Correspondence of genotypes of sporadic *Yersinia enterocolitica* bioserotype 4/O:3 strains from human and porcine sources

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SUMMARY

The sources and transmission routes of sporadic *Yersinia enterocolitica* bioserotype 4/O:3 infections in Finland were studied. A total of 212 human strains were compared with 334 non-human strains, including 163 strains from pig slaughterhouses, 164 strains from retail outlets and 7 strains from pet animals. All strains were characterized using pulsed-field gel electrophoresis (PFGE) with *Not*I enzyme. When the 194 human and 287 non-human strains of 22 identical *Not*I profiles were further characterized with *Apa*I and *Xho*I enzymes, 126 genotypes (DI = 0·94) were distinguished. Of all 212 human strains, 80% were genetically indistinguishable from the strains found in samples of pig origin when characterized with the three enzymes. A major contamination source of sporadic *Y. enterocolitica* 4/O:3 infections was revealed to be edible pig offal: 71% of the human strains were indistinguishable from the strains isolated from tongues, livers, kidneys and hearts of pigs. These results reveal that in Finland contaminated pig offal is an important vehicle in the transmission of *Y. enterocolitica* bioserotype 4/O:3 from slaughterhouses to humans.

INTRODUCTION

Yersinia enterocolitica is a highly heterogeneous species and has therefore been divided into various bioserotypes, only a few of which are associated with human disease [1]. *Y. enterocolitica* belonging to bioserotype 4/O:3 is a common cause of human yersiniosis and present in regions where pig reservoirs prevail [1]. This type accounts for most of the *Y. enterocolitica* infections globally [2]. All pathogenic *Y. enterocolitica* strains carry a plasmid which is essential for virulence [1]. In Finland, the annual incidence rates of reported *Y. enterocolitica* infections have ranged between 564–873 cases per 5 million persons during 1995–9 [3]. The most common symptom is

diarrhoea, typically occurring in young children and secondary immunologically induced sequelae, such as reactive arthritis, are not uncommon, especially in HLA-B27-positive individuals [1]. Strains of bioserotype 4/O:3 generally cause sporadic infections which cannot be traced to a vehicle or event [1] and so far only one outbreak of serotype O:3 strains associated with household preparation of chitterlings has been reported [4].

The epidemiology of *Y. enterocolitica* infections is poorly understood. Strains of bioserotype 4/O:3 are frequently isolated from swine, suggesting that these are an important reservoir for the organism [5] but transmission from pigs to humans has not been proven. In case-control studies, food, particularly undercooked pork, has been shown to be a primary source of infections [6, 7], although pathogenic isolates have seldom been recovered from pork or any other

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foods, with the exception of fresh pig tongues [8]. *Y. enterocolitica* bioserotype 4/O:3 is thus far the only pathogenic bioserotype found in food samples in Finland, and it has only been isolated from foods originating from pigs with the highest prevalence in samples of pig offal, including tongue, heart, liver and kidney [9, 10].

In order to identify reservoirs of infection, transmission vehicles and associations between clinical cases, several DNA-based methods have been used for subtyping of *Y. enterocolitica* bioserotype 4/O:3 [11–13], pulsed-field gel electrophoresis (PFGE) being among the most sensitive [14]. Genetic diversity is limited in bioserotype 4/O:3 [15], but with PFGE using *Not*I, *Apa*I and *Xho*I enzymes, this group can be efficiently divided into several genotypes with a discrimination index of 0.93 [16]. In this study, we have used this method to identify sources and possible transmission routes of sporadic *Y. enterocolitica* 4/O:3 infections.

MATERIALS AND METHODS

Strains of bioserotype 4/O:3

A total of 546 strains of *Y. enterocolitica* belonging to bioserotype 4/O:3 isolated during 1995–9 were studied (Table 1). Of these strains, 212 were from humans and 334 from non-human sources, including 163 strains from slaughterhouses, 164 from retail outlets and 7 from pet animals. Human strains were isolated from faecal samples of patients with diarrhoea from different parts of Finland. Porcine and environmental strains were recovered from samples collected from 9 pig slaughterhouses and 17 retail outlets. The pet strains were isolated from faeces from dogs with diarrhoea, and from asymptomatic cats. The strains were biotyped according to the revised scheme of Wauters et al. [17] and serotyped by slide agglutination using O:3 antiserum (Denka Seiken, Tokyo, Japan).

Detection of *yadA* gene in the strains of bioserotype 4/O:3 with PCR

The pathogenicity of the strains was determined with the nested PCR method targeting the *yadA* gene on the virulence plasmid according to Kapperud et al. [18]. Four small colonies (< 2 mm) from pure blood agar culture were boiled in 100 μl of water for 10 min, and 2 μl of this suspension was used as a template in the first PCR step.

