

Occurrence of pathogenic *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* in small wild rodents

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SUMMARY

Rodents are a potential source of pathogenic *Yersinia enterocolitica* and *Y. pseudotuberculosis*. In order to study this, 190 rodents were captured and sampled on seven pig farms ($n=110$), five chicken farms ($n=55$) and six other locations ($n=25$) in Sweden. Pigs from three of the pig farms were also sampled ($n=60$). Pathogenic *Y. enterocolitica* was detected by TaqMan PCR in about 5% of rodent samples and 18% of pig samples. Only rodents caught on pig farms tested positive for the pathogen. *Y. enterocolitica* bioserotype 4/O:3 strains isolated from the rodent and pig samples were compared by pulsed-field gel electrophoresis and revealed a high degree of similarity, which was confirmed by random amplified polymorphic DNA. *Y. pseudotuberculosis* was only detected in one rodent sample. Thus, rodents may be vectors for the transmission of pathogenic *Y. enterocolitica* to pigs, acting as carriers rather than a reservoir, and should therefore remain an important issue in hygiene control measures on farms.

Key words: Epidemiology, transmission, *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, zoonotic foodborne diseases.

INTRODUCTION

Yersiniosis is a zoonotic gastrointestinal infection reported in humans worldwide. In the European Union it is the third most frequently reported zoonosis after campylobacteriosis and salmonellosis. Most reported infections are caused by *Yersinia enterocolitica* with only a few being due to *Yersinia pseudotuberculosis* [1]. The symptoms of yersiniosis are age-dependent and in children aged <5 years the most common symptom is non-specific gastroenteritis. For other age groups acute mesenteric lymphadenitis, septicaemia

and sequelae such as arthritis, erythema nodosum and Reiter's syndrome have also been reported [2]. The most frequent *Y. enterocolitica* bioserotypes pathogenic to humans are 1B/O:8, 2/O:5, 27, 2/O:9, 3/O:3 and 4/O:3, with the latter predominating in cases reported worldwide. Pathogenicity in *Y. enterocolitica* is linked to the presence of genes situated on both the chromosome and the plasmid [3]. Since the presence of the plasmid is unstable, PCR primers and probes have frequently been directed towards a chromosomally located gene, for example the *ail* (attachment invasion locus) gene, as a more reliable target for detection [4–6]. Recently, two TaqMan PCR methods, targeting different locations on the *ail* gene in *Y. enterocolitica* and *Y. pseudotuberculosis*, respectively, were developed [6, 7]. These methods were used for detection of the pathogens in this study.

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The source of yersiniosis is considered to be contaminated food, especially pork and pork products [8, 9]. Pigs are the only food animals that regularly harbour the pathogen [10–12] and are considered the main reservoir [13, 14]. To develop control measures and lower the incidence of *Y. enterocolitica* or maintain *Yersinia*-free herds, better knowledge is needed of possible sources of contamination at the farm level. Wild rodents may be one such source, since these pests often have free access to pig houses. Previous studies have shown that *Y. enterocolitica* is common in wild rodents, but isolation of the most widespread human pathogenic bioserotype, 4/O:3, is rare [15–17].

Y. pseudotuberculosis is a foodborne pathogen causing repeated outbreaks in certain countries in the Northern Hemisphere, e.g. Finland, Canada, Russia and Japan [18]. Although wild birds, rodents and pigs appear to be major reservoirs, this pathogen seems to generally circulate in the environment between water, soil and wild animals [18, 19]. In Finland there are almost annually occurring outbreaks of *Y. pseudotuberculosis* [18], some of which have been traced to carrots or iceberg lettuce, possibly contaminated by wild animals [20–22]. *Y. pseudotuberculosis* has also been isolated from pigs and pork products and in some of these cases rodents have been suspected of carrying and spreading the infection [23].

Due to inefficient methods, pathogenic strains of *Y. enterocolitica* are often difficult to isolate, especially from environmental samples. The underlying reason is that in these samples the pathogen is present in low numbers together with a high amount of background flora, and sufficiently selective culture media are not available [24]. Instead, PCR methods can be utilized and currently the use of probe-based real-time PCR has improved the specificity of the analysis. However, in certain studies, it is necessary to isolate the bacterium; e.g. in outbreak investigations or when tracing a source of contamination it is crucial to compare the genotypes of the isolates involved. At present the gold standard for genotyping pathogenic *Y. enterocolitica* and *Y. pseudotuberculosis* is pulsed-field gel electrophoresis (PFGE) [20, 23, 25–27]. Random amplified polymorphic DNA (RAPD) is a rapid genetic fingerprinting method that can be used in addition to PFGE. It has previously been tested on foodborne *Y. enterocolitica* spp. [28].

