing.

Ethical statement

In Finland, no ethical permit is needed for snap trapping because the Finnish Act on the Use of Animals for Experimental Purposes (62/2006) and the Finnish Animal Experiment Board (16th May, 2007) do not classify snap-trapping as an animal experiment. All trappings were done with the permission of landowners. A permit (23/5317/2001) for capturing protected species (mainly shrews) was granted by the Finnish Ministry of Environment. Other species captured are not protected in Finland, and none of the captured species is included in the Red List of Finnish Species.

Culture methods

The intestinal samples (including feces) and tongue samples were cut into small pieces with scissors and suspended in 4.5 ml of peptone-mannitol-broth (PMB). Direct culturing after 4-h incubation at room temperature and cold enrichment for 7, 14 and 21 days at 4ºC were carried out according to Laukkanen et al. (2008). After each incubation step, 100 µl of the PMB was spread on two selective agar plates: cefsulodin-irgasan-novobiosin (CIN; Oxoid, Basingstoke, UK) and MacConkey (Scharlau, Barcelona, Spain) plates. After cold enrichment the PMB was alkaline-treated with 0.25% potassium hydroxide (KOH) solution for 20 s before spreading on selective agar plates. Small colonies (1 mm in diameter) with a deep red center surrounded by a colorless zone on CIN and small colorless colonies on MacConkey were collected after 20–24 h incubation at 30ºC and after further incubation at room temperature for 24 h. The typical colonies were inoculated on tryptic-soy-agar (TSA; Difco, Maryland, USA) plates that were incubated at 30ºC for 24 h to create

pure cultures for further identification. Up to four colonies on the TSA plates were tested for oxidase reaction (Pro-Lab Diagnostics, Richmond Hill, Canada) and oxidase-negative isolates were further inoculated on urea agar slants. Urea-positive isolates were identified using the API 20E test (BioMérieux, Marcy l'Etoile, France) incubated at 25ºC for 18–20 h.

Characterization

All suspected *Yersinia* spp. isolates identified by API 20E were biotyped using the following tests according to Wauters et al. (1987): esculin hydrolysis, tween-esterase activity and pyrazinamidase production, indole production, and salicin, xylose and trehalose fermentation. Suspected *Y. pseudotuberculosis* isolates were biotyped with the raffinose and melibiose fermentation and citrate utilization tests (Tsubokura and Aleksic 1995). Furthermore, the isolates were characterized using a multiplex PCR assay targeting the 16S rRNA and *ail* genes of *Y. enterocolitica* according to Wannet et al. (2001). All suspected *Y. pseudotuberculosis* isolates were confirmed by PCR targeting a specific region of the *inv* of *Y. pseudotuberculosis* (Nakajima et al. 1992). All *Y. enterocolitica* isolates confirmed by PCR targeting the 16S rRNA region and *inv*-positive *Y. pseudotuberculosis* isolates were serotyped by slide agglutination using commercial antisera O:1, O:2, O:3, O;5, O:8 and O:9 for *Y. enterocolitica* and O:1-O:6 for *Y. pseudotuberculosis* (Denka Seiken, Tokyo, Japan). The presence of the virulence plasmid was studied by PCR targeting the *vir*F gene on the virulence plasmid (pYV) (Nakajima et al. 1992, Joutsen et al. 2013). The virulence gene *ail* from six *Y. enterocolitica* biotype 1A isolates (4 sucrose-positive and 2 sucrose-negative ones) and one *Y. kristensenii* isolate was confirmed by sequencing in both directions. Sequencing data were analysed with Bionumerics (Applied Maths).

Statistical analysis

The statistical analysis was conducted with SPSS software version 24 (IBM, Armonk, NY, USA). The differences in the yearly proportion of *Yersinia* spp. positive voles, mice and shrews separately, and in the isolation from oral or intestinal samples were analysed with Pearson´s Chi-square test.

