

Transmission of *Yersinia pseudotuberculosis* in the Pork Production Chain from Farm to Slaughterhouse[∇]

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The transmission of *Yersinia pseudotuberculosis* in the pork production chain was followed from farm to slaughterhouse by studying the same 364 pigs from different production systems at farm and slaughterhouse levels. In all, 1,785 samples were collected, and the isolated *Y. pseudotuberculosis* strains were analyzed by pulsed-field gel electrophoresis. The results of microbial sampling were combined with data from an on-farm observation and questionnaire study to elucidate the associations between farm factors and the prevalence of *Y. pseudotuberculosis*. Following the same pigs in the production chain from farm to slaughterhouse, we were able to show similar *Y. pseudotuberculosis* genotypes in live animals, pluck sets (containing tongue, tonsils, esophagus, trachea, lungs, diaphragm, liver, and kidneys), and carcasses and to conclude that *Y. pseudotuberculosis* contamination originates from the farms, is transported to slaughterhouses with pigs, and transfers to pluck sets and carcasses in the slaughter process. The study also showed that the high prevalence of *Y. pseudotuberculosis* in live pigs predisposes carcasses and pluck sets to contamination. When production types and capacities were compared, the prevalence of *Y. pseudotuberculosis* was higher in organic production than in conventional production and on conventional farms with high rather than low production capacity. We were also able to associate specific farm factors with the prevalence of *Y. pseudotuberculosis* by using a questionnaire and on-farm observations. On farms, contact with pest animals and the outside environment and a rise in the number of pigs on the farm appear to increase the prevalence of *Y. pseudotuberculosis*.

Yersinia pseudotuberculosis is a food-borne pathogen that can cause serious illness in humans (15, 29). Symptoms of the illness, which include fever and acute abdominal pain caused by mesenteric lymphadenitis, are often clinically indistinguishable from those of acute appendicitis (32, 33). Systemic complications, such as erythema nodosum and reactive arthritis, are also relatively common (13, 15). In the last few years, the incidence of *Y. pseudotuberculosis* per 100,000 inhabitants in Finland has been 0.6 to 5 (20–22). Recent *Y. pseudotuberculosis* outbreaks in Finland have been linked to vegetables, e.g., carrots and iceberg lettuce (15, 16, 29), but worldwide milk, water, and pork have also been suspected sources of *Y. pseudotuberculosis* infections (10, 28, 36).

Y. pseudotuberculosis is frequently found in tonsils and intestinal contents of clinically healthy pigs at slaughterhouses around the world. The prevalence of *Y. pseudotuberculosis* in the tonsils and intestinal contents of fattening pigs has ranged from 0.03% to 6% (3, 11, 25, 38) and from 0.6% to 3% (3, 12, 19, 34, 35, 37), respectively. *Y. pseudotuberculosis* has also been isolated from pork (6), indicating a possible route from pigs to humans.

Differences in pig husbandry practices can affect the prevalence of such pathogens as *Yersinia*. The prevalence of *Yersinia enterocolitica* has been shown to be higher in specialized slaughter pig production than in conventional farrow-to-finish production and in conventional production than in organic production (27, 31). Because *Y. pseudotuberculosis* has been isolated from soil and a multitude of wild animals (8, 9, 24, 26), the prevalence of *Y. pseudotuberculosis* may be increased in production systems, such as organic pig production, where pigs have contact with the outside environment.

Pigs are often asymptomatic carriers of *Y. pseudotuberculosis*, and infected pigs cannot immediately be identified in the slaughter process. Understanding factors affecting the prevalence of *Y. pseudotuberculosis* is therefore important in order to identify potential measures to control the occurrence of *Y. pseudotuberculosis* both on farms and at slaughterhouses. To this end, we followed 364 individual fattening pigs from the farms to the slaughterhouse and showed using pulsed-field gel electrophoresis analysis of isolated strains that carcasses and pluck sets are contaminated with *Y. pseudotuberculosis* strains that pigs acquired on the farms. We also analyzed the associations between different farm factors and the prevalence of *Y. pseudotuberculosis* in different pig production systems at farm and slaughterhouse levels.