Table 1. *Origin of strains of* Y. enterocolitica *bioserotype 4/O:3 isolated during 1995–9*

Samples	Year of isolation	No. of strains	No. of *yadA*-positive strains
Pig slaughterhouses	1995–9		
Tonsils		62	49
Faeces		6	6
Carcass		38	37
Heart		10	9
Kidney		19	17
Liver		13	12
Equipment		8	6
Sludge		3	3
Air		4	4
Retail outlets	1996–9		
Pig tongue		138	135
Pig heart		3	3
Pig kidney		8	8
Pork		15	14
Pet animals	1998–9		
Dogs		5	5
Cats		2	2
Humans	1996–9	212	205
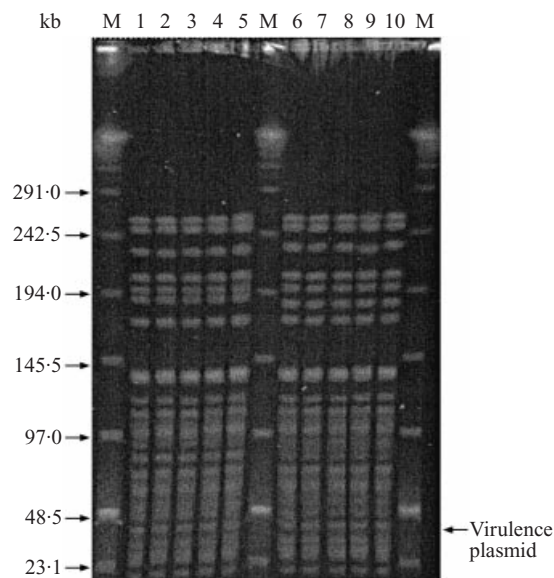
Fig. 1. The two most common *Not*I profiles, NA1 and NB1, found in human infections and in samples of pig origin and in pet animals. Lanes: M, Low Range Molecular weight marker; 1–5, NA1 profile; 6–10, NB1 profile; 1, pig tonsil strain; 2, pig liver; 3, pork; 4, cat; 5, human; 6, tonsil; 7, air from a pig slaughterhouse; 8, pig heart from a retail outlet; 9, dog; 10, human.

DNA isolation, digestion with *Not*I, *Apa*I and *Xho*I enzymes, and pulsed-field gel electrophoresis

DNA isolation, digestion with different enzymes and

Table 2. *Distribution of* NotI *profiles of 546* Y. enterocolitica *bioserotype 4/O:3 strains recovered from humans, pig slaughterhouses, retail outlets and pet animals*

NotI profile	Humans Faeces (n = 212)	Pig slaughterhouses (n = 163)* Tonsils (n = 62)	Faeces (n = 6)	Carcass (n = 38)	Heart (n = 10)	Kidney (n = 19)	Liver (n = 13)	Equipment (n = 8)	Sludge (n = 3)	Air (n = 4)	Retail outlets (n = 164) Pig tongue (n = 138)	Pig heart (n = 3)	Pig Kidney (n = 8)	Pork (n = 15)	Pet animals Dog faeces (n = 5)	Cat faeces (n = 2)	Total no. of strains (n = 546)
NA1	86	25	2	22	4	6	5	3	1	2	56	2	3	8	1	2	228
NA2		1															1
NA3	11	1	1		1						4						18
NA4	8	1		1	1	1	2	1			15		1	1			32
NA5				1													1
NA6											1						1
NA7	4										2						6
NA8	6					1		1	1		9						18
NA9											3						3
NA10											1						1
NA11											1						1
NA12	3										1		1	1			6
NA13		1															1
NA14											1		1				2
NA15							1										1
NA16				1													1
NA17	10										2	1					13
NA18	3			1							1						5
NA19	1	1		1							1						4
NA20	1																1
NA21	1																1
NA22	1																1
NA23	1																1
NA24	1																1
NA25	1																1
NB1	24	12	2	3		3		2		1	21	1		2	1		72
NB2	10	1		1	1	1	4										18
NB3		2			1						3						6
NB4	1	1															2
NB5	6	1								1	1					3	12
NB6		1															1
NB7		1															1
NB8		1															1
NB9	1	2		4	1												8

[continued overleaf

Genotypes of Y. enterocolitica 4/O:3 strains   39

Table 2 (cont.)

