Different enteropathogenic Yersinia strains found in wild boars and domestic pigs

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Different Enteropathogenic *Yersinia* Strains Found in Wild Boars and Domestic Pigs

Maria Fredriksson-Ahomaa,[1] Silke Wacheck,[2] Rebecca Bonke,[2] and Roger Stephan[3]

Abstract

Yersinia enterocolitica and *Yersinia pseudotuberculosis* strains isolated from wild boars and fattening pigs were characterized and compared with each other. In wild boars, *ail*-positive *Y. enterocolitica* strains belonged to bioserotypes 4/O:3 (36%, 5/14), 2/O:9 (29%, 4/14), and 2/O:5,27 (21%, 3/14). Additionally, two *ail*-positive strains were untypable. Among fattening pigs, the bioserotype 4/O:3 was dominating (91%, 71/78), and bioserotypes 2/O:5,27 (8%, 6/78) and 2/O:9 (1%, 1/78) were rare. *inv*-positive *Y. pseudotuberculosis* strains of serotypes O:1 and O:2 were isolated only from wild boars. Antimicrobial resistance patterns between wild boar and fattening pig strains differed. Most of the *ail*-positive *Y. enterocolitica* strains carried *yst*, *hreP,* and *virF* genes. Several genotypes of *Y. enterocolitica* strains were obtained by PFGE using *Not*I, *Apa*I, *Xho*I, and *Spe*I enzymes. All genotypes of wild boar strains differed from fattening pig strains. Especially strains of bioserotype 4/O:3 were clearly different with all four enzymes. These results show that wild boar strains differed from domestic pig strains. More wild boar strains should be isolated to show that wild boars and domestic pigs are reservoirs for different *Y. enterocolitica* and *Y. pseudotuberculosis* strains.

Introduction

Yersinia enterocolitica and *Yersinia pseudotuberculosis* are foodborne pathogens that can cause acute gastroenteritis and mesenteric lymphadenitis mimicking appendicitis (Fredriksson-Ahomaa *et al.*, 2010). Pigs are considered to be the main reservoir of human pathogenic *Y. enterocolitica* strains carrying this pathogen frequently in the tonsils at slaughter (Martínez *et al.*, 2009, 2010a, 2010b). *Y. pseudotuberculosis* has also been isolated from fattening pigs but with lower prevalences as *Y. enterocolitica* (Martínez *et al.*, 2009, 2010a, 2010b). Pathogenic *Y. enterocolitica* and *Y. pseudotuberculosis* can easily be detected and identified using polymerase chain reaction (PCR) targeting the chromosomally encoded virulence genes *ail* and *inv*, respectively (Fredriksson-Ahomaa *et al.*, 2007; Bhaduri *et al.*, 2009; Niskanen *et al.*, 2009). In Switzerland, the detection rate of *ail*-positive *Y. enterocolitica* in fattening pigs (Fredriksson-Ahomaa *et al.*, 2007) and wild boars (Fredriksson-Ahomaa *et al.*, 2009) was 88% and 35%, respectively, using PCR. The detection rate of *inv*-positive *Y. pseudotuberculosis* in fattening pigs (not published) and wild boars (Fredriksson-Ahomaa *et al.*, 2009) was 10% and 20%, respectively.

The reservoirs of human pathogenic *Y. enterocolitica* and *Y. pseudotuberculosis* are mostly still poorly known (Fredriksson-Ahomaa *et al.*, 2010). Only *Y. enterocolitica* strains belonging to bioserotype 4/O:3 and *Y. pseudotuberculosis* strains of bioserotype 2/O:3 have repeatedly been isolated from fattening pigs at slaughter in Europe (Martínez *et al.*, 2009, 2010a, 2010b). A high genetic similarity between human and porcine *Y. enterocolitica* 4/O:3 strains has been shown by PFGE using *Not*I, *Apa*I, and *Xho*I enzymes, indicating that pigs are an important reservoir for human pathogenic *Y. enterocolitica* 4/O:3 strains (Fredriksson-Ahomaa *et al.*, 2006). Pigs might have a role as a source in human *Y. pseudotuberculosis* 2/O:3 infections, although this link has not yet been confirmed.