The objectives of this study were to investigate the presence of pathogenic *Y. enterocolitica* and *Y. pseudotuberculosis* in wild rodents caught at various locations in Sweden, including pig farms, using TaqMan

PCR for detection and conventional culture for isolation of the two pathogens; and two fingerprinting methods to identify and compare the genotypes of the isolates recovered.

MATERIALS AND METHODS

Sampling procedures

Between December 2005 and December 2007, rodent traps were set at 28 locations in Sweden: on 16 pig farms, five chicken farms, and seven other non-farm-related locations. A total number of 207 rodents were caught at 20 of the 28 trapping locations, while none were caught at the remaining eight locations. Eight were discarded from the analysis due to technical problems. Both live and snap traps (traps that instantly kill the rodent) were set at points with signs of rodent activity, such as burrows or droppings. Number of traps used and number of days the traps were set at specific locations varied depending on the area and supply of rodents, i.e. between 10 and 50 traps and between 2 days and several weeks. Traps were checked every day. Most of the trapping locations ($n=24$) were in central/southeast Sweden (Mälardalen region) but four of the pig farms were located in southern and southwest Sweden (Småland/Halland region). The capture and euthanasia of rodents were approved by the Swedish Ethical Committee for Scientific Experiments (protocol C247/5).

Both rodents and pigs were examined for the presence of pathogenic *Y. enterocolitica* and *Y. pseudotuberculosis*. The occurrence in rodents was studied by examining colon tissue samples from 199 rodents and lymph nodes from 128 of the same rodents. Nine colon samples were lost during processing. The sampled rodents originated from seven pig farms ($n=110$), five chicken farms ($n=55$) and six other locations ($n=25$). Information regarding the capture locations and rodent species caught is listed in Table 1. At necropsy, performed on the day of capture, about 1–1.5 cm of proximal colon and superficial cervical lymph nodes from all rodents were aseptically removed and collected in separate 1.5-ml microcentrifuge tubes. Samples were immediately transported to the laboratory and either processed on the same day or stored at $-80\text{ }^{\circ}\text{C}$ until analysed. Histological examination of intestines, kidney, liver, lungs and spleen was performed at the Department of Pathology and Wildlife Diseases, National Veterinary Institute (Uppsala, Sweden).

Table 1. Locations where rodents were sampled and proportion of rodents that tested positive by TaqMan PCR for pathogenic *Y. enterocolitica*

No.	Type of location	Description of location	Proportion of infected mice	Proportion of infected rats	Infected rodents as a proportion of other species*	Total proportion of infected rodents
1	Pig farm	Piglet-producing herd	1/22	0/0	0/1	1/23
2		Piglet-producing herd	0/2	0/0	0/0	0/2
3		Integrated pig farm, outdoors	1/8	0/0	0/4	1/12
4		Integrated pig farm	0/10	0/0	0/2	0/12
5		Integrated pig farm	0/6	0/11	0/0	0/17
6		Fattening herd	0/1	7/24	0/0	7/25
7		Fattening herd	0/19	0/0	0/0	0/19
		Σ=	2/68	7/35	0/7	9/110
8	Chicken farm	Young chickens	0/31	0/0	0/0	0/31
9		Chickens	0/7	0/0	0/4	0/11
10		Chickens, reared outdoors	0/5	0/0	0/0	0/5
11		Chickens, reared outdoors	0/2	0/0	0/0	0/2
12		Chickens and livestock	0/1	0/5	0/0	0/6
		Σ=	0/46	0/5	0/4	0/55
13	Others	City park pond	0/0	0/6	0/0	0/6
14		Sewage treatment plant	0/0	0/0	0/2	0/2
15		City mill	0/0	0/7	0/0	0/7
16		Ruminant veterinary clinic	0/6	0/0	0/0	0/6
17		Supermarket	0/0	0/2	0/0	0/2
18		Other locations	0/0	0/1	0/1	0/2
		Σ=	0/6	0/16	0/3	0/25
	Total Σ=		2/120	7/56	0/14	9/190

* Thirteen yellow-necked mice, *Apodemus flavicollis* and one water vole, *Microuis agrestis* (location 18).

The occurrence of pathogenic *Y. enterocolitica* in pigs was studied for three of the seven pig farms where rodents were caught, i.e. locations 3, 5 and 6 (Table 1); fattening pigs at locations 3 and 6, and growers at location 5. On each of the three farms, 20 individual live pigs were sampled by Amies culture swabs (Copan Innovation, Italy). The swabs were rubbed over the rectal mucosa covering an area of about 2 cm × 2 cm. Swabs were stored in Amies agar gel medium with charcoal (Copan Innovation) at 8 °C until cultivation.