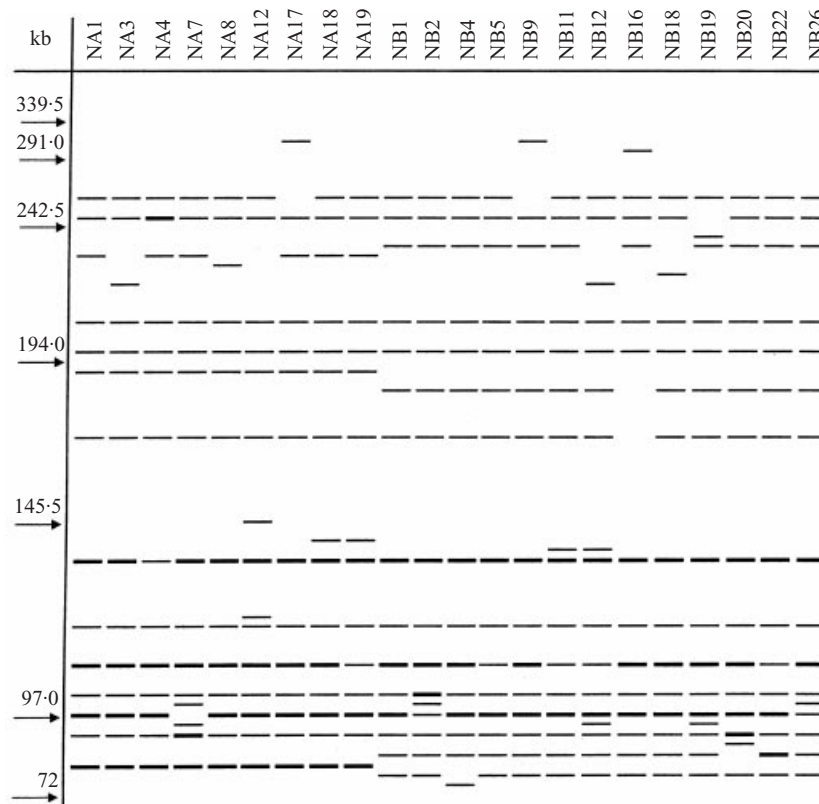| NotI profile	Humans Faeces (n = 212)	Pig slaughterhouses (n = 163)[a]									Retail outlets (n = 164)				Pet animals		Total no. of strains (n = 546)
		Tonsils (n = 62)	Faeces (n = 6)	Carcass (n = 38)	Heart (n = 10)	Kidney (n = 19)	Liver (n = 13)	Equipment (n = 8)	Sludge (n = 3)	Air (n = 4)	Pig tongue (n = 138)	Pig heart (n = 3)	Pig Kidney (n = 8)	Pork (n = 15)	Dog faeces (n = 5)	Cat faeces (n = 2)	
NB10		1															1
NB11	2	2									1						5
NB12	1	1															2
NB13		2															2
NB14		1															1
NB15		1									1						2
NB16	2	1				1											4
NB17											1			1			2
NB18	1														1		2
NB19	3			1													4
NB20	2										2						4
NB21				2							1				1		4
NB22	7					3	1		1		1				1		14
NB23											1						1
NB24											2						2
NB25						1					1						2
NB26	2	1			1												4
NB27						2	1				1						4
NB28	1																1
NB29	1																1
NB30	1																1
NB31	2																2
NB32	3																3
NB33	1																1
NB34	2																2
NB35	1																1
NB36											1						1
NB37		1															1
NB38											1						1
NB39											1						1

* Number of samples in parenthesis.

Fig. 2. Twenty-two different *Not*I profiles of *Y. enterocolitica* 4/O:3 isolates recovered from human and non-human sources.

PFGE were performed according to Fredriksson-Ahomaa et al. [16]. All human and non-human strains that gave identical DNA profiles with *Not*I enzyme were further characterized with *Apa*I and *Xho*I enzymes as described previously [16]. Strains were considered to be different if any band differences in fragments over 70 kb was observed.

RESULTS

A total of 515 out of 546 strains were *yadA* positive (Table 1). A fragment of about 40 kb was observed in PFGE patterns of *yadA*-positive strains (Fig. 1) but not in *yadA*-negative strains when *Not*I enzyme was used. The plasmid did not interfere with the PFGE patterns produced by any enzymes in this study since only fragments over 70 kb were used for strain discrimination.

In all, 64 different PFGE profiles were obtained when 546 strains of *Y. enterocolitica* bioserotype 4/O:3 were characterized with PFGE using *Not*I enzyme (Table 2). The discrimination index was 0·80 [19]. The two most common *Not*I profiles, NA1 and NB1, were found in human infections, in samples of pig origin and in pet animals (Fig. 1). Altogether, 194

(92%) of 212 human strains were indistinguishable from 140 (86%) of 163 strains from slaughterhouses, 140 (85%) of 164 strains from retail outlets and all 7 strains from pet animals. These 481 strains belonging to 22 genotypes were further characterized with *Apa*I and *Xho*I enzymes (Fig. 2). The number of different genotypes increased from 64 to 126, and the discrimination index rose from 0·80 to 0·94 when all the human and non-human strains with identical *Not*I profiles were further characterized with *Apa*I and *Xho*I. Of the 126 genotypes, 68 were found among human strains and 97 in strains of pig origin. A total of 170 (80%) of 212 human strains belonging to 39 genotypes were genetically indistinguishable from strains of pig origin when characterized with all three enzymes (Table 3).