Pathogenic *Y. enterocolitica* and *Y. pseudotuberculosis* strains have recently been isolated from wild boars; however, their impact on the epidemiology of human yersiniosis is unclear (Fredriksson-Ahomaa *et al.*, 2010). In recent years, wild boar population has exploded all over Europe. At the same time, outdoor pig farming has become popular, which may increase the risk of transmission of zoonotic bacteria like enteropathogenic *Yersinia* between wild boars and domestic pigs. However, the epidemiological link between wild boars and domestic pigs is unknown. In this study, enteropathogenic

[1]Department of Food Hygiene and Environmental Health, Faculty of Veterinary Medicine, University of Helsinki, Helsinki, Finland.
[2]Faculty of Veterinary Medicine, Institute of Food Hygiene, Ludwig-Maximilians University, Munich, Germany.
[3]Vetsuisse Faculty, Institute for Food Safety and Hygiene, University of Zurich, Zurich, Switzerland.

Table 1. Origin of Enteropathogenic *Yersinia* Strains

Animal	Number of			Number of strains belonging to					
				Yersinia enterocolitica *bioserotype*				Yersinia pseudotuberculosis *serotype*	
	animals studied	*positive animals*	*strains*	*2/O:5,27*	*2/O:9*	*4/O:3*	*NT*	*O:1*	*O:2*
Wild boars	153	18	18	3	4	5	2	3	1
Fattening pigs	212	72	78	6	1	71	0	0	0

NT, not typable.

Yersinia strains isolated from wild boars were characterized and compared with strains found in domestic pigs.

Materials and Methods

Enteropathogenic Yersinia *strains used for characterization*

In total, 14 *ail*-positive *Y. enterocolitica* strains and 4 *inv*-positive *Y. pseudotuberculosis* strains isolated from wild boars were characterized and compared with 78 *ail*-positive *Y. enterocolitica* strains isolated from fattening pigs (Table 1). The wild boar strains originated from tonsils of animals sampled during October 2007 and March 2008 in Switzerland (Fredriksson-Ahomaa *et al.*, 2009). The domestic pig strains were from tonsils of fattening pigs sampled during February and March 2006 in Switzerland (Fredriksson-Ahomaa *et al.*, 2007).

Bio- and serotyping of enteropathogenic Yersinia *strains*

The biotype of *ail*-positive *Y. enterocolitica* strains was determined using pyrazinamidase and tween esterase activity, esculin hydrolysis, indole production, and salicin, xylose, and trehalose fermentation tests (Wauters *et al.*, 1987). Serotyping was carried out with slide agglutination using commercial *Y. enterocolitica* O:3, O:5, O:9, and O:27 antisera (Sifin) and *Y. pseudotuberculosis* O:1 to O:4 antisera (MastGroup).

Antimicrobial resistance of enteropathogenic Yersinia *strains*

Antimicrobial resistance analysis was performed with disc-diffusion test according to CLSI (2002) except that the incubation temperature was 30°C. Mueller-Hinton broth and agar (Oxoid) were used as the test media and commercially available antimicrobial test disks (Oxoid). The agar plates were incubated at 30°C for 16 to 18 h. Antimicrobial agents commonly used in either treatment of pig disease or as growth promoters or those used in the treatment of human clinical disease were selected for testing. The following 16 antimicrobials were tested: ampicillin (10 μg), amoxicillin/clavulanic acid (20/10 μg), aztreonam (30 μg), cefotaxim (30 μg), ciprofloxacin (5 μg), chloramphenicol (30 μg), colistin (25 μg), erythromycin (15 μg), furazolidon (50 μg), gentamicin (10 μg), nalidixic acid (30 μg), streptomycin (10 μg), tetracycline (30 μg), trimethoprim (5 μg), trimethoprim/sulfamethoxazole (1.25/23.75 μg), and sulfamethoxazole (25 μg). Breakpoints to establish resistance were based on CLSI recommendations for *Enterobacteriaceae*.

Detection of different genes in enteropathogenic Yersinia *strains*

Ten different genes were studied by real-time PCR based on SYBRGreen according to Fredriksson-Ahomaa *et al.* (2007). *Y. enterocolitica* strains were identified using the 16S rRNA gene (Sen, 2000) and *Y. pseudotuberculosis* strains using *inv* gene (Thoerner *et al.*, 2003). Three chromosomal virulence genes, *ail* (Nakajima *et al.*, 1992; Lambertz *et al.*, 2008), and *yst* (Ibrahim *et al.*, 1997), *hreP* (Heusipp *et al.*, 2001), and *virF* (Nakajima *et al.*, 1992) gene located on the virulence plasmid were studied. Additionally, serotypes O:3 and O:9 of *Y. enterocolitica* were determined using the *rfb*C (Weynants *et al.*, 1996) and *per* (Jacobsen *et al.*, 2005) genes, respectively, and lactamase genes, *blaA* and *blaB*, were studied (Stock *et al.*, 1999).