Sample preparation and culture

The rodent colon tissue samples and the superficial cervical lymph nodes were treated with 150–250 μl of 0.9% NaCl solution and the tissues were thoroughly mashed with a Pasteur pipette to achieve a homogeneous mixture. Samples were then vortexed and centrifuged (50 g) for 10 min. Sub-portions (10 μl) of the supernatant from each sample were spread onto

Cefsulodin Irgasan Novobiocin (CIN) agar plates (Oxoid, CM 653 and SR 109) and the remaining volume was used for DNA extraction (see below). The pig swabs were streaked directly onto CIN agar plates. All plates were incubated at 30 °C for 21 ± 3 h. If no typical colonies had appeared after 24 h, the CIN agar plates were incubated another 21 ± 3 h. Small red ‘bull’s-eye’ colonies were considered presumptive pathogenic *Y. enterocolitica*. Up to four colonies per plate were subcultured and transferred to individual tubes containing brain heart infusion (BHI) broth (Oxoid, CM0225) mixed with 17% glycerol and stored at –80 °C until characterization.

Positive control strains

In the TaqMan PCR methods, strain SLV-408 (CCUG 45643) of *Y. enterocolitica* 4/O:3 and strain TAVA 81 of *Y. pseudotuberculosis* were used as reference and control strains. Strain SLV-408 was also used as positive control when performing the

bioserotyping and PFGE. Sterile distilled water was used as a negative control in the PCR.

DNA extraction and TaqMan PCR

DNA was extracted from the tissue samples on the remaining supernatants (as described above) with the DNeasy Blood and Tissue kit (Qiagen GmbH, Germany). Moreover, following subculture on BHI agar of the isolated colonies recovered from the rodents and pigs, DNA was prepared by transfer of a loop of the bacteria to 200 μ l sterile distilled water with 20 μ l of 0.8 M NaOH solution added. The tubes were incubated at 75 °C for ~10 min and 48 μ l of equal volumes of 0.8 M HCl and 0.1 M Tris (pH 8.3), were added [6]. In some cases and when preparing DNA for RAPD analysis, again the DNeasy Blood and Tissue kit was used according to the manufacturer's protocol for Gram-negative bacteria. For the detection of pathogenic *Y. enterocolitica* and *Y. pseudotuberculosis*, two TaqMan probe-based PCR methods targeting different sites of the *ail* gene were applied as described previously [6, 7]. A positive amplification control was included in the analysis [6]. In cases of PCR inhibition, which occurred more frequently in the lymph node samples than the colon samples, samples were diluted 1:10 and re-tested. Monitored by the amplification control, this was sufficient to eliminate the effect of any inhibitory substances present.

Presumptive colonies isolated from the rodent and pig samples were initially examined by PCR for identification of pathogenic *Y. enterocolitica*. PCR-negative colonies were further analysed by TaqMan PCR for identification of *Y. pseudotuberculosis*.

Phenotypic analysis

Isolates testing positive in TaqMan PCR for pathogenic *Y. enterocolitica* were biotyped according to a reduced variant of the scheme by Wauters *et al.* [29], which included tests for lipase, salicin, esculin, xylose, trehalose, Voges–Proskauer and pyrazinamidase, performed as described previously [27]. To reveal presence or absence of the virulence plasmid, Congo Red-brain heart infusion agarose plates (CR-BHO) were used [30]. The biotyped isolates were serotyped by a slide agglutination test with the commercial antisera O:3 and O:9 (Reagensia AB, Sweden).

Sequencing

PCR products obtained from three *Y. enterocolitica* isolates (68, 104, 200; see Table 2), three *Y. enterocolitica* TaqMan-positive colon samples from rodents, and one *Y. pseudotuberculosis* TaqMan-positive rodent colon sample, were sequenced at Uppsala Genome Centre (Rudbeck Laboratory, Sweden). The PCR products were purified with QIA quick PCR Purification kit (Qiagen GmbH). Sequencing was performed in both directions.