MATERIALS AND METHODS

Sampling. A total of 15 farms, five organic (median, 338 fattening pigs), five conventional with production capacity of under 1,000 fattening pigs (median, 350) per year, and five conventional farms with production capacity of 1,000 fattening pigs or more (median, 2,600) per year, were selected from southwestern

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TABLE 1. *Yersinia pseudotuberculosis* in conventionally and organically produced pigs at farm level

Production type	Value for rectal swab samples			
	n	No. (%) positive	95% CI ^a	
			Random sampling ^b	Clustering at farms ^c
Organic	121	23 (19)	12–27	5–48
Conventional (total)	243	6 (3)	1–6	0–14
High capacity ^d	125	6 (5)	2–11	0–27
Low capacity ^e	118	0 (0)	0–3	NC ^f
Total	364	29 (8)	6–12	2–21

^a CI, confidence interval.
^b Ninety-five percent confidence interval of prevalence when sampling was assumed to be randomized (exact binomial estimates).
^c Ninety-five percent confidence interval of prevalence when the fact that 21 to 26 pigs were sampled from each farm was considered (Fleiss quadratic 95% confidence interval).
^d Production capacity of 1,000 fattening pigs or more per year.
^e Production capacity of fewer than 1,000 fattening pigs per year.
^f NC, could not be calculated.

Finland and sampled between June 2003 and January 2005. The organic farms are registered as organic and inspected according to European Union regulations (2). On each farm, 21 to 26 pigs were sampled for *Y. pseudotuberculosis*. Samples were collected at the farm and slaughterhouse, and all samples were logistically connected to the corresponding pig. At the farm, rectal swabs were obtained from pigs, and the pigs were ear tagged for further sampling at the slaughterhouse. The time between farm and slaughterhouse sampling was 1 to 2 weeks. At the slaughterhouse, intestinal content, tonsils, pluck set (containing tongue, tonsils, esophagus, trachea, heart, lungs, diaphragm, liver, and kidneys), and carcass swabs were collected after meat inspection. Rectal swabs were obtained using sterile cotton wool sticks, and the samples were transferred into tubes containing 10 ml of PMB (phosphate-buffered saline supplemented with 1% mannitol and 0.15% bile salts). Intestinal content was collected from an incision into a bowel with a sterile spoon, and pluck sets were sampled by swabbing lungs, heart, liver, and kidneys with a 7.5- by 7.5-cm gauze square moistened with 10 ml of peptone water. Peptone water-moistened gauze was also used for swabbing thoracic and pelvic cavities of both halves of the carcass. Samples were stored cold during transportation and delivered to the laboratory on the same day. Analyses were started immediately after delivery or on the following day.

Determination of *Y. pseudotuberculosis*. A total of 1,785 microbiological samples were examined using two different enrichment methods. Samples were examined using selective enrichment in irgasan-ticarillin-potassium chloride (ITC broth base [Merck, Darmstadt, Germany] supplemented with ticarillin and irgasan [Abtek Biologicals Ltd., United Kingdom] and 1 mg/ml KClO₃) broth and cold enrichment in PMB for 7 and 14 days. In brief, samples were diluted 1:9 in PMB and mixed thoroughly. A 1-ml volume of PMB was inoculated in 9 ml of ITC broth and incubated at 25°C for 2 to 3 days. PMB broth was cold enriched at 4°C for 7 and 14 days. Alkali treatment (0.5 ml of the sample mixed with 4.5 ml of 0.25% KOH solution for 20 s before cultivation) was used after 14 days of cold enrichment. A quantity of 100 µl of sample was streaked after each enrichment step onto a cefsulodin-irgasan-novobiocin (CIN) agar plate (*Yersinia* Selective Agar Base and *Yersinia* Selective Supplement; Oxoid, Basingstoke, United Kingdom) and incubated at 30°C for 18 to 20 h and further at 22°C for 24 h. From each CIN agar plate, one to five suspect colonies were streaked onto tryptic soy agar (Difco, Maryland) plates for pure culture. All isolates on tryptic soy agar plates were tested for urea hydrolysis using a urea agar slant, and isolates that hydrolyzed urea were identified and tested for pathogenicity using a PCR assay targeting the chromosomal virulence gene *inv* and the *virF* gene in a virulence plasmid (pYV) according to the method of Nakajima et al. (18) with modifications as described by Niskanen et al. (25). All *inv*- and *virF*-positive isolates were identified as pathogenic *Y. pseudotuberculosis* and further tested using the API 20 E test (BioMérieux, Marcy l’Etoile, France) according to the manufacturer’s instructions, with the exception of incubation at 25°C for 18 to 20 h.