Results

We investigated samples from 1840 wild small mammals for the presence of *Yersinia* spp. including voles (1171), mice (376) and shrews (293), trapped in 2001-2007 from 37 trapping locations in Finland (Table 1). The sample size in each trapping location in each year is presented in Table 2. Most of the material comes from 30 trapping locations along two transect lines crossing Southern Finland from the western coast to the eastern border (Figure 1). The isolates were characterized and the presence of virulence gene *ail* studied to assess the distribution of different *Yersinia* strains among wild small mammals captured at several locations over several years. From 1840 small mammals screened, a total of 641 *Yersinia* spp. isolates was obtained from 227 (12%) wild small mammals (Table 3). *Yersinia*-positive animals were trapped from 78% (29/37) of the trapping locations. *Yersinia* spp. were isolated more commonly ($p<0.001$) from intestinal samples (60%, 407 isolates from 678 samples) than from oral samples (15%, 234 isolates from 1603 samples).

Y. enterocolitica was the most common species found in 142 (8%) animals (Table 3). Almost all (358/359) *Y. enterocolitica* isolates belonged to the non-pathogenic biotype 1A and only one isolate from intestinal sample of a field vole belonged to bioserotype 2/O:9, which is associated with human and animal yersiniosis (Table 4). Some (14%; 50/359) *Y. enterocolitica* isolates were sucrose negative and thus first identified as *Y. kristensenii* using API 20E; however, they were confirmed to be *Y. enterocolitica* 1A after biotyping and PCR targeting the 16S rRNA region specific for *Y. enterocolitica*. Biotype 1A isolates were of serotypes O:2, O:2,3, O:3, O:3,9, O:5,

O:8 or O:9. Using API 20E, 13 isolates from four shrews were identified as *Y. pseudotuberculosis* (Table 3). However, only eight of these isolates, obtained from intestinal samples of two common shrews were confirmed as *Y. pseudotuberculosis* after PCR targeting the species-specific region of the *inv* gene (Table 4). All *Y. pseudotuberculosis* isolates belonged to biotype 1 and serotype O:2.

In total, 82 (23%) out of 358 *Y. enterocolitica* 1A isolates and 12 (6%) out of 213 *Y. kristensenii* isolates carried the chromosomal virulence gene *ail*. The *ail*-positive *Y. enterocolitica* 1A and *Y. kristensenii* isolates were isolated from 41 and four small mammals, respectively. *Y. enterocolitica* 1A carrying the *ail* gene was isolated in most of the species studied (Table 5). The house mouse and harvest mouse were the only species without any *ail*-positive *Yersinia* isolates but their sample sizes were also the smallest. The *ail*-positive isolates were most frequently found in field voles (9%) and sibling voles (10%), especially in 2005, which was a cyclic peak year of voles. The proportion of *Yersinia*-positive voles was significantly higher ($p<0.001$) during the years 2005 and 2007 compared to other years, and also to other peak year of 2002. There were no significant differences in the proportion of *Yersinia* spp. carrying animals between other years when voles were trapped. *Y. kristensenii* carrying the *ail* gene was isolated from three bank voles and one common shrew, which were all trapped in the same area in 2005. Small mammals carrying the *ail*-positive *Yersinia* were trapped in 12 different locations (32%) (Table 5). Field voles excreting *ail*-positive *Yersinia* were found in seven (28%) trapping locations (Table 5). The prevalence of *ail*-positive *Yersinia* in the animals varied from 1% to 14% at the 12 positive trapping locations (Figure 2). Most of the *ail*-positive isolates (84%) were found in the intestines.

The partially sequenced *ail*-gene fragments (111 bp) from six *Y. enterocolitica* 1A isolates and one *Y. kristensenii* isolate were identical to each other and had 98.8% similarity with previously isolated *ail* gene from *Y. enterocolitica* biotype 1A (GenBank: FN812732.1).