PFGE

The *Y. enterocolitica* 4/O:3 strains isolated from the rodent ($n=5$) and pig samples ($n=10$) were compared by PFGE. SLV-408 (CCUG 45643) was included as a control strain. The PFGE Standardized Laboratory Protocol for Molecular Subtyping of *Escherichia coli* O:157:H7, non-typhoidal *Salmonella* serotypes and *Shigella sonnei* [31, 32] was used according to the protocol with the following modifications: 300 μ l instead of 2 ml cell suspension buffer, 100 μ l instead of 200 μ l restriction enzyme mixture containing 6.5 μ g BSA and 1 μ l restriction enzyme instead of 5 μ l. To compensate for the smaller amount of enzyme, incubation time was extended to 4 h instead of 1.5–2 h as suggested in the protocol. Restriction enzymes *NotI* and *XbaI* (New England Biolabs, USA) were chosen and additional typing was performed for some of the isolates with *ApaI*. *Salmonella* serotype Braenderup H9812 was used as a standard [33]. Electrophoresis was performed in 0.5 \times TBE buffer with 1–25 s switching time for 22 h at 14 °C with a CHEF-DR III instrument (Bio-Rad Laboratories, USA). Gels (Agarose NA, 17-0554-02; Amersham Biosciences, UK) were stained with GelRed [Biotium (www.biotium.com)] and visualized with Quantity One software (Bio-Rad). PFGE patterns were analysed by visual examination of banding differences, and also analysed with GelCompare II software [Applied Maths (www.applied-maths.com)].

RAPD

All strains isolated from rodents and pigs were also analysed by RAPD. Ready-To-Go RAPD Analysis Beads (Pharmacia Biotech, USA) were used. The protocol provided by the manufacturer was used with the primers provided (5'-GGTGCGGGAA-3' and 5'-GTTTCGCTCC-3'). PCR products were loaded on a 1% agarose gel stained with ethidium bromide and

Table 2. Results of phenotypic and genotypic characterization of *Y. enterocolitica* 4/O:3 strains isolated from rodents and pigs in this study

Epidemiological data				Phenotypic and genotypic description				
Isolate no.	Source of origin	Location no*	Year	CR-BHO†	PFGE <i>NotI</i>	PFGE <i>XbaI</i>	PFGE <i>ApaI</i>	RAPD
58	Brown rat	6	2006	+++	A	1	a	2
67	Brown rat	6	2006	+++	B	1	b	2
68	Brown rat	6	2006	+++	B	1	b	2
200	Brown rat	6	2007	+++	B	1	n.t.	2
104	House mouse	1	2006	+++	B	1	n.t.	2
8	Pig	3	2007	++	B	1	n.t.	2
13	Pig	3	2007	+++	B	1	n.t.	2
18	Pig	3	2007	+++	B	1	b	2
502	Pig	6	2007	+++	B	1	b	2
504	Pig	6	2007	+++	B	1	n.t.	2
506	Pig	6	2007	+++	B	1	n.t.	2
510	Pig	6	2007	+++	B	1	n.t.	2
516	Pig	6	2007	+++	B	1	b	2
520	Pig	6	2007	+++	B	1	n.t.	2
522	Pig	6	2007	+++	B	1	n.t.	2
SLV408	Pig	—	—	n.t.	B	1	n.t.	2

n.t., Not tested; CR-BHO, Congo Red-brain heart infusion agarose plates.

* Described in Table 1.

† CR-BHO results; numbers of colonies indicated as ++, 10–50; +++, >150 [27].

visualized digitally with GelDoc 2000 (Bio-Rad) and Quantity One software (Bio-Rad). Isolates differing in size and numbers of bands were assigned to different RAPD types.

RESULTS

Pathogenic *Y. enterocolitica* in rodents and pigs

The TaqMan PCR screening of the 190 rodent samples for presence of pathogenic *Y. enterocolitica* revealed that nine (5%) of 190 colon samples analysed, from rodents caught at locations 1, 3 and 6, were PCR-positive for pathogenic *Y. enterocolitica* (Table 1). All lymph node samples were negative. All PCR-positive samples originated from rodents caught on pig farms located at distances ranging 20–65 km from each other.

Conventional culture on the 190 rodent colon samples identified five colonies recovered from five individual rodents caught at locations 1 and 6 as *Y. enterocolitica* 4/O:3 by bioserotyping (Table 2). The results were confirmed by PCR. Thus, of the nine rodent samples that initially tested positive by PCR, five *Y. enterocolitica* 4/O:3 strains were obtained.

From the pig swabs, 11 presumptive colonies from 11 individual pigs were confirmed as pathogenic *Y. enterocolitica* by TaqMan PCR, 4/20 pigs from location 3, and 7/20 pigs from location 6. Bioserotyping identified 10 of these colonies as 4/O:3 (Table 2), while one showed inconsistent serotyping results. Further analysis indicated that this particular strain of 4/O:3 was contaminated with *Citrobacter freundii*, and it was excluded from further analysis. The CR-BHO agarose plate analyses indicated that all 15 rodent and pig isolates identified as 4/O:3 harboured the virulence plasmid. Histological examination revealed no certain signs of infection in any of the rodents.