Serotyping. Isolates were serotyped with slide agglutination using commercial O:1 to O:6 antisera (Denka Seiken, Tokyo, Japan).

TABLE 2. *Yersinia pseudotuberculosis* in conventionally and organically produced pigs at slaughterhouse level

Production type	Intestinal content				Tonsil				Pluck set				Carcass			
	n	No. (%) pos. ^b	95% CI ^a		n	No. (%) pos.	95% CI		n	No. (%) pos.	95% CI		n	No. (%) pos.	95% CI	
			Random sampling ^c	Clustering at farms ^d			Random sampling ^c	Clustering at farms ^d			Random sampling ^c	Clustering at farms ^d			Random sampling ^c	Clustering at farms ^d
Organic	119	11 (9)	5–16	2–31	119	28 (24)	17–33	4–62	120	5 (4)	1–9	0–24	120	10 (8)	4–15	1–37
Conventional (total)	239	13 (5)	3–9	0–28	231	6 (3)	1–6	1–9	234	1 (0.4)	0–2	0–3	239	0 (0)	0–2	NC ^e
High capacity ^f	121	13 (11)	6–19	0–49	117	4 (3)	1–9	0–18	119	0 (0)	0–3	NC	121	0 (0)	0–3	NC
Low capacity ^g	118	0 (0)	0–3	NC	114	2 (2)	0–6	0–6	115	1 (1)	0–6	0–6	118	0 (0)	0–3	NC
Total	358	24 (7)	5–10	2–20	350	34 (10)	7–14	3–27	354	6 (2)	1–4	0–7	359	10 (3)	2–5	0–14

^a CI, confidence interval.
^b Pos., positive.
^c Ninety-five percent confidence interval of prevalence when sampling was assumed to be randomized (exact binomial estimates).
^d Ninety-five percent confidence interval of prevalence when the fact that 21 to 26 pigs were sampled from each farm was considered (Fleiss quadratic 95% confidence interval).
^e NC, could not be calculated.
^f Production capacity of 1,000 fattening pigs or more per year.
^g Production capacity of fewer than 1,000 fattening pigs per year.

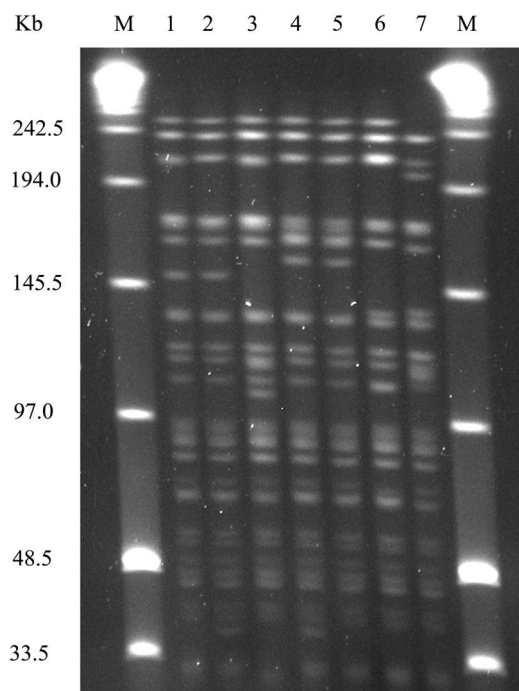


FIG. 1. Seven different SpeI profiles (lanes 1 to 7) of *Yersinia pseudotuberculosis* strains obtained. M designates midrange PFGE markers.

PFGE. A total of 286 isolates were characterized by pulsed-field gel electrophoresis (PFGE) as described by Niskanen et al. (25) using SpeI and NotI restriction enzymes (New England Biolabs, Ipswich, MA). One isolate from each isolation step, i.e., one to three isolates per sample, was characterized, except on farm A, where all isolates were analyzed. Restriction patterns were analyzed visually and with the assistance of BioNumerics (Applied Maths, Sint-Martens-Latem, Belgium) version 4.61. Applied Dice coefficient correlation was applied to identify similarities among PFGE types, and a dendrogram was constructed with the unweighted-pair group method using arithmetic averages. The position tolerance was set to 1.40%, with the optimization value at 1.50%.