Altogether, 114 (54%) of 212 human strains were indistinguishable from tonsil strains. A total of 56 (59%) of 95 strains found in pig slaughterhouses from carcasses, offal and the environment were of human genotypes and were indistinguishable from strains found in tonsils. Furthermore, 75 (35%) and 110 (52%) strains found in human infections were indistinguishable from strains recovered in pig slaughterhouses on carcasses and edible offal (heart,

Table 3. *Sources of 39 different genotypes found in 170 human strains of* Y. enterocolitica *bioserotype 4/O:3 from sporadic infections*

Genotype	PFGE-patterns obtained by *Not*I, *Apa*I and *Xho*I	No. of human strains	Sources in pig slaughterhouses (No. of strains)	Sources in retail outlets (No. of strains)	Pet animals (No. of strains)
G1	NA1/AA1/HA1	4	Tonsil (3)	Pig tongue (7), pork (1)	
G2	NA1/AA1/HA2	1		Tongue (4), pork (1)	
G3	NA1/AA1/HA3	5	Tonsil (2), faeces (1), heart (1), liver (1), kidney (1), conveyor (1)	Tongue (5), pig heart (1), pig kidney (1)	
G4	NA1/AA2/HA2	15	Tonsil (4), heart (1)	Tongue (4)	
G5	NA1/AA2/HA3	47	Tonsil (8), faeces (1), carcass (19), heart (2), liver (3), kidney (4), brisket saw (1), sludge (1), air (1)	Tongue (24), pork (5), kidney (2)	Cat (1)
G6	NA1/AA2/HA4	1	Carcass (2), liver (1), air (1)	Tongue (4)	
G7	NA1/AA2/HA8	1		Tongue (1), heart (1)	
G8	NA1/AA2/HA10	1	Tonsil (2)	Pork (1)	
G9	NA1/AA2/HA15	3	Tonsil (1)		
G10	NA1/AA9/HA3	3	Tonsil (1)		
G11	NA1/AA10/HA3	2		Tongue (1)	
G12	NA3/AA15/HA3	9	Tonsil (1), heart (1)	Tongue (4)	
G13	NA4/AA1/HA3	2	Liver (1), kidney (1)	Tongue (11), pork (1)	
G14	NA4/AA2/HA3	6	Tonsil (1), carcass (1), heart (1), liver (1), computer keyboard (1)	Tongue (4), kidney (1)	
G15	NA7/AA3/HA15	3		Tongue (2)	
G16	NA8/AA4/HA4	1		Tongue (7)	
G17	NA8/AA17/HA2	1		Tongue (2)	
G18	NA8/AA17/HA3	4	Kidney (1), hooks (1), sludge (1)		
G19	NA12/AA13/HA3	3		Kidney (1), pork (1)	
G20	NA17/AA2/HA3	5		Tongue (2), kidney (1)	

Genotype	PFGE-patterns obtained by *Not*I, *Apa*I and *Xho*I	No. of human strains	Sources in pig slaughterhouses (No. of strains)	Sources in retail outlets (No. of strains)	Pet animals (No. of strains)
G21	NA18/AA30/HA19	3	Carcass (1)	Tongue (1)	
G22	NA19/AA2/HA3	1		Tongue (1)	
G23	NB1/AB1/HB1	8	Tonsil (8), carcass (1), kidney (3), computer keyboard (1), conveyor (1), air (1)	Tongue (15), heart (1)	Dog (1)
G24	NB1/AB1/HB7	4		Tongue (1)	
G25	NB1/AB2/HB2	2		Tongue (1)	
G26	NB1/AB4/HB1	1	Carcass (1)		
G27	NB1/AB4/HB2	1		Pork (1)	
G28	NB1/AB7/HB1	4		Tongue (1)	
G29	NB2/AB15/HB1	6	Tonsil (1), carcass (1), heart (1), liver (4), kidney (1)		
G30	NB4/AB28/HB1	1	Tonsil (1)		
G31	NB5/AB4/HB1	5		Tongue (1)	
G32	NB11/AB24/HB12	2	Tonsil (1)		
G33	NB12/AB24/HB12	1	Tonsil (1)		
G34	NB16/AB22/HB13	2	Tonsil (1)		
G35	NB18/AB18/HB2	1		Pork (1)	
G36	NB19/AB1/HB1	3	Carcass (1)		
G37	NB20/AB2/HB2	1		Tongue (2)	
G38	NB22/AB19/HB1	6	Liver (1), kidney (3), sludge (1)	Tongue (1), pork (1)	
G39	NB26/AB14/HB1	1	Tonsil (1), heart (1)		