Table 2. Number of Resistant Enteropathogenic *Yersinia* Strains in Wild Boars and Fattening pigs

Antimicrobials	Wild boars						Fattening pigs		
	Y. enterocolitica *(14)*				Y. pseudotuberculosis *(4)*		Y. enterocolitica *(78)*		
	2/O:5,27 (3)	*2/O:9 (4)*	*4/O:3 (5)*	*NT (2)*	*O:1 (3)*	*O:2 (1)*	*2/O:5,27 (6)*	*2/O:9 (1)*	*4/O:3 (71)*
Ampicillin	3	4	5	2	0	0	6	1	71
Amoxicillin/ clavulanic acid	2	4	5	0	0	0	5	0	0
Erythromycin	3	4	5	2	3	1	6	1	71
Streptomycin	1	3	1	0	0	0	0	0	5
Sulphametoxazol	0	0	0	0	0	0	0	0	2
Trimethoprim	0	0	0	0	0	0	0	0	1
Trimethoprim/sulfamethoxazole	0	0	0	0	0	0	0	0	1

Number of strains is given in parentheses.

Characterization of enteropathogenic Yersinia *strains using PFGE*

Genotyping was done using *Not*I, *Apa*I, *Xho*I, and *Spe*I enzymes for *Y. enterocolitica* (Najdenski *et al.*, 1994) and *Not*I, *Xho*I, and *Spe*I enzymes for *Y. pseudotuberculosis* (Niskanen *et al.*, 2002). DNA was isolated using CHEF Genomic DNA Plug Kits (Bio-Rad). The plugs were lysed for 4–6 h at 37°C in lysozyme solution and overnight at 50°C in proteinase K solution. The plugs were washed six times in wash buffer before restriction digestion. The DNA was digested overnight with 10 U of *Not*I and with 20 U of *Apa*I, *Xho*I, and *Spe*I enzymes according to the manufacturer's instructions (New England Biolabs). The restriction fragments were separated through a 1.0% gel (pulsed-field-certified agarose; BioRad) in 0.5×TBE with a CHEF Mapper XA system (BioRad). Lambda Ladder PFG marker (New England Biolabs) was used as a size standard. Pulse times were ramped from 1 to 25 sec over 22 h for *Not*I and *Apa*I, from 1 to 20 sec over 20 h for *Spe*I and from 1 to 18 sec over 18 h for *Xho*I. The gels were stained with ethidium bromide, destained with the running buffer, and photographed with a Gel Doc EQ system (BioRad). Isolates were considered to be different when a one-band difference between fragments over 70 kb was observed (Fredriksson-Ahomaa *et al.*, 2006).

Results and Discussion

Bioserotype 4/O:3 of *ail*-positive *Y. enterocolitica* strains was identified in 5 (36%) out of 14 wild boar strains, and this type was the most dominant type (91% of the 78 strains) in domestic pigs at slaughter (Table 1). Bioserotypes 2/O:9 and 2/O:5,27 were identified in four (29%) and three (21%) wild boar strains, respectively. These types were very rare in fattening pigs found only in 1% and 8% of the strains, respectively. Additionally, two *ail*-positive *Y. enterocolitica* strains from wild boars could be neither bio- nor serotyped. Bioserotype 4/O:3 is widely distributed and the most common type causing human yersiniosis in Europe (EFSA, 2009). It is also the most common type in European pig population (Martínez *et al.*, 2009, 2010a, 2010b). Bioserotype 2/O:9 is the second most common type identified in human yersiniosis in Europe followed by bioserotype 2/O:5,27. Bioserotypes 2/O:5,27 and 2/O:9 have only sporadically been isolated from domestic pigs in Europe except England, where bioserotypes 2/O:9 and 2/O:5,27 are more common than 4/O:3 (Martínez *et al.*, 2010b). More wild boar strains have to be isolated to get more information about the distribution of different bioserotypes in wild boar population.

Y. pseudotuberculosis strains were only isolated from wild boars in Switzerland. However, it has recently been shown that *Y. pseudotuberculosis* can also be found in domestic pigs but that the prevalence is clearly lower than the prevalence of *Y. enterocolitica* (Martínez *et al.*, 2009, 2010a, 2010b). Further studies are needed to show that wild boars are a more important reservoir for *Y. pseudotuberculosis* than domestic pigs. Two different serotypes (O:1 and O:2) were identified among four *inv*-positive *Y. pseudotuberculosis* strains (Table 1). Serotype O:3, which is the dominant type in domestic pigs in Europe, was not found in wild boars (Martínez *et al.*, 2009, 2010a, 2010b). A high isolation rate of *Y. pseudotuberculosis* strains belonging to a wide variety of serotypes in fattening pigs has recently been reported in England (Martínez *et al.*, 2010b). It was speculated that this might be attributed to wild animals having contact with pigs reared outside.