Y. pseudotuberculosis in rodents and pigs

The TaqMan PCR analyses for detection of *Y. pseudotuberculosis* resulted in one positive sample of 190 rodent colon samples analysed, whereas all 128 lymph node samples analysed tested negative. The positive sample came from a house mouse caught on a pig farm (location 3). No isolate was obtained. At autopsy, this mouse showed hyperaemic mucous membranes of the caecum and, histologically,

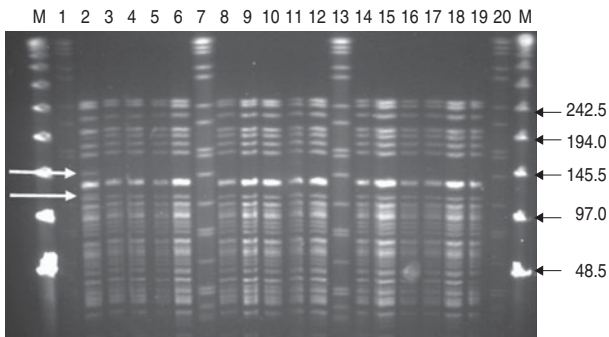


Fig. 1. PFGE (*NotI*) profiles for isolates of *Y. enterocolitica* 4/O:3 from rodents and pigs. M, Lambda marker. Lanes 1, 7, 13, 20, Standard *Salmonella* Braenderup H9812; lanes 2–5, rat isolates from location 6; lane 6, mouse isolate from location 1; lanes 8–10, pig isolates from location 3; lanes 11, 12, 14–18, pig isolates from location 6; lane 19, control strain SLV408. White arrows to the left indicate differences between pulsotypes A and B. Black arrows to the right indicate size of lambda marker bands.

moderate to severe acute multifocal hepatitis and splenitis. None of the isolates obtained from pigs tested positive in TaqMan PCR.

Sequencing

When the amplified 163-bp PCR products were sequenced, almost complete base-pair sequences, i.e. 160, 159, 163 and 159 bp, were obtained, from the three rodent *Y. enterocolitica* 4/O:3 strains and from one of the colon tissue samples, respectively. A similarity search in GenBank (www.ncbi.nlm.nih.gov) showed that they were identical to the corresponding parts of the deposited *ail* genes of *Y. enterocolitica* AY004311 and AJ605740. For the remaining two colon samples, the 52-bp short sequence obtained from one was identical to corresponding parts of AY004311, whereas the other sequence was of too poor quality to be analysed. The 159-bp sequence from the colon sample that tested positive for *Y. pseudotuberculosis* was identical to the *ail* gene of *Y. pseudotuberculosis* (acc. nos: CP001048, CP000720, BX936398).

PFGE and RAPD analyses

Results from the genotyping obtained by PFGE and RAPD are summarized in Table 2. PFGE analysis of 15 strains isolated from rodents ($n=5$) and pigs ($n=10$) generated one profile when cleaved with restriction enzyme *XbaI* and two profiles A and B when cleaved with restriction enzyme *NotI* (Fig. 1). The use of *ApaI*, which was applied for a selection of six of the

isolates, produced two pulsotypes, a and b. The two groups of pulsotypes obtained with *ApaI* corresponded to the two groups of pulsotypes obtained with *NotI*. Just one rodent isolate showed the pulsotypes A and a, while all pig isolates, four rodent isolates and the control strain showed the pulsotypes B and b. Analyses of the same six isolates by RAPD showed one type of banding pattern for each of the two primers used. Thus, the discriminatory power was the same as for *XbaI*.

DISCUSSION

In this study rodents were collected at different locations in Sweden (including pig farms) and their potential role as carriers of pathogenic strains of *Y. enterocolitica* was investigated. *Y. enterocolitica* bioserotype 4/O:3, which is the most common bioserotype reported in human yersiniosis throughout the world, was detected in about 5% at all locations, and on pig farms, in 8% of the rodents. The pathogen was detected in both mice and rats, but only in those caught on pig farms. The proportion of positive rats (20%) on pig farms is comparable to other studies that showed a prevalence in black rats on pig farms of between 14% and 17% [15, 34]. However, in earlier studies, isolates of *Y. enterocolitica* from rodents were only serotyped and not biotyped and it is therefore uncertain whether those isolates were human pathogenic yersiniae [15, 34, 35]. In an early study where O:3 isolates recovered from field vole (*Microtus agrestis*) were biotyped, the biotypes obtained differed from those recognized as being human pathogens [17]. Over the years, knowledge of the pathogenic determinants of the pathogen has increased and new techniques have been introduced, so that detection of the pathogenic bioserotypes is now both rapid and specific. Simultaneously, the workload in performing biochemical tests for biotyping has been reduced. We found it useful to first apply a TaqMan PCR method for screening the rodent samples, to obtain an early indication of presence/absence of the pathogen in a sample. TaqMan PCR and biochemical reactions were then applied on the presumptive colonies appearing on CIN agar [29], and then isolates were serotyped and the virulence plasmid-associated phenotypes of the colonies were determined with CR-BHO agarose [30]. Besides reducing the time involved, this strategy made the confirmation steps more efficient in identification of the human pathogenic bioserotypes of the bacterium.