Discussion

There are only few studies on the prevalence of *Yersinia* spp. in wild animals in Europe including wild boars and deer but not wild small mammals (Fredriksson-Ahomaa et al. 2011, Joutsen et al. 2013, Bancerz-Kisiel et al. 2015). In our study, the prevalence of *Yersinia* spp. in Finnish wild small mammal populations from several locations across southern Finland was studied during a seven-year period. While most of the *Yersinia* isolates belonged to non-pathogenic species, especially *Y. kristensenii* or to non-pathogenic *Y. enterocolitica* of biotype 1A, pathogenic *Y. pseudotuberculosis* of bioserotype 1/O:2 and *Y. enterocolitica* of bioserotype 2/O:9, both of which carried the virulence plasmid (pYV), were also isolated. *Y. pseudotuberculosis* was only isolated from common shrews, suggesting shrews may be carriers for this enteropathogenic species causing yersiniosis in both humans and animals. Multiple *Y. pseudotuberculosis* epidemics related to vegetables have occurred in Finland (Nuorti et al. 2004, Jalava et al. 2006, Kangas et al. 2008, Rimhanen-Finne et al. 2009). Wild small mammals with access to vegetables during harvest or storage were suggested to be the potential source of contamination in one of these *Y. pseudotuberculosis* –inflicted carrot epidemics (Jalava et al. 2006). *Y. pseudotuberculosis* was isolated from a pooled sample of common shrew intestines from one of these epidemics-related farms (Kangas et al. 2008). Long-term storage of carrots in cold temperatures favors the growth of enteropathogenic *Yersinia* spp. if the vegetables are contaminated by small mammals during the harvest or storage. The common shrew was the only species in our study known to excrete *Y. pseudotuberculosis* in its feces. Common shrew is, unlike rodent species in this study, an insectivore and eats a lot of worms (Churchfield 1990). *Y. pseudotuberculosis* thrives in cool soils, and most probably worms continuously filtering soil accumulate bacteria, further contaminating shrews.

The only pathogenic *Y. enterocolitica* isolate carrying the virulence plasmid belonged to bioserotype 2/O:9 and was found in a field vole. This bioserotype is the second most common cause of human yersiniosis but the reservoir remains unknown (EFSA 2007, Moriki et al. 2010). All these enteropathogenic *Yersinia* carrying the virulence plasmid were isolated from intestinal samples showing that wild small mammals sporadically excrete these pathogens in their feces. This may suggest that wild small mammals play a previously unknown role in the epidemiology of human yersiniosis by shedding pathogenic strains into the environment. In a previous study, it was shown that laboratory mice can develop a persistent but asymptomatic *Y. pseudotuberculosis* infection in the colon when infected with low doses of pathogen and shed the pathogen (Fahlgren et al. 2014). Wild small mammals carrying and possibly amplifying and excreting pathogenic *Yersinia* spp. may produce a risk if coming into contact e.g. with non-carrier domestic animals, food storages or irrigation water sources. It is very typical in the strongly seasonal Finnish climate that wild small rodents, and even shrews, invade human settlements in late autumn – early winter when the first frosts appear. For example, the epidemic peak of hantaviral disease caused by Puumala hantavirus, takes place in October – January in Finland (Brummer-Korvenkontio et. al. 1999, Kallio et al. 2009). However, further studies are needed to clarify the persistence of *Yersinia* infection and shedding of enteropathogenic *Yersinia* spp. in small mammals.

Yersinia was an occasional finding in all wild small mammals, and *Y. enterocolitica* biotype 1A and *Y. kristensenii* strains carrying the *ail* gene were isolated especially from voles. Bank and field voles as well as common shrews are the most common small mammal species in Finland and they were the most common species trapped during our study. Voles exhibit strong multiannual density fluctuations, often called cycles, in Finland (Korpela et al. 2013). Therefore, the annual numbers of trapped animals varied quite widely. Consequently the numbers of positive animals varied

multiannually, particularly because the sample sizes were low in cyclic decline years (2003, 2006). The proportions of animals carrying *ail*-positive *Yersinia* spp. were higher during the peak years while during years of lowest densities no *ail*-positive isolates were detected. However, with the prevalence of peak year *ail*-positives, it was not very probable to find positives in low-density years. Furthermore, the prevalence of animals excreting *ail*-positive *Yersinia* isolates was high in some trapping areas (locations 4, 9, 24 and 34) suggesting that horizontal transmission of the *ail* gene between the pathogenic and non-pathogenic *Y. enterocolitica* and between *Y. enterocolitica* and *Y. kristensenii* isolates may have occurred in wildlife in these areas. This will be studied further. *ail*-positive small mammals were found in the study localities across Finland, suggesting there is no geographic clustering of them.