liver, kidneys), respectively. In addition, 140 (66%) and 66 (31%) human strains were indistinguishable from strains from retail outlets of pig offal (tongues, kidneys and hearts) and pork, respectively. In all, 151 (71%) of the human strains were genetically indistinguishable from strains isolated from pig tongues, hearts, livers and kidneys from slaughterhouses and retail outlets.

The most common genotypes (G4, G5, G12, G14, G23, G29, G38), representing 46% (97/212) of the strains in human infections, were found in pig slaughterhouses from tonsils, carcasses, livers, hearts, kidneys, equipment, sludge and air, and at the retail level from pig tongues, hearts, kidneys and pork (Table 3). Two common genotypes, G5 and G23, found in human infections were also found in a cat and dog, respectively. The possible transmission routes of different genotypes, G1–G39, found in sporadic *Y. enterocolitica* bioserotype 4/O:3 infections were direct transmission from pigs to man and indirect transmission from pigs to man via contaminated pork, especially pig offal, and via contaminated environment and pet animals (Fig. 3).

DISCUSSION

A total of 80% of human strains of *Y. enterocolitica* were indistinguishable from strains of pig origin from slaughterhouses and retail outlets when characterized with *Not*I, *Apa*I and *Xho*I enzymes, supporting the hypothesis that the pig is the main source of sporadic human *Y. enterocolitica* bioserotype 4/O:3 infections. Several possible transmission routes exist from pigs to humans. One transmission link may be direct contact with pigs, a common risk for pig farmers and slaughterhouse workers. The transmission of *Y. enterocolitica* 4/O:3 from pigs directly to humans has not thus far been proven, however, an increased frequency of antibodies against *Y. enterocolitica* O:3 among slaughterhouse workers and pig farmers has been reported [20, 21].

Many human genotypes were recovered from pig slaughterhouses. Pig tonsils harbouring *Y. enterocolitica* 4/O:3 appear to be an important source of contamination in pig slaughterhouses since 59% of the strains recovered from pig carcasses, offal and the slaughterhouse environment belonging to human genotypes were indistinguishable from tonsil strains. The yersinia-positive tonsils may contaminate the carcass, offal and the environment during the slaughtering process. Contamination of offal is un-

avoidable when the tonsils are removed along with the tongue, liver, kidneys and heart, and all are placed together on a conveyor or hung on a hook [22]. We detected *yadA*-positive *Y. enterocolitica* on 98% of tongues, 38% of livers, 86% of kidneys and 63% of hearts in earlier studies [9, 10]. Pig faeces also represent another source of contamination in slaughterhouses [22]. Sealing off the pig rectum with a plastic bag prior to evisceration has been shown to decrease intestinal spread to the carcass [23] and this method is now widely used in Denmark, Norway and Sweden [22].

Y. enterocolitica 4/O:3 can be transmitted from pig slaughterhouses to meat processing plants and then to retail level via contaminated pig carcasses and offal. We established that 35% and 52% of the human strains were indistinguishable from strains found in pig slaughterhouses on carcasses and offal, respectively. Many of the human genotypes identified in slaughterhouses were also found in retail outlets, demonstrating a possible transmission route from slaughterhouses to retail outlets, especially due to contaminated pork and edible offal.

Human genotypes were recovered from pig tongues, hearts, kidneys and pork at the retail level, suggesting that *Y. enterocolitica* infections of bioserotype 4/O:3 may also be of food origin. Contaminated pig offal and pork may be an important transmission vehicle of this bacterium from retail outlets to man, since 66% and 31% of the strains isolated from human infections were identical to strains isolated from offal and pork, respectively. Data from case-control studies strongly supports the association between yersiniosis and the eating of raw or undercooked pork products [6, 7]. Raw meat, particularly pork, is not eaten in Finland, except for occasional nibbling on raw minced pork while preparing pork dishes. Cross-contamination of cooked foods or foods not normally harbouring *Y. enterocolitica* is the more probable route of infection.

