AEM
Journals.ASM.org

Piglets Are a Source of Pathogenic *Yersinia enterocolitica* on Fattening-Pig Farms

Sonja Virtanen, Laura Salonen, Riikka Laukkanen-Ninios, Maria Fredriksson-Ahomaa, and Hannu Korkeala

Department of Food Hygiene and Environmental Health, Faculty of Veterinary Medicine, University of Helsinki, Helsinki, Finland

To study the origin and spread of *Yersinia enterocolitica* among pigs, fecal and blood samples were repeatedly taken on a fattening farm. A few piglets were found to be already infected on breeding farms. After the piglets were mixed, the infection spread through the whole unit. Eventually, all the pigs excreted the pathogen.

Yersinia enterocolitica is a food-borne human pathogen that is reported to cause 3.5 cases per 1,000,000 population in the United States annually and 16.5 cases per 1,000,000 population in the European Union (6, 23). Yersiniosis occurs most often in young children (4). Common symptoms include diarrhea, fever, and abdominal pain (15). In older children and adults, the disease may be confused with appendicitis. Unpleasant sequelae, such as reactive arthritis and erythema nodosum, may occur (5). Pigs are considered to be the major reservoir of the pathogen (2, 10, 18). Undercooked pork and other pig products have been reported to be the main source of human infections, according to epidemiological investigations (3, 9, 13, 15, 16).

Y. enterocolitica is carried by infected finishing pigs from farms to slaughterhouses (22). The prevalence of human pathogenic *Y. enterocolitica* is high in pigs at slaughter (7). The carcasses become contaminated with the pathogen in the slaughterhouses by contamination from tonsils and intestinal contents (8, 22). The level of carcass contamination can be reduced with such hygiene practices as removing the head and bagging the rectum (1, 20, 26, 28), but these alone fail to completely eliminate the carcass contamination. Therefore, reducing the occurrence of the pathogen at the farm level is also needed. The aim of this study was to investigate the transmission of *Y. enterocolitica* from breeding farms to fattening units under conventional farming conditions.

In total, 76 pigs were monitored on a fattening farm over the whole fattening period. The farm consisted of 14 units, and each unit contained eight pens. The number of pigs in each pen ranged from 8 to 10. Each unit had a separate ventilation system and operated on an all-in all-out system. Units were cleaned and disinfected between each lot. Two separate units were monitored in this study, unit 1 in winter and unit 2 in summer. The pigs originated from 16 farrowing farms. Two of the farrowing farms delivered piglets to both units. Piglets were about 12 weeks old when they entered the fattening farm. The weight of the piglets ranged from 23.6 kg to 51.3 kg at the beginning of the study (mean, 36.1 kg, and standard deviation, 6.25). Pigs were allocated into 16-m^2 pens and matched according to their sizes and sexes. A moderate amount of straw was used as bedding. Repeated fresh fecal samples were taken from the same earmarked pigs at 2-week intervals. Blood samples were collected for serology at the beginning and at the end of the study. Samples from feed and drinking water were taken during the first sampling.

Ten grams of feed pellets and feces were mixed into 90-ml peptone-mannitol broth (PMB), and 100 μl was plated (i) immediately, (ii) after 7 days, and (iii) after 14 days of cold enrichment at 4°C onto cefsulodin-Irgasan-novobiocin (CIN) agar (Oxoid, Cambridge, United Kingdom) and incubated at 28°C for 24 h and further at room temperature for 24 h (21). With the 14-day cold enrichment, an alkali treatment with 0.25% KOH solution was used before plating (21). Typical colonies were cultured on tryptic soy agar (TSA) (Difco, Lawrence, KS). The pathogenicity of the isolates was confirmed by using PCR that detected the virulence genes *ail* (24) and *virF* (17). The isolates were biotyped (31) and serotyped using commercial antisera (Denka Seiken, Tokyo, Japan).