Y. enterocolitica 4/O:3 has not been reported in free-living rodents, but it has been isolated from rats living in proximity to pigs [36]. The results in the present study support these findings in that all 4/O:3-positive rodents identified were caught on pig farms and that rodents collected at other locations were found to be negative. Pathogens were predominately found in rats in this study. However, a few mice caught on pig farms also carried the pathogen. To our knowledge this is the first reported isolation of *Y. enterocolitica* 4/O:3 from a house mouse. The fact that only rodents caught near pigs tested positive indicates that rather than being reservoirs, rodents are more likely to act as carriers of bacteria they contract from infected pigs and their environment. However, since the number of trapped animals on other locations was generally lower than on pig farms, this assumption should be made with some caution. On one farm, rat faeces were visible both inside and outside pig pens. Rats have been video-recorded feeding from the floor in pig pens [37], showing that faecal-oral transmission of the bacteria is likely to occur between pigs and rodents. The bacterium was isolated from rats caught in two consecutive years on the same farm, showing that colonization of rats is not an exceptional event. Generally, the farms with positive rodents were farms where rodents seemed to be abundant, based on information from farmers and visual signs of their presence. All farms in this study, like most Swedish farms, applied pest control by the use of rodenticides, but control of the rodent population was insufficient in some cases. Based on the data derived from this study, a high abundance of wild rodents in pig farms should always be regarded as a risk factor for maintaining pathogenic *Y. enterocolitica* infection in pigs. A recommendation to pig producers is to always emphasize pest control, including construction and maintenance of functional barriers.

PFGE revealed two pulsotypes among the 4/O:3 strains isolated from rodents and pigs in this study. One of the pulsotypes originated from a single strain isolated from one of the rats, while the DNA profiles of the remaining strains deriving from four rodent isolates were indistinguishable and similar to those derived from the pig isolates. In an attempt to improve the discriminatory power, in addition to using the two restriction enzymes *NotI* and *XbaI*, the restriction enzyme *ApaI* was applied as suggested by Fredriksson-Ahomaa *et al.* [26]. The use of RAPD with two sets of primers showing identical patterns

confirmed the similarity among the isolates. However, no additional differentiation was reached. This is in agreement with previous studies where the usefulness of RAPD in differentiating between *Yersinia* strains was poor [28]. In Japan Hayashidani *et al.* [38] isolated the highly virulent bioserotype 1B/O:8 of *Y. enterocolitica* from rodents and pigs and revealed similar pulsotypes in the rodent and pig isolates, suggesting a common source of contamination. In contrast to *Y. enterocolitica* 4/O:3, bioserotype 1B/O:8 can be found in the environment and has repeatedly been isolated from free-living wild rodents of different species [35]. While rodents may be regarded as reservoirs for 1B/O:8 [35, 39], thereby also constituting a direct risk for public health, rodents carrying 4/O:3 strains appear more likely to be vectors for pathogen transmission between pigs within a pig herd, as indicated by the present study and others [34, 36].

Y. pseudotuberculosis was detected in only one of the rodent samples examined (1/190) and in none of the 60 pig samples, indicating a low prevalence of this pathogen in these animals in Sweden. Similarly, *Y. pseudotuberculosis* is only rarely reported as a source of human infection in Sweden and no human outbreaks have been reported. In contrast, recent studies have shown that in Finland, pigs most probably play a role as a reservoir of human *Y. pseudotuberculosis* infections [40] and that pest animals may be responsible for spreading the bacterium on Finnish pig farms [23]. However, *Y. pseudotuberculosis* is difficult to detect by available detection methods and therefore can easily be overlooked. It often persists in low numbers and it is debatable whether the direct detection approach applied in the present study was sensitive enough to reveal the pathogen.