Association between *Y. pseudotuberculosis* and farm factors. Data on farm management practices were collected from the farms with a questionnaire and on-farm observations, as described earlier (30), to assess different features associated with the presence of *Y. pseudotuberculosis* in pigs. SPSS 12.0.1 (SPSS Inc., Chicago, IL) was used to calculate correlations between farm factors and the prevalence of *Y. pseudotuberculosis*-positive pigs on different farms. In addition, a two-level (farm and pig) multivariate logistic regression model was constructed with MLwiN 2.02 (Centre for Multilevel Modeling, University of Bristol, United Kingdom). Building on the finding that fattening pigs can carry *Y. enterocolitica* in tonsils without shedding it into feces at the farm (23) and assuming that the same applies to *Y. pseudotuberculosis*, the association between *Y. pseudotuberculosis* and farm factors was tested with combined on-farm rectal swab results and tonsil results from the slaughterhouse. A pig was considered positive if either or both the rectal and tonsil samples were positive. A pig was excluded from analyses if either the rectal or the tonsil sample was missing. A total of 14 pigs were excluded from the analyses.

RESULTS

Altogether 103 of 1,785 samples investigated (6%) were *Y. pseudotuberculosis* positive including farm and slaughterhouse samples (Tables 1 and 2). *Y. pseudotuberculosis* was isolated from 62 pigs (17%) in either rectal, intestinal, or tonsil samples. *Y. pseudotuberculosis* was isolated from six farms (40%), i.e., 3 of 10 conventional (30%) and three of five organic (60%) farms. The within-farm prevalence of *Y. pseudotuberculosis* on

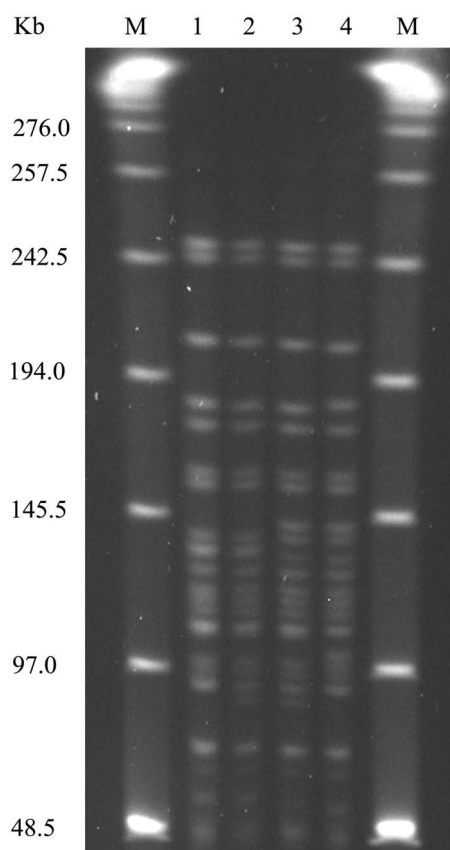


FIG. 2. Four different NotI profiles (lanes 1 to 4) of *Yersinia pseudotuberculosis* strains obtained. M designates midrange PFGE markers.

all farms in rectal swabs, intestinal contents, tonsils, pluck sets, and carcass samples varied from 0% to 56%, 0% to 52%, 0% to 75%, 0% to 21%, and 0% to 38%, respectively.

All *Y. pseudotuberculosis* isolates were melibiose negative and agglutinated with O:3 antiserum. Altogether seven and four different PFGE patterns were obtained from 286 *Y. pseudotuberculosis* isolates harboring *inv* and *virF* genes using SpeI and NotI enzymes, respectively (Fig. 1 and 2). Eight different genotypes (gI to gVIII) were gained by combining SpeI and NotI profiles. Genotypes gII and gIII were found from three and two farms, respectively (Table 3). The other genotypes were found from only one farm each. From three farms, only one genotype was recovered.

All of the genotypes isolated from pluck sets and carcasses were also isolated from pigs of the same farm (Table 3). In addition, genotypes obtained from carcasses were also found in rectal swab, intestinal content, or tonsil samples from the same individual pigs (Table 4). Four of the six *Y. pseudotuberculosis*-positive pluck sets were from pigs in which the same *Y. pseudotuberculosis* genotype was also obtained from other samples.