Pathogenic *Y. enterocolitica* strains attach and invade the host tissue cells with the help of *ail* (Pierson and Falkow 1993). The *ail*-positive *Y. enterocolitica* 1A and *Y. kristensenii* isolates are possibly able to invade the intestinal cells of small mammals and persist there despite a lack of the virulence plasmid needed for full virulence and infection. However, further studies are needed to evaluate the full virulence of these *ail*-positive isolates including the functionality of the *ail* gene.

The *ail* gene is typically associated only with pathogenic *Y. enterocolitica* strains (Miller et al. 1989). *ail*-positive *Y. enterocolitica* has sporadically been found in wild animals, food and humans with diarrhea (Sihvonen et al. 2009, Cheyne et al. 2010, Kraushaar et al. 2011, Liang et al. 2014, Bancerz-Kisiel et al. 2015). PCR detection of *ail* is commonly used in the detection of pathogenic *Y. enterocolitica* (Fredriksson-Ahomaa and Korkeala 2003, Lambertz et al. 2008), but the presence of *ail* also in non-pathogenic *Y. enterocolitica* and other *Yersinia* spp. questions the usefulness of this gene alone in PCR detection.

The identification of *Yersinia* species was challenging during our study. Differentiating between sucrose-negative *Y. enterocolitica* and *Y. kristensenii* isolates was impossible using only API 20E, and PCR targeting the 16S rRNA was needed for correct identification. Identification of *Y. pseudotuberculosis* was also impossible with API 20E and thus confirmation was performed using a PCR targeting the species-specific region of the *inv* gene in *Y. pseudotuberculosis*. The identification of environmental presumptive *Y. pseudotuberculosis* strains has been shown to be potentially incorrect when using biochemical tests (Niskanen et al. 2009). Typically, correctly identified *Y. pseudotuberculosis* isolates can be serotyped and they carry the virulence plasmid. All *Y. pseudotuberculosis* isolates in our study were of bioserotype 1/O:2, which is one of the bioserotypes found in wild birds and boars in Europe (Niskanen et al. 2003, Fredriksson-Ahomaa et al. 2011). The *Y. pseudotuberculosis* isolates also carried the virulence plasmid indicating full pathogenicity of these isolates.

Conclusions

Y. pseudotuberculosis of bioserotype 1/O:2 was isolated from two common species of shrew and *Y. enterocolitica* 2/O:9 from one species of field vole, indicating that wild small mammals could carry enteropathogenic *Yersinia* spp. and thus could also act as possible contamination sources of vegetables. Non-pathogenic *Y. enterocolitica* of biotype 1A frequently carried the *ail* gene typically found only in pathogenic *Y. enterocolitica* of biotypes 1B and 2 to 5, hampering the identification of pathogenic *Y. enterocolitica* isolates. Surprisingly, the *ail* gene was also detected sporadically in non-pathogenic *Yersinia* species *Y. kristensenii*.

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Disclosure statement

No competing financial interests exist.

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Figure 1. Trapping sites in Finland. The number refers to trapping locations in the Table 2 and the size of the circle to the number of trapped animals.

Figure 2. Trapping sites with *ail*-positive *Yersinia* spp. carrying animals in Finland. The number refers to trapping locations in the Table 2 and the size of the circle to the percentage of *ail*-positive *Yersinia* spp. carrying animals.

Table 1. Number of screened animals per species and year

Animal species	Number of locations	Number of trapped animals							
		All	2001	2002	2003	2004	2005	2006	2007
Voles (Arvicolinae)	34	1171	8	765	16	18	228	76	60
Field vole *(Microtus agrestis)*	25	281	7	214	0	4	34	9	13
Bank vole *(Myodes glareolus)*	33	811	1	503	16	14	163	67	47
Sibling vole *(Microtus levis)*	7	79	0	48	0	0	31	0	0
Mice (Murinae)	29	376	26	63	2	184	64	13	24
Striped field mouse *(Apodemus agrarius)*	6	52	0	2	0	30	20	0	0
Yellow-necked mouse *(Apodemus flavicollis)*	19	225	21	51	2	114	32	1	4
Harvest mouse *(Micromys minutus)*	18	46	5	4	0	17	6	7	7
House mouse *(Mus musculus)*	8	53	0	6	0	23	6	5	13
Shrews (Soricidae)	29	293	4	106	1	86	51	15	30
Common shrew *(Sorex araneus)*	29	293	4	106	1	86	51	15	30
All	37	1840	38	934	19	288	343	104	114