Contaminated pig offal was revealed to be the primary transmission vehicle of this bacterium to man, since 71% of the human strains were indistinguishable from strains isolated from tongues, livers, kidneys and hearts. Cross-contamination from offal will occur directly or indirectly via equipment, air and food handlers in slaughterhouses, retail outlets and residential kitchens. As a psychrotrophic microbe, *Y. enterocolitica* is able to multiply along the cold-chain from the slaughterhouse to the home refrigerator. Two common genotypes, G5 and G23, found in human infections were often recovered from offal, but also from a cat and a dog, respectively. Thus, another
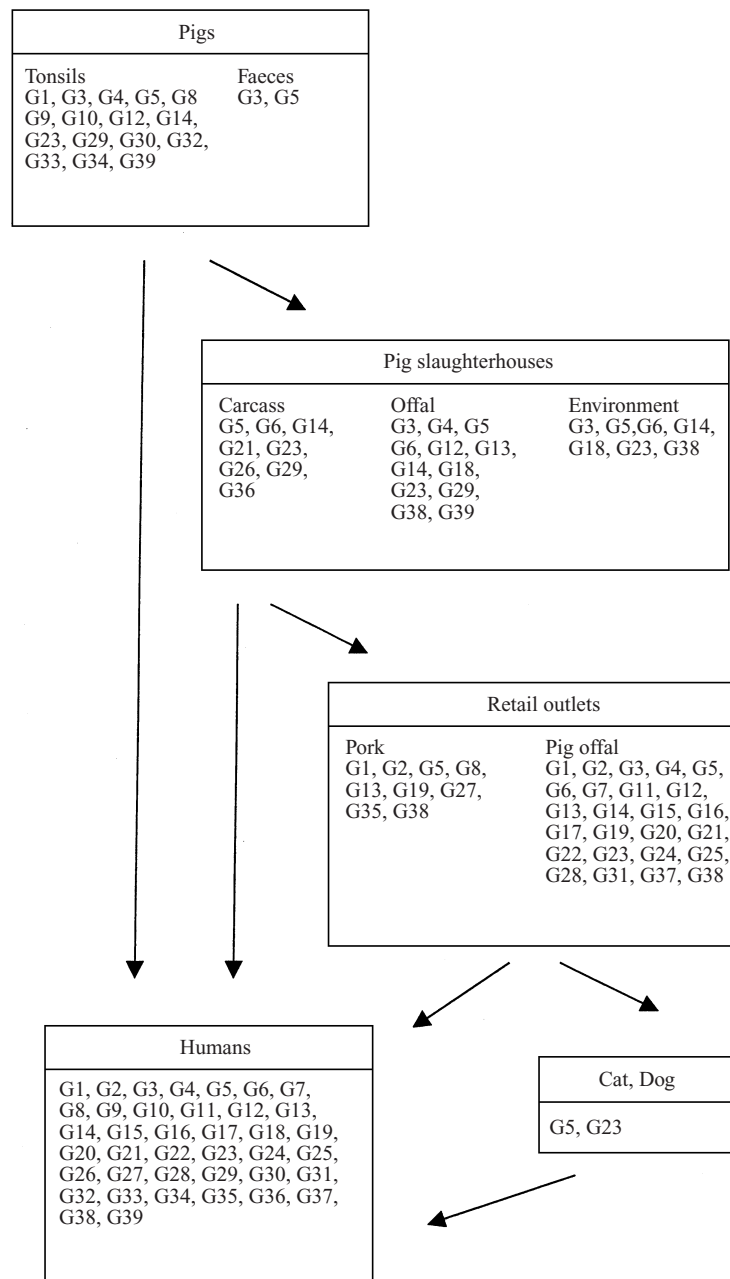
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┌─────────────────────────────────────┐
│                 Pigs                 │
├──────────────────────┬──────────────┤
│ Tonsils              │ Faeces       │
│ G1, G3, G4, G5, G8   │ G3, G5       │
│ G9, G10, G12, G14,   │              │
│ G23, G29, G30, G32,  │              │
│ G33, G34, G39        │              │
└──────────────────────┴──────────────┘

┌────────────────────────────────────────────────────┐
│                 Pig slaughterhouses                 │
├──────────────┬──────────────┬──────────────────────┤
│ Carcass      │ Offal        │ Environment          │
│ G5, G6, G14, │ G3, G4, G5   │ G3, G5,G6, G14,      │
│ G21, G23,    │ G6, G12, G13,│ G18, G23, G38        │
│ G26, G29,    │ G14, G18,    │                      │
│ G36          │ G23, G29,    │                      │
│              │ G38, G39     │                      │
└──────────────┴──────────────┴──────────────────────┘

┌────────────────────────────────────────────┐
│               Retail outlets                │
├──────────────────┬─────────────────────────┤
│ Pork             │ Pig offal               │
│ G1, G2, G5, G8,  │ G1, G2, G3, G4, G5,     │
│ G13, G19, G27,   │ G6, G7, G11, G12,       │
│ G35, G38         │ G13, G14, G15, G16,     │
│                  │ G17, G19, G20, G21,     │
│                  │ G22, G23, G24, G25,     │
│                  │ G28, G31, G37, G38      │
└──────────────────┴─────────────────────────┘