All strains were sensitive to aztreonam, cefotaxim, ciprofloxacin, chloramphenicol, colistin, gentamicin, and nalidixic acid, and resistant to erythromycin using disc diffusion method. However, there were some differences in the resistance patterns of *Y. enterocolitica* strains from wild boars and fattening pigs and between the *Y. enterocolitica* and *Y. pseudotuberculosis* strains among seven antimicrobials (Table 2). The resistance to amoxicillin/clavulanic acid differed between *Y. enterocolitica* 4/O:3 and 2/O:9 strains from wild boars and fattening pigs. All *Y. enterocolitica* 4/O:3 and 2/O:9 strains from wild boars showed resistance to amoxicillin/clavulanic acid, whereas the strains from fattening pigs were sensitive. One explanation may be that the β-lactamase A was not inhibited by clavulanic acid in wild boar strains due to some differences in cell wall between wild boar and domestic pig strains (Sharma *et al.*, 2004). Additionally, out of 71 domestic pig

Table 3. Different Genes Detected in Enteropathogenic *Yersinia* Strains Isolated from Wild Boars and Fattening pigs

	Wild boars						*Fattening pigs*		
	Y. enterocolitica *(14)*				Y. pseudotuberculosis *(4)*		Y. enterocolitica *(78)*		
Gene	*2/O:5,27 (3)*	*2/O:9 (4)*	*4/O:3 (5)*	*NT (2)*	*O:1 (3)*	*O:2 (1)*	*2/O:5,27 (6)*	*2/O:9 (1)*	*4/O:3 (71)*
16S rRNA	3	4	5	2	0	0	6	1	71
rfbC	0	0	5	0	0	0	0	0	71
per	0	4	0	0	0	0	0	1	0
blaA	3	4	5	2	0	0	6	1	71
blaB	3	4	5	2	0	0	6	1	71
inv	0	0	0	0	3	1	0	0	0
ail	3	4	5	2	0	0	6	1	71
yst	3	4	5	0	0	0	6	1	71
hreP	3	4	5	0	0	0	6	1	71
virF	3	4	5	0	3	1	6	1	49

Number of strains is given in parentheses.

Table 4. Different Genotypes of Enteropathogenic *Yersinia* Strains

	Wild boars						*Fattening pigs*		
	Y. enterocolitica *(14)*				Y. pseudotuberculosis *(4)*		Y. enterocolitica *(78)*		
Genotype	*2/O:5,27 (3)*	*2/O:9 (4)*	*4/O:3 (5)*	*NT (2)*	*O:1 (3)*	*O:2 (1)*	*2/O:5,27 (6)*	*2/O:9 (1)*	*4/O:3 (71)*
GE[a] 1–7	0	0	0	0	0	0	0	0	71
GE 8–10	0	0	5	0	0	0	0	0	0
GE 51–52	0	0	0	0	0	0	6	0	0
GE 53	3	0	0	0	0	0	0	0	0
GE 91	0	0	0	0	0	0	0	1	0
GE 92–93	0	4	0	0	0	0	0	0	0
GP[b] 1–4	0	0	0	0	3	1	0	0	0

Number of strains is given in parentheses.
[a]GE, genotype for *ail*-positive *Y. enterocolitica*.
[b]GP, genotype for *inv*-positive *Y. pseudotuberculosis*.

strains studied, 2 strains were resistant to sulphametoxazol—of them, one to trimethoprim as well as trimethoprim/sulphametoxazole (Table 2). Resistance to trimethoprim and trimethoprim/sulphametoxazole seems not to be a cause of major concern. All *Y. pseudotuberculosis* strains were sensitive to ampicillin, which can be explained by the inability to produce β-lactamases due to the lack of the *blaA* and *blaB* genes (Table 3). All *Y. enterocolitica* strains were resistant to ampicillin and carried both β-lactamase genes.

All *Y. enterocolitica* strains belonging to human pathogenic bioserotypes carried *yst* and *hreP* genes associated with the virulence (Ibrahim *et al.*, 1997; Heusipp *et al.*, 2001), which shows that the presence of these genes correlates well with the bioserotypes associated with yersiniosis (Table 3). These genes were missing in two *ail*-positive *Y. enterocolitica* strains from wild boars, which could not be classified into any bioserotype. This indicates that these two strains may have a lower pathogenicity. This may also indicate that the presence of *ail* is not a sufficient virulence marker alone to detect and identify human pathogenic strains. The *virF* gene located on the virulence plasmid was detected in all *Y. pseudotuberculosis* strains and in all *Y. enterocolitica* strains of pathogenic bioserotypes isolated from wild boars. This gene was not detected in 31% of *Y. enterocolitica* 4/O:3 strains recovered from pigs. The virulence plasmid is needed for the full virulence, but it can easily be lost during culturing (Li *et al.*, 1998). Further studies are needed to prove that the virulence plasmid is more unstable in *Y. enterocolitica* 4/O:3 strains from domestic pigs.