The prevalence of *Y. pseudotuberculosis* was higher in all sample types in organic pork production than in conventional pork production (Tables 1 and 2). A significant difference was present in the total number of positive rectal swab (chi-square test, $P < 0.001$), tonsil ($P < 0.001$), pluck set ($P < 0.010$), and carcass ($P < 0.001$) samples between the organic and conven-

TABLE 3. *Yersinia pseudotuberculosis* genotypes and numbers of positive samples from *Yersinia pseudotuberculosis*-positive farms

Farm	Farm type	Genotype(s) (no. of positive samples) from sample type:					
		Rectal swab ^a	Intestinal content	Tonsils	Pluck set	Carcass	Total
A	Organic	gII (12), ^A gIV (2), ^{A,B} gVII (1), ^B gVIII (1)	gII (8)	gII (7), gIV (1), gVIII (1)	gII (4), gIV (1)	gII (6), gIV (2), gVIII (1)	gII (36), gIV (6), gVII (1), gVIII (3)
D	Organic	gIII (6)	gIII (1)	gIII (17), gV (1)		gIII (1)	gIII (25), gV (1)
F	Conventional	gIII (6)	gIII (13)	gII (3), gIII (1)			gII (3), gIII (20)
K	Conventional			gVI (1)			gVI (1)
M	Organic	gI (3)	gI (2)	gI (1)			gI (6)
O	Conventional			gII (1)	gII (1)		gII (2)

^a The same capital superscript letter indicates that the two different genotypes were isolated from the same sample.

tional production types when sampling was assumed to be randomized but not between farms from the two systems when the fact that 21 to 26 pigs were sampled from each farm was considered (Mann-Whitney U test). The prevalence of *Y. pseudotuberculosis* was higher in rectal swab, intestinal content, and tonsil samples on conventional farms with high production capacity ($\geq 1,000$ pigs) than on those with low production capacity ($< 1,000$ pigs) (Tables 1 and 2). The difference between farms with low and high production capacities in conventional production was significant in the total numbers of positive rectal swab (chi-square test, $P < 0.050$) and intestinal content ($P < 0.001$) samples when sampling was assumed to be randomized but not when the fact that 21 to 26 pigs were sampled from each conventional farm was considered (Mann-Whitney U test).

In correlation and logistic regression analyses, contact with pets and pest animals and the outside environment was associated with a high prevalence of *Y. pseudotuberculosis* on farms (Tables 5 and 6). In correlation analyses, organic production and farm management practices, such as large group size on conventional farms and the use of troughs for drinking, were also associated with high prevalence (Table 5).

Y. pseudotuberculosis was isolated from 76 samples after 7 days of cold enrichment in PMB (Table 7). An additional 27

positive samples were found after an enrichment of 14 days in PMB and alkali treatment. *Y. pseudotuberculosis* was isolated from 48 samples with both (7- and 14-day) cold enrichment steps. Plating onto CIN agar after ITC enrichment did not recover any new positive samples, and *Y. pseudotuberculosis* was isolated from only 12 samples. None of the isolation steps produced all genotypes: 7 days of enrichment produced seven of eight different genotypes (gI to gV, gVII, and gVIII) and 14 days of enrichment produced seven of eight different genotypes (gI to gVI and gVIII). ITC enrichment recovered only two genotypes (gII and gIII).

DISCUSSION

In this study, we were able to demonstrate the transmission of *Y. pseudotuberculosis* from pigs to carcasses and pluck sets by using PFGE analyses of isolated strains. On all farms where *Y. pseudotuberculosis* was isolated from both on-farm and slaughterhouse samples, the same genotypes were found at both farm and slaughterhouse levels, indicating that the contamination detected in the slaughterhouse originates from the farms in question. All carcass-positive pigs harbored the same *Y. pseudotuberculosis* genotype in either rectal, intestinal, or tonsil samples, and it is therefore likely that *Y. pseudotuberculosis* contamination in the carcass derives from *Y. pseudotuberculosis* carriage by the same pig during the fattening period. Cross-contamination at least with pluck sets at the slaughterhouse is also possible, since two of the six pluck set-positive pigs were not *Y. pseudotuberculosis* positive in rectal, intestinal, or tonsil samples. To our knowledge, this is the first time that farms have been demonstrated to be a source of *Y. pseudotuberculosis* contamination for carcasses and pluck sets. However, since not all *Y. pseudotuberculosis* strains from contaminated pluck sets could be linked to a certain pig, the potential role of the slaughterhouse environment in cross-contamination with *Y. pseudotuberculosis* should be further explored.