The genotyping of the isolated strains was done by using multiple-locus variable-number tandem-repeat analysis (MLVA) (12, 29). The size of the end products was determined by capillary electrophoresis with an ABI 310 genetic analyzer (Applied Biosystems, Foster City, CA) in the GS STR Pop4 G5 module. Serum samples were tested by using a commercially available enzyme-

TABLE 1 *Yersinia* antibodies in serum samples and MLVA types of *Yersinia enterocolitica* isolates from fecal samples of piglets taken the day after arriving at unit 1 of the fattening farm

Farm of origin of piglets	No. of piglets	% of piglets seropositive (no. of positive pigs/no. of pigs studied)	MLVA type(s)[a] of the isolates (no. of *Y. enterocolitica*-positive piglets)
1	9	38 (3/8)	A (5), B (1)
2	3	0 (0/3)	A (1)
3	4	67 (2/3)	B (2)
4	3	0 (0/3)	ND
5	3	0 (0/3)	C (1)
6	4	0 (0/4)	B (1)
7	6	50 (3/6)	C (4), A (1)
8	3	0 (0/3)	ND
9	2	0 (0/2)	ND
10	3	0 (0/2)	A (1)

[a] MLVA types are A (6-1-12-13-12-3), B (6-1-12-29-7-3), and C (3-1-20-27-8-3). ND, *Y. enterocolitica* not detected.

Received 12 December 2011 **Accepted** 25 January 2012
Published ahead of print 3 February 2012
Address correspondence to Sonja Virtanen, sonja.virtanen@helsinki.fi.
Supplemental material for this article may be found at http://aem.asm.org/.

doi:10.1128/AEM.07805-11

TABLE 2 MLVA types of *Yersinia enterocolitica* isolates from fecal samples of pigs in each pen during follow-up in summer and winter

Unit	Pen	No. of pigs	MLVA type(s) (% of strains) at[a]: Day 1	Wk 2	Wk 4	Wk 6	Wk 8	Wk 10	Wk 13
1 (winter)	I	9	**C (50),** A (33), B (17)	**A (80),** C (20)	**A (88),** C (13)	**A (60),** C (40)	**A (100)**	**A (100)**	**A (100)**
	II	9	**A (75),** B (25)	**A (83),** C (17)	**A (88),** C (13)	**A (89),** C (11)	**A (100)**	**A (100)**	**A (67),** B (33)
	III	10	A (50), B (50)	A (33), B (33), C (33)	**B (40),** A (30), C (30)	**B (78),** C (22)	**B (67),** C (33)	**A (60),** B (30), C (10)	**B (67),** A (33)
	IV	8	**C (67),** B (33)	**B (71),** A (14), C (14)	**B (71),** A (14), C (14)	**B (67),** C (33)	NF	**A (67),** B (33)	**B (50),** A (25), C (25)
	V	4	**A (100),**	**A (100),**	**A (100)**	**A (100)**	NF	**A (100)**	NF
2 (summer)	VI	9	NS	**D (60),** A (40)	**D (88),** F (13)	**D (100)**	**D (100)**	NF	NS
	VII	9	NS	**E (67),** A (33)	**E (67),** A (33)	**E (75),** A (25)	**E (50),** A (17), D (17), F (17)	**E (100)**	NS
	VIII	10	NS	**A (56),** F (22), D (11), E (11)	**E (45),** A (36), F (18)	**E (56),** A (33), F (11)	NF	NF	NS
	IX	8	NS	**D (50),** A (25), F (25)	**D (50),** F (38), E (13)	**D (75),** F (25)	**D (100)**	NF	NS

[a] MLVA types are A (6-1-12-13-12-3), B (6-1-12-29-7-3), C (3-1-20-27-8-3), D (5-1-15-10-6-3), E (7-1-10-15-4-3), and F (9-1-9-7-6-3). The dominant strains in the pens are shown in boldface. NF, *Y. enterocolitica* not found; NS, not studied.

linked immunosorbent assay (ELISA) kit (Pigtype Yopscreen, Labor Diagnostik, Leipzig, Germany) according to the manufacturer's instructions.

Eight piglets which had originated from three farms had antibodies against *Yersinia* when they arrived at unit 1 in the fattening farm (Table 1). In a previous study, pigs were not found to be seropositive until the age of 102 to 107 days (25). After pigs receive oral contamination, antibodies are shown to take as long as 12 days to develop (27), which indicates that the piglets in our study had certainly encountered the pathogen on their original farms. The day after their arrival, 17 piglets in the same unit already excreted pathogenic *Y. enterocolitica* in their feces. Eventually, the pathogen was isolated in the fecal samples of every pig in both units during the fattening period. Only pathogenic bioserotype 4/O:3 strains were detected.