All genotypes of wild boar strains differed from domestic pig strains (Table 4). Especially wild boar strains belonging to bioserotype 4/O:3 were clearly different from domestic pig strains with all four enzymes. The PFGE profiles of bioserotype 2/O:5,27 and 2/O:9 strains isolated from wild boars differed only slightly from domestic pig strains (Fig. 1). Bhaduri *et al.* (2009) have recently demonstrated that *Y. enterocolitica* O:3 and O:5 strains isolated from fattening pigs in the United States are highly similar within the serotype. Further, the genotypes of *Y. enterocolitica* 4/O:3 from human and porcine sources in Europe have shown to be very homogeneous supporting a link between domestic pigs and human yersiniosis (Fredriksson-Ahomaa *et al.*, 2006). Strains from wild boars, domestic pigs, and humans isolated during the same time period are needed to study the molecular epi-

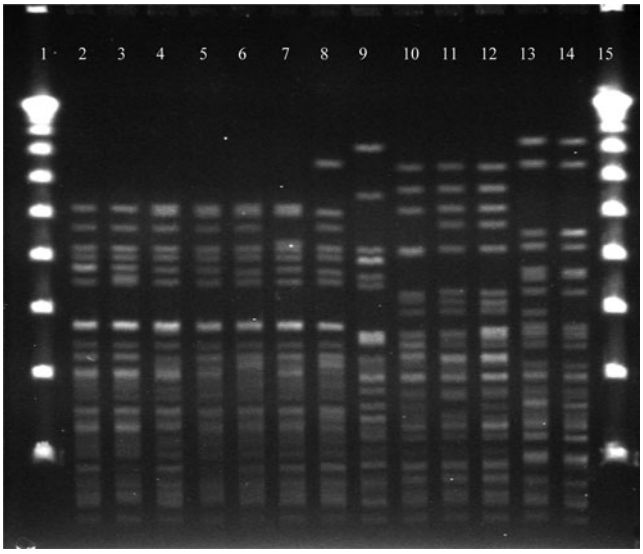

FIG. 1. Different *Not*I profiles of *Yersinia enterocolitica* strains from wild boars and fattening pigs. 1 and 15: Lambda Ladder marker; 2–8: *Not*I patterns (GE 1–7) of *Y. enterocolitica* 4/O:3 strains from fattening pigs; 9: *Not*I pattern (GE 8) of *Y. enterocolitica* 4/O:3 strains from wild boars; 10 and 11: *Not*I patterns (GE 51–52) of *Y. enterocolitica* 2/O:5,27 strains from fattening pigs; 12: *Not*I pattern (GE 53) of *Y. enterocolitica* 2/O:5,27 strains from wild boars; 13: *Not*I pattern (GE 91) of the *Y. enterocolitica* 2/O:9 strain from a fattening pig; 14: *Not*I pattern (GE 93) of *Y. enterocolitica* 2/O:9 strains from wild boars.

demiological link between wild boars and domestic pigs, and the role of these strains in human yersiniosis.

Y. pseudotuberculosis strains belonging to two serotypes presented all different genotypes (Table 4). There was also a clear difference between the three O:1 strains demonstrating a high genetic diversity among wild boar strains belonging to serotype O:1. The genotypes of serotype O:3 strains isolated from domestic pigs in Finland have shown to have a very limited genetic diversity (Niskanen *et al.*, 2002). More research is needed to elucidate the role of wild boars and domestic pigs in the transmission of *Y. pseudotuberculosis* between animals and from animals to humans.

In conclusion, *ail*-positive *Y. enterocolitica* strains isolated from wild boars were different from domestic pig strains. More wild pig strains have to be collected to prove that wild boars and domestic pigs are reservoirs for different strains of human pathogenic *Y. enterocolitica.*

Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
Maria Fredriksson-Ahomaa, D.V.M.
Department of Food Hygiene and Environmental Health
Faculty of Veterinary Medicine
University of Helsinki
P.O. Box 66
Helsinki FI-00014
Finland

E-mail: maria.fredriksson-ahomaa@helsinki.fi