All of the positive carcasses originated from farms A and D, where the prevalence of *Y. pseudotuberculosis* in pigs (rectal swabs, intestinal content, and tonsils) was high. On the other hand, on farm F, where the prevalence was also very high, no *Y. pseudotuberculosis* was recovered from carcasses or pluck sets. Apparently, the high prevalence in live pigs predisposes carcasses and pluck sets to contamination, but strict hygiene practices in slaughterhouses can reduce contamination.

TABLE 4. Distribution of *Yersinia pseudotuberculosis* genotypes in carcass- or pluck set-positive pigs

Farm and pig	Genotype(s) from sample type:				
	Rectal swab	Intestinal content	Tonsils	Pluck set	Carcass
A					
273	gII		gII		gII
274	gIV, gVII				gIV
275	gII, gVIII			gII	gVIII
276		gII			gII
280				gII	
286	gII		gII		gII
287	gII, gIV	gII		gIV	gIV
289	gII				gII
291	gII		gII	gII	
292	gII			gII	gII
297		gII			gII
D					
204			gIII		gIII
O					
304				gII	

TABLE 5. Farm factors associated with the prevalence of *Yersinia pseudotuberculosis* in herds with correlation analyses

Variable	All farms			Conventional			Organic		
	<i>n</i>	<i>r</i> ^a	<i>P</i>	<i>n</i>	<i>r</i> ^a	<i>P</i>	<i>n</i>	<i>r</i> ^a	<i>P</i>
Moderate or large numbers of rodents in the piggery	15	0.931	0.000	10	NC ^c	NC	5	0.984	0.002
Low stocking density (m ² /pig)	15	0.806	0.000	10	-0.084	0.817	5	0.845	0.072
Number of mo that pigs have access to outdoor area	15	0.704	0.003	10	NC	NC	5	0.598	0.286
Pest and pet animals hygiene score ^b	15	0.673	0.006	10	0.278	0.436	5	0.628	0.257
Slaughter pigs have access to outdoor areas	15	0.647	0.009	10	NC	NC	5	0.482	0.411
Birds have access to piggery	15	0.636	0.011	10	0.650	0.050	5	0.590	0.295
Dogs have access to piggery	9	0.722	0.028	5	-0.250	0.685	4	0.985	0.015
Slaughter pigs drink from trough	15	0.560	0.030	10	0.550	0.099	5	0.590	0.295
Organic production	15	0.529	0.043	10	NC	NC	5	NC	NC
Moist area around drinker	15	0	1.000	10	0.002	0.999	5	0.984	0.002
Large group size	15	-0.202	0.471	10	0.939	0.047	5	-0.546	0.341

^a Pearson correlation coefficient.

^b From the work of Siekkinen et al. (30).

^c NC, could not be calculated.

The prevalence of *Y. pseudotuberculosis* was higher in organically produced pigs than in conventionally produced pigs, but the wide range of within-farm prevalence suggests that there are some farm-specific factors that affect the prevalence of *Y. pseudotuberculosis* on farms, even within the same production system. These farm factors seem to include contact with animals and with the outside environment. *Y. pseudotuberculosis* has been isolated from many different animals and soil (8, 9, 24, 26), and some *Y. pseudotuberculosis* strains from wild animals, the environment, and pigs have been shown to have the same patterns in restriction endonuclease analysis of virulence plasmids (9). Pest animals and the environment are therefore a possible initial source of *Y. pseudotuberculosis* for pigs on farms. However, some authors have proposed that, e.g., *Y. enterocolitica* would more likely move from pigs to rodents than from rodents to pigs (1, 17). Irrespective of the initial source of *Y. pseudotuberculosis*, pest animals seem to have a substantial role in spreading and maintaining the *Y. pseudotuberculosis* contamination on the farm. The higher prevalence of *Y. pseudotuberculosis* on organic farms may be explained by the large number of pest and pet animal contacts of organically farmed pigs. Pigs in conventional farming are kept indoors year round, having fewer outdoor contacts than organically farmed pigs and therefore fewer possibilities of receiving *Y. pseudotuberculosis* from the environment.