Table 2. Sample sizes and number of *Yersinia* and *ail*-positive *Yersinia* carrying animals in 2001-2007

Trapping location (No. of animals)	Trapping year							No. (%) of animals positive for	
	2001	2002	2003	2004	2005	2006	2007	*Yersinia* spp.	*ail*-pos. *Yersinia*
1. [a] Mustasaari (46)	0	5	0	7	***34*** [b]	0	0	6 (13.0)	2 (4.3)
2. Laihia (79)	0	**10**[c]	0	12	**57**	0	0	4 (5.1)	0
3. Ilmajoki (41)	0	16	0	5	**20**	0	0	3 (7.3)	0
4. Nurmo (27)	0	***6***	0	13	6	2	0	2 (7.4)	2 (7.4)
5. Alavus (93)	0	***89***	0	4	0	0	0	2 (2.2)	2 (2.3)
6. Ähtäri (47)	0	**40**	0	7	0	0	0	1 (2.1)	0
7. Multia (63)	0	28	0	**20**	**15**	0	0	4 (6.3)	0
8. Uurainen (23)	0	**15**	0	8	0	0	0	1 (4.3)	0
9. Laukaa (49)	0	***33***	0	16	0	0	0	11 (22.4)	8 (16.3)
10. Hankasalmi (2)	0	2	0	0	0	0	0	0	0
11. Pieksamäki (49)	0	42	0	**7**	0	0	0	1 (2.0)	0
12. Virtasalmi (65)	0	***65***	0	0	0	0	0	7 (10.8)	3 (4.6)
13. Rantasalmi (34)	0	**31**	0	1	2	0	0	3 (8.8)	0
14. Savonlinna (12)	0	***11***	0	1	0	0	0	2 (16.7)	1 (8.3)
15. Punkaharju (7)	0	6	0	1	0	0	0	0	0
16. Parikkala (13)	0	0	0	**13**	0	0	0	2 (15.4)	0
17. Rautjärvi (24)	0	10	6	8	0	0	0	0	0
18. Joutseno (29)	0	11	0	0	***18***	0	0	3 (10.3)	1 (3.4)
19. Lappeenranta (91)	0	***71***	3	**10**	7	0	0	4 (4.4)	1 (1.1)
20. Savitaipale (60)	0	**42**	0	18	0	0	0	11 (18.3)	0
21. Valkeala (44)	0	29	0	13	2	0	0	0	0
22. Jaala (20)	0	10	0	3	7	0	0	0	0
23. Asikkala (54)	0	50	1	3	0	0	0	0	0

24 Hauho (22)	0	21	1	0	0	0	0	0	0
25. Valkeakoski (67)	0	**50**	0	**17**	0	0	0	6 (9.0)	0
26. Vesilahti (61)	0	55	1	**4**	1	0	0	1 (1.6)	0
27. Hämeenkyrö (50)	0	**41**	0	**9**	0	0	0	6 (12.0)	0
28. Sastamala (55)	0	40	0	***7***	**8**	0	0	3 (5.5)	2 (3.6)
29. Kokemäki (123)	**38**	**71**	7	**7**	0	0	0	6 (4.9)	0
30. Ulvila (71)	0	34	0	**26**	**11**	0	0	5 (7.0)	0
31.[d] Himanka (107)	0	0	0	0	**38**	**45**	**24**	39 (36.4)	0
32.[d] Toholampi (55)	0	0	0	0	0	30	**25**	11 (20.0)	0
33.[d] Ylihärmä (22)	0	0	0	0	0	7	***15***	10 (45.5)	1 (4.5)
34.[d] Myrkky (156)	0	0	0	0	***117***	9	**30**	63 (40.4)	21 (13.5)
35.[d] Loppi (31)	0	0	0	0	0	**11**	***20***	7 (22.6)	1 (3.2)
36. Mäntsälä (22)	0	0	0	22	0	0	0	0	0
37. Suitia (26)	0	0	0	**26**	0	0	0	3 (11.5)	0
Total (1840)	38	934	19	288	343	104	114	227 (12.3)	45 (2.4)

[a] The number refers to trapping locations marked on Figures 1 and 2.