┌───────────────────────────────┐    ┌────────────────┐
│            Humans             │    │   Cat, Dog     │
├───────────────────────────────┤    ├────────────────┤
│ G1, G2, G3, G4, G5, G6, G7,   │    │ G5, G23        │
│ G8, G9, G10, G11, G12, G13,   │    └────────────────┘
│ G14, G15, G16, G17, G18, G19, │
│ G20, G21, G22, G23, G24, G25, │
│ G26, G27, G28, G29, G30, G31, │
│ G32, G33, G34, G35, G36, G37, │
│ G38, G39                      │
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Fig. 3. Possible transmission routes of different genotypes, G1–G39, found in sporadic *Y. enterocolitica* bioserotype 4/O:3 infections.

transmission link may be pet animals, which are fed raw contaminated offal and may subsequently transmit yersinia via faeces to humans, especially to small children. Transmission from pets to humans has not been proven to date, but Fenwick et al. [24] have shown that dogs can carry *Y. enterocolitica* bioserotype 4/O:3 asymptomatically and excrete this organism in the faeces for weeks.

A total of 29 genotypes, including 42 (20%) human strains, were not present in slaughterhouses or at retail level. Reasons for this may be that the missing genotypes are so rare that we have been unable to locate them or that the number of samples is too small. It is also possible that infections arising from the strains belonging to these genotypes are obtained from abroad or that they are not of pig origin but from some unknown source. The biodiversity among the strains of pig origin was observed to be higher than that among the human strains. Fifty-eight genotypes, containing 87 (27%) strains, found in slaughterhouses and at retail level were not recovered from humans. A possible explanation may be that the

strains belonging to these genotypes have a lower virulence, thus causing only mild infections and needing a higher infective dose.

Most of the strains in this study were *yadA* positive and were therefore expected to carry the virulence plasmid. This plasmid is needed for expression of full pathogenicity but can be lost during isolation, especially at a temperature of 37 °C, and during prolonged storage [25]. However, only 3% (7/212) of the human strains and 7% (24/334) of the non-human strains were *yadA*-negative.

The strains were considered different when even a one-band difference was noted, because the genetic variation within *Y. enterocolitica* 4/O:3 has been demonstrated to be limited [13, 15, 26], the genotypes have been shown to be stable *in vitro* [27], and the same band difference rule has been used to differentiate *Y. enterocolitica* strains in all previous PFGE studies [13–15, 26, 27]. The *Not*I enzyme proved to have a good discriminatory power (DI = 0·80) in this study when compared with other methods used in earlier epidemiological studies [11, 12]. To increase discriminatory power, the strains with identical *Not*I profiles were further characterized with *Apa*I and *Xho*I. The discrimination index of 0·94 obtained in this study is slightly higher than in our earlier study [16].

The possible transmission of human genotypes from pig tonsils to offal and carcasses and from offal and pork to humans has been demonstrated. Prevention of *Y. enterocolitica* infection should be directed at reducing the spread of the bacterium from the pig oral cavity to offal, carcasses and the environment during slaughter. According to the European Union legislation (64/433/EEC), the tonsils and tongue must be removed from the head prior to meat inspection, and the head must be attached to the carcass during the inspection. However, this study shows how important it is to remove the head containing the highly contaminated tonsils and tongue before evisceration and to inspect and handle them in a separate room with separate tools to reduce the human infections received from contaminated offal and carcasses.