Farm management practices, such as large group size on conventional farms or the use of troughs for drinking, can spread the infection from one pig to another at the piggery:

pigs in a pen can contaminate the water in the trough with feces, spreading *Y. pseudotuberculosis* to other pigs in a manner similar to that for *Salmonella* strains (4, 39). Moreover, one carrier can infect numerous other pigs with *Y. pseudotuberculosis* in pig-to-pig contacts. Production capacity also seems to affect the prevalence of *Y. pseudotuberculosis*: in this study the prevalence was higher on conventional farms with high production capacity than on low-capacity farms. In a previous study (1), high within-farm prevalence of *Yersinia* was associated with poor hygiene conditions, but no such relationship was seen in our study or in an on-farm observation and questionnaire study by Siekkinen et al. (30), which evaluated the same organic and conventional pig farms as those in our study.

On-farm management of pet and pest animals seems to be an integral component in maintaining *Y. pseudotuberculosis*-negative farms. The possibility of spreading *Y. pseudotuberculosis* contamination via troughs and pig-to-pig contacts should also be minimized. Because of the significance of pest animals and outdoor contacts in the introduction or maintenance of *Y. pseudotuberculosis* contamination on farms, producing and maintaining *Y. pseudotuberculosis*-free pig farms, especially in an organic production system, can be difficult. Slaughter hygiene and slaughter methods are therefore important in preventing carcass contamination.

The two cold enrichment steps used were almost equally effective, and neither of the isolation steps can be omitted

TABLE 6. Results of a two-level multivariate logistic regression analysis for farm factors associated with the prevalence of *Yersinia pseudotuberculosis* in herds^a

Variable	Estimate	Odds ratio	95% CI of odds ratio	<i>P</i>
Constant	-4.353	0.01	0.003-0.051	0.000
Birds have access to piggery	2.575	13.13	1.966-87.72	0.008
Number of mo that pigs have access to outdoor area	0.269	1.31	1.041-1.646	0.021

^a Herd variance (σ^2_{farm}), 1.660 (standard error = 1.016), *P* value = 0.103 (Wald test); intraclass correlation coefficient = 0.492. CI, confidence interval.

TABLE 7. Number of *Yersinia pseudotuberculosis*-positive samples after cold enrichment

Sample material	<i>n</i>	No. (%) of positive samples in different isolation steps		Total no. (%) of positive samples
		7 days in PMB and CIN	14 days in PMB, KOH, and CIN	
Rectal swab	364	28 (8)	20 (5)	29 (8)
Intestinal content	358	20 (6)	17 (5)	24 (7)
Tonsils	350	21 (6)	22 (6)	34 (10)
Pluck set	354	3 (1)	6 (2)	6 (2)
Carcass	359	4 (1)	9 (3)	10 (3)
Total	1,785	76 (4)	74 (4)	103 (6)

without a marked reduction in isolation rate. ITC enrichment performed poorly in the isolation of *Y. pseudotuberculosis*. ITC enrichment is part of the International Organization for Standardization standard for isolation of *Y. enterocolitica* (14) and has been used efficiently in isolation of this pathogen (5), but no data on the use of ITC in isolation of *Y. pseudotuberculosis* are available. In agreement with our results, Niskanen et al. (25) reported that all *Y. pseudotuberculosis* strains from tonsils were isolated after cold enrichment when direct plating, overnight enrichment in tryptic soy broth, and selective enrichment in modified Rappaport broth for 3 days were used. A possible reason for poor performance in ITC enrichment is weak growth of *Y. pseudotuberculosis* with strongly selective media. CIN, for example, has been shown to inhibit the growth of *Y. pseudotuberculosis* (7), and two selective media (ITC and CIN) may have overwhelmed the bacteria.

Following the same pigs from farm to slaughterhouse, we were able to conclude that *Y. pseudotuberculosis* contamination originates from the farms, is transported to slaughterhouses with pigs, and transfers to pluck sets and carcasses in the slaughter process. The prevalence of *Y. pseudotuberculosis* was higher in organic production than in conventional production and on conventional farms with high rather than low production capacity. We were also able to associate specific farm factors with the prevalence of *Y. pseudotuberculosis* by using questionnaires and on-farm observations. On farms, contact with pest animals and the outside environment and the rise in the number of pigs on the farm appear to increase the prevalence of *Y. pseudotuberculosis*.

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