[b] Bold and italic numbers include samples of *ail*-positive animals.

[c] Bold numbers include *Yersinia*-positive animals.

[d] Localities 31-35 were selected due to known earlier *Yersinia* spp. occurrence.

Table 3. Distribution of different *Yersinia* spp. in voles, mice and shrews

Animal species (number of animals)	Number of *Yersinia*-positive animals using PCR (number of isolates)							
	Yersinia spp.	*Yersinia enterocolitica*	*Yersinia kristensenii*	*Yersinia mollaretii/ bercovieri*	*Yersinia frederiksenii*	*Yersinia rohdei*	*Yersinia intermedia*	*Yersinia pseudotuber-culosis*
Voles (1171)	152 (421)	103 (259)	57 (144)	7 (14)	2 (4)	0	0	0
Field vole (281)	41 (116)	36 (93)	8 (18)	2 (5)	0	0	0	0
Bank vole (811)	98 (277)	58 (144)	46 (122)	4 (7)	2 (4)	0	0	0
Sibling vole (79)	13 (28)	9 (22)	3 (4)	1 (2)	0	0	0	0
Mice (376)	50 (138)	30 (73)	12 (29)	12 (27)	2 (4)	1 (2)	2 (3)	0
Striped field mouse (52)	13 (36)	9 (22)	4 (11)	1 (1)	1 (1)	0	1 (1)	0
Yellow-necked mouse (225)	30 (81)	18 (44)	6 (13)	9 (22)	0	0	1 (2)	0
Harvest mouse (46)	2 (6)	0	1 (3)	0	1 (3)	0	0	0
House mouse (53)	5 (15)	3 (7)	1 (2)	2 (4)	0	1 (2)	0	0
Common shrew (293)	25(82)	9 (27)	15 (40)	0	1 (2)	0	0	2 (8)
All	227 (641)	142 (359)	84 (213)	19 (41)	5 (10)	1 (2)	2 (3)	2 (8)

Table 4. Identification of 641 *Yersinia* spp. isolated from voles, mice and shrews

Yersinia spp.	Biotype	Number of isolates (number of animals)			
		Identified by API 20E	16S rRNA[a] positive	*ail*[b] positive	*inv*[c] positive
Y. enterocolitica		359 (142)	359 (142)	83 (41)	ND[d]
sucrose positive	1A	308 (119)	308 (119)	53 (24)	
	2	1 (1)	1 (1)	1 (1)	
sucrose negative	1A	50 (26)	50 (26)	29 (18)	
Y. kristensenii		213 (84)	0 (84)	12 (4)	ND
Y. mollaretii/bercovieri		41 (19)	0 (19)	0 (19)	ND
Y. rohdei		2 (1)	0 (1)	0 (1)	ND
Y. frederiksenii		10 (5)	0 (5)	0 (5)	ND
Y. intermedia		3 (2)	0 (3)	0 (3)	ND
Y. pseudotuberculosis		13 (4)	ND	ND	8 (2)
All isolates		641 (227)	359 (142)	95 (45)	

[a] 16S rRNA sequence studied only in *Y. enterocolitica*

[b] *ail* sequence typically found only in *Y. enterocolitica* strains of biotypes 1B, 2–5