ACKNOWLEDGEMENTS

REFERENCES

1. Bottone EJ. *Yersinia enterocolitica*: overview and epidemiologic correlates. Microb Infect 1999; **1**: 323–33.
2. Ostroff S. *Yersinia* as an emerging infection: epidemiologic aspects of yersiniosis. Contrib Microbiol Immunol 1995; **13**: 5–10.
3. National Public Health Institute (Finland). Infectious diseases in Finland 1995–1999. Helsinki. KTL B4/2000.
4. Lee A, Gerber RA, Lonsway DR, et al. *Yersinia enterocolitica* O:3 infections in infants and children, associated with the household preparation of chitterlings. N Engl J Med 1990; **322**: 984–7.
5. Doyle MP. Pathogenic *Escherichia coli*, *Yersinia enterocolitica*, and *Vibrio parahemolyticus*. Lancet 1990; **336**: 111–5.
6. Tauxe RV, Wauters G, Goossens V, et al. *Yersinia enterocolitica* infections and pork: the missing link. Lancet 1987; **i**: 1129–32.
7. Ostroff SM, Kapperud G, Hutwagner LC, et al. Sources of sporadic *Yersinia enterocolitica* infections in Norway: a prospective case-control study. Epidemiol Infect 1994; **112**: 133–41.
8. de Boer E. Isolation of *Yersinia enterocolitica* from foods. Contrib Microbiol Immunol 1995; **13**: 71–3.
9. Fredriksson-Ahomaa M, Hielm S, Korkeala H, High prevalence of *yadA*-positive *Yersinia enterocolitica* in pig tongues and minced meat at retail level in Finland. J Food Prot 1999; **62**: 123–7.
10. Fredriksson-Ahomaa M, Korte T, Korkeala H. Contamination of carcasses, offals and the environment with *yadA*-positive *Yersinia enterocolitica* in a pig slaughterhouse. J Food Prot 2000; **63**: 31–5.
11. Kapperud G, Nesbakken T, Aleksic S, Mollaret HH. Comparison of restriction endonuclease analysis and phenotypic typing methods for differentiation of *Yersinia enterocolitica* isolates. J Clin Microbiol 1990; **28**: 1125–31.
12. Mendoza MC, Alzugaray R, Landeras E, González-Hevia MA. Discriminatory power and application of ribotyping of *Yersinia enterocolitica* O:3 in an epidemiological study. Eur J Clin Microbiol Infect Dis 1996; **15**: 220–6.
13. Asplund K, Johansson T, Siitonen A. Evaluation of pulsed-field gel electrophoresis of genomic restriction fragments in the discrimination of *Yersinia enterocolitica* O:3. Epidemiol Infect 1998; **121**: 579–86.
14. Iteman I, Guiyoule A, Carniel E. Comparison of three molecular methods for typing and subtyping pathogenic *Yersinia enterocolitica* strains. J Med Microbiol 1996; **45**: 48–56.

15. Buchrieser C, Weagant SD, Kaspar CW. Molecular characterisation of *Yersinia enterocolitica* by pulsed-field gel electrophoresis and hybridisation of DNA fragments to *ail* and pYV probes. Appl Environ Microbiol 1994; **60**: 4371–9.

16. Fredriksson-Ahomaa M, Autio T, Korkeala H. Efficient subtyping of *Yersinia enterocolitica* bio-serotype 4/O:3 with pulsed-field gel electrophoresis. Lett Appl Microbiol 1999; **29**: 308–12.

17. Wauters G, Kandolo K, Janssens M. Revised bio-grouping scheme of *Yersinia enterocolitica*. Contrib Microbiol Immunol 1987; **9**: 14–21.

18. Kapperud G, Vardund T, Skjerve E, Hornes E, Michaelsen T. Detection of pathogenic *Yersinia enterocolitica* in foods and water by immunomagnetic separation, nested polymerase chain reactions, and colorimetric detection of amplified DNA. Appl Environ Microbiol 1993; **59**: 2938–44.

19. Hunter PR, Gaston MA. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. J Clin Microbiol 1988; **26**: 2465–6.

20. Merilahti-Palo R, Lahesmaa R, Granfors K, Gripenberg-Lerche C, Toivanen P. Risk of yersinia infection among butchers. Scand J Infect Dis 1991; **23**: 55–61.

21. Seuri M, Granfors K. Antibodies against *Yersinia* among farmers and slaughterhouse workers. Scand J Work Environ Health 1992; **18**: 128–32.

22. Borch E, Nesbakken T, Christensen H. Hazard identification in swine slaughter with respect to foodborne bacteria. Int J Food Microbiol 1996; **30**: 9–25.

23. Nesbakken T, Nerbrink E, Røtterud OJ, Borch E. Reduction of *Yersinia enterocolitica* and *Listeria* spp. on pig carcasses by enclosure of the rectum during slaughter. Int J Food Microbiol 1994; **23**: 197–208.

24. Fenwick SG, Madie P, Wilks CR. Duration of carriage and transmission of *Yersinia enterocolitica* biotype 4, serotype O:3 in dogs. Epidemiol Infect 1994; **113**: 471–7.

25. Portnoy DA, Martinez RJ. Role of plasmid in the pathogenicity of *Yersinia* species. Curr Top Microbiol Immunol 1985; **118**: 29–51.

26. Saken E, Roggenkamp A, Aleksic S, Heesemann J. Characterisation of pathogenic *Yersinia enterocolitica* serogroups by pulsed-field gel electrophoresis of genomic *Not*I restriction fragments. J Med Microbiol 1994; **41**: 329–38.

27. Najdenski H, Iteman I, Carniel E. Efficient subtyping of pathogenic *Yersinia enterocolitica* strains by pulsed-field gel electrophoresis. J Clin Microbiol 1994; **32**: 2913–20.