In conclusion, the results obtained in our study suggest that rodents, primarily the brown rat and to a lesser extent the house mouse, are possible vectors for transmission of *Y. enterocolitica* 4/O:3 on pig farms. Since there is no evidence of rodents acting as reservoirs of the infection, they should mainly be considered as posing a risk for maintaining and spreading the bacteria within a farm, especially between different batches of pigs in all-in/all-out systems.

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DECLARATION OF INTEREST

None.

REFERENCES

1. Anon. The community summary report on trends and sources of zoonoses, zoonotic agents, antimicrobial resistance and foodborne outbreaks in the European Union in 2007. *EFSA Journal* 2009; **223**: 3–312.
2. Bottone EJ. *Yersinia enterocolitica*: the charisma continues. *Clinical Microbiology Reviews* 1997; **10**: 257–276.
3. Fredriksson-Ahomaa M, Stolle A, Korkeala H. Molecular epidemiology of *Yersinia enterocolitica* infections. *FEMS Immunology and Medical Microbiology* 2006; **47**: 315–329.
4. Jourdan AD, Johnson SC, Wesley IV. Development of a fluorogenic 5' nuclease PCR assay for detection of the *ail* gene of pathogenic *Yersinia enterocolitica*. *Applied and Environmental Microbiology* 2000; **66**: 3750–3755.
5. Thisted Lambertz S, et al. A combined culture and PCR method for detection of pathogenic *Yersinia enterocolitica* in food. *International Journal of Food Microbiology* 2000; **57**: 63–73.
6. Thisted Lambertz S, et al. Real-time PCR method for detection of pathogenic *Yersinia enterocolitica* in food. *Applied and Environmental Microbiology* 2008; **74**: 6060–6067.
7. Thisted Lambertz S, Nilsson C, Hallanvuoto S. TaqMan-based real-time PCR method for detection of *Yersinia pseudotuberculosis* in food. *Applied and Environmental Microbiology* 2008; **74**: 6465–6469.
8. Ostroff SM, et al. Sources of sporadic *Yersinia enterocolitica* infections in Norway: a prospective case-control study. *Epidemiology and Infection* 1994; **112**: 133–141.
9. Tauxe RV, et al. *Yersinia enterocolitica* infections and pork: the missing link. *Lancet* 1987; **329**: 1129–1132.
10. Bhaduri S, Wesley IV, Bush EJ. Prevalence of pathogenic *Yersinia enterocolitica* strains in pigs in the United States. *Applied and Environmental Microbiology* 2005; **71**: 7117–7121.
11. Fredriksson-Ahomaa M, Stolle A, Stephan R. Prevalence of pathogenic *Yersinia enterocolitica* in pigs slaughtered at a Swiss abattoir. *International Journal of Food Microbiology* 2007; **119**: 207–212.
12. Lindblad M, et al. Microbiological baseline study of swine carcasses at Swedish slaughterhouses. *Journal of Food Protection* 2007; **70**: 1790–1797.
13. Hurvell B, Glatthard V, Thal E. Isolation of *Yersinia enterocolitica* from swine at an abattoir in Sweden. *Contributions to Microbiology and Immunology* 1979; **5**: 243–248.
14. Wauters G. Carriage of *Yersinia enterocolitica* serotype 3 by pigs as a source of human infection. *Contributions to Microbiology and Immunology* 1979; **5**: 249–252.
15. Aldova E, Cerny J, Chmela J. Findings of yersinia in rats and sewer rats. *Zentralblatt fur Bakteriologie Mikrobiologie und Hygiene – Abt 1 Orig A* 1977; **239**: 208–212.
16. Kaneko K, Hashimoto N. Occurrence of *Yersinia enterocolitica* in wild animals. *Applied and Environmental Microbiology* 1981; **41**: 635–638.
17. Kapperud G. *Yersinia enterocolitica* in small rodents from Norway, Sweden and Finland. *Acta Pathologica et Microbiologica Scandinavica – Section B, Microbiology and Immunology* 1975; **83 B**: 335–342.
18. Hallanvuoto S, Foodborne Yersinia. Identification and molecular epidemiology of isolates from human infections (dissertation). Helsinki, Finland: University of Helsinki, 2009, 132 pp.
19. Fukushima H, Gomyoda M. Intestinal carriage of *Yersinia pseudotuberculosis* by wild birds and mammals in Japan. *Applied and Environmental Microbiology* 1991; **57**: 1152–1155.
20. Jalava K, et al. An outbreak of gastrointestinal illness and erythema nodosum from grated carrots contaminated with *Yersinia pseudotuberculosis*. *Journal of Infectious Diseases* 2006; **194**: 1209–1216.
21. Rimhanen-Finne R, et al. *Yersinia pseudotuberculosis* causing a large outbreak associated with carrots in Finland, 2006. *Epidemiology and Infection* 2008; **4**: 1–6.
22. Nuorti JP, et al. A widespread outbreak of *Yersinia pseudotuberculosis* O:3 infection from iceberg lettuce. *Journal of Infectious Diseases* 2004; **189**: 766–774.
23. Laukkanen R, et al. Transmission of *Yersinia pseudotuberculosis* in the pork production chain from farm to slaughterhouse. *Applied and Environmental Microbiology* 2008; **74**: 5444–5450.
24. Fredriksson-Ahomaa M, Korkeala H. Low occurrence of pathogenic *Yersinia enterocolitica* in clinical, food, and environmental samples: a methodological problem. *Clinical Microbiology Reviews* 2003; **16**: 220–229.
25. Asplund K, Johansson T, Siitonen A. Evaluation of pulsed-field gel electrophoresis of genomic restriction fragments in the discrimination of *Yersinia enterocolitica* O:3. *Epidemiology and Infection* 1998; **121**: 579–586.
26. Fredriksson-Ahomaa M, Autio T, Korkeala H. Efficient subtyping of *Yersinia enterocolitica* bioserotype 4/O:3 with pulsed-field gel electrophoresis. *Letters in Applied Microbiology* 1999; **29**: 308–312.