[c] *inv* sequence studied only in *Y. pseudotuberculosis*

[d] ND Not determined

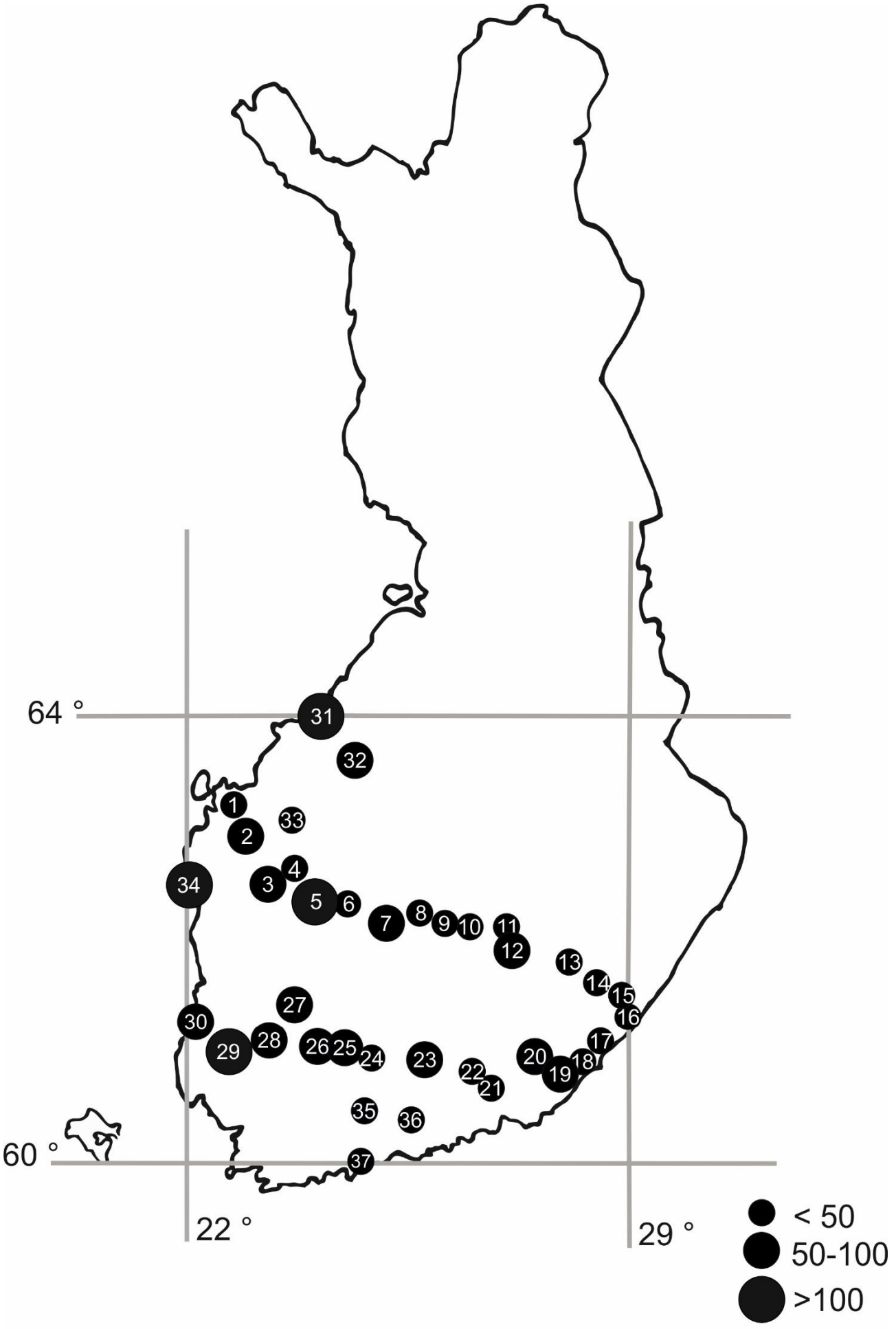
Table 5. Distribution of *ail*-positive *Yersinia enterocolitica* and *Yersinia kristensenii* in voles, mice and shrews

Animal species[a]	Number of animals	Number of *ail*-positive				
		Animals	*Yersinia enterocolitica*		*Yersinia kristensenii*	Trapping locations
			Biotype 1A	Biotype 2		
Field vole	281	25 (8.9%) [b]	25	1	0	7 (28.0%) [c]
Bank vole	811	6 (0.7%)	3	0	3	3 (9.1%)
Sibling vole	79	8 (10.1%)	8	0	0	1 (14.3%)
Striped field mouse	52	1 (1.9%)	1	0	0	1 (16.7%)
Yellow-necked mouse	225	3 (1.3%)	3	0	0	2 (10.5%)
Common shrew	293	2 (0.7%)	1	0	1	1 (3.4%)
All animals	1741	45 (2.5%)	41	1	4	12 (31.6%)