27. **Thisted Lambertz S, Danielsson-Tham ML.** Identification and characterization of pathogenic *Yersinia enterocolitica* isolates by PCR and pulsed-field gel electrophoresis. *Applied and Environmental Microbiology* 2005; **71**: 3674–3681.
28. **Blixt Y, et al.** Interlaboratory random amplified polymorphic DNA typing of *Yersinia enterocolitica* and *Y. enterocolitica*-like bacteria. *International Journal of Food Microbiology* 2003; **83**: 15–26.
29. **Wauters G, Kandolo K, Janssens M.** Revised biogrouping scheme of *Yersinia enterocolitica*. *Contributions to Microbiology and Immunology* 1987; **9**: 14–21.
30. **Bhaduri S, et al.** Simple assay of calcium dependency for virulent plasmid-bearing clones of *Yersinia enterocolitica*. *Journal of Clinical Microbiology* 1990; **28**: 798–800.
31. **PulseNet.** One-day (24–28 h) standardized laboratory protocol for molecular subtyping of *Escherichia coli* O157:H7, non-typhoidal *Salmonella* serotypes, and *Shigella sonnei* by pulsed field gel electrophoresis (PFGE). In: PulseNet PFGE Manual, 2004.
32. **Ribot EM, et al.** Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* for PulseNet. *Foodborne Pathogens and Disease* 2006; **3**: 59–67.
33. **Hunter SB, et al.** Establishment of a universal size standard strain for use with the PulseNet standardized pulsed-field gel electrophoresis protocols: converting the national databases to the new size standard. *Journal of Clinical Microbiology* 2005; **43**: 1045–1050.
34. **Pokorna V, Aldova E.** Finding of *Yersinia enterocolitica* in *Rattus rattus*. *Journal of Hygiene, Epidemiology, Microbiology and Immunology* 1977; **21**: 104–105.
35. **Iinuma Y, et al.** Isolation of *Yersinia enterocolitica* serovar O8 from free-living small rodents in Japan. *Journal of Clinical Microbiology* 1992; **30**: 240–242.
36. **Kaneko KI, et al.** Occurrence of *Yersinia enterocolitica* in house rats. *Applied and Environmental Microbiology* 1978; **36**: 314–318.
37. **Akande O.** A study on wild rat behaviour and control on a pig farm (Master's thesis). Uppsala, Sweden: Swedish University of Agricultural Sciences, 2008, 53 pp.
38. **Hayashidani H, et al. (eds).** Molecular genetic typing of *Yersinia enterocolitica* serovar O:8 isolated in Japan. In: *The Genus Yersinia*. Secaucus, NJ, USA: Kluwer Academic Publishers, 2003, pp. 363–365.
39. **Hayashidani H, et al.** Potential sources of sporadic human infection with *Yersinia enterocolitica* serovar O:8 in Aomori Prefecture, Japan. *Journal of Clinical Microbiology* 1995; **33**: 1253–1257.
40. **Niskanen T, Fredriksson-Ahomaa M, Korkeala H.** *Yersinia pseudotuberculosis* with limited genetic diversity is a common finding in tonsils of fattening pigs. *Journal of Food Protection* 2002; **65**: 540–545.