[a] Only species with *ail*-positive *Yersinia* spp. isolations

[b] Percentage of *ail*-positive animals in a species

[c] Percentage of trapping locations with *ail*-positive animal species out of locations where species was captured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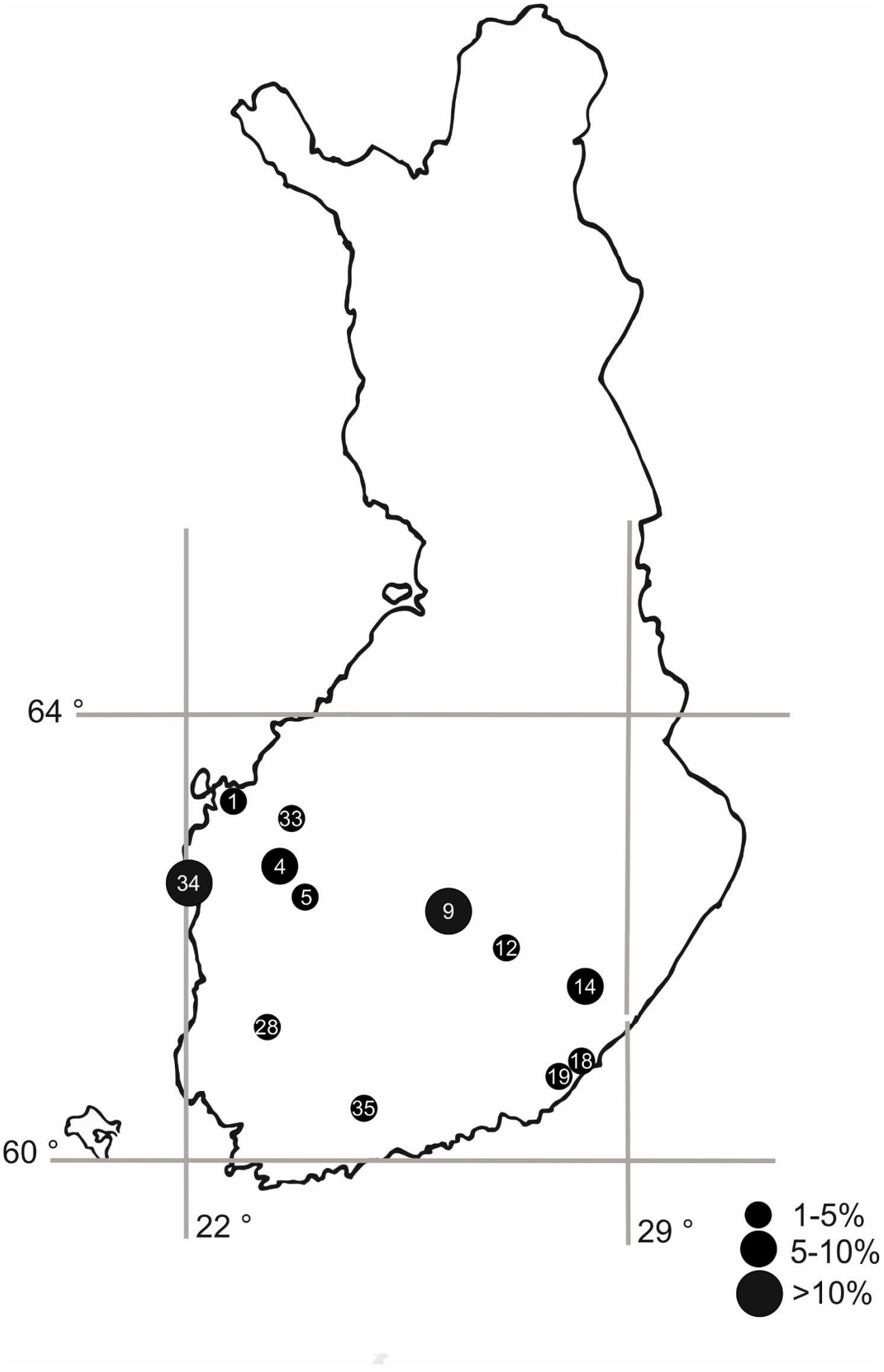
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