Three different MLVA types, types A (6-1-12-13-12-3), B

TABLE 3 Fecal excretion of pathogenic *Yersinia enterocolitica* and prevalence of *Yersinia* antibodies in the pigs during follow-up

Unit	Farm of origin of piglets	No. of pigs	No. of fattening pigs excreting *Y. enterocolitica* (% fecal prevalence) at wk: 2	4	6	8	10	13	% of pigs seropositive at wk 13
1 (winter)	1	9	6	7	6	0	3	0	NS[a]
	2	3	2	1	2	1	2	0	NS
	3	4	3	4	3	1	0	3	NS
	4	3	3	3	3	1	2	1	NS
	5	3	2	3	3	1	0	1	NS
	6	4	2	4	3	2	3	0	NS
	7	6	5	5	2	0	3	3	NS
	8	3	1	3	2	1	3	1	NS
	9	2	0	2	2	1	2	1	NS
	10	3	2	3	3	0	1	1	NS
Unit 1 total	1–10	40	26	35	29	8	19	11	
2 (summer)	1	6	4	6	4	1	0	NS	75
	2	3	2	3	3	0	0	NS	100
	11	3	3	3	3	1	0	NS	50
	12	6	0	3	5	4	1	NS	80
	13	2	2	2	2	0	0	NS	100
	14	7	6	7	5	0	0	NS	80
	15	6	2	4	6	3	0	NS	80
	16	3	2	3	3	0	0	NS	100
Unit 2 total	1, 2, 11–16	36	21	32	31	9	1		82
Total (units 1 and 2)	1 to 16	76	47 (62)	67 (88)	60 (79)	17 (22)	20 (26)	11 (28)	

[a] NS, not studied.

(6-1-12-29-7-3), and C (3-1-20-27-8-3), were isolated from the fecal samples of the pigs in unit 1 (Table 2; also see Table S1 in the supplemental material). Genotypes A, B, and C were isolated from piglets from farms 1, 3, and 7, respectively (Table 1). These genotypes were later isolated from the other pigs in the fattening unit. However, the strains differed between the two units. In unit 2, four different MLVA genotypes, types A (6-1-12-13-12-3), D (5-1-15-10-6-3), E (7-1-10-15-4-3), and F (9-1-9-7-6-3), were found (Table 2; also see Table S2 in the supplemental material). The origin of the genotypes remained unclear because the first samples were collected 2 weeks after the piglets arrived at the farm and the infection had sufficient time to spread among the pigs.

MLVA type A was found in both units. This genotype originated from farm 1, which had delivered piglets to both units. The origin of type A strains was further confirmed when additional fecal samples from pigs from farm 1 were collected separately and type A strains alone were found. Farm 1 seems to be a reservoir of this genotype, and apparently the piglets from this farm continuously transmit the contamination when delivered to different fattening farms. The same MLVA types were present in each unit from the beginning of the study until the end, and no new genotypes emerged during the follow-up (Table 2). Samples from feed and drinking water were negative for *Y. enterocolitica*.

The prevalence of pathogenic *Y. enterocolitica* in the feces of fattening pigs varied between 22% and 88% for different sampling times (Table 3). An increase in the prevalence in feces up until 16 weeks of age of the pigs was noted, which was followed by a decrease over time until the end of the follow-up. This pattern is in agreement with previous studies (11, 14, 25). Despite the low fecal prevalence at the end of the period, most of the sampled pigs were seropositive at the time of slaughter. The level of contamination remained higher in unit 1, which was monitored in winter. *Y. enterocolitica* has previously been reported to be isolated more frequently during cold months (19, 30), which may be one explanation for the difference between the two units. During the follow-up, the incidence of *Y. enterocolitica* in fecal samples of the pigs was 100%, which demonstrates that the pathogen spreads thoroughly within a pig herd.

The results of this study show that piglets from certain breeding farms transmit *Y. enterocolitica* strains into a fattening farm and the infection subsequently spreads throughout the whole unit. In order to reduce the level of this pathogen in pig production, mixing piglets from *Y. enterocolitica*-positive farms with piglets negative for *Y. enterocolitica* should be avoided and prevention methods should be targeted at piglet production units.

ACKNOWLEDGMENTS

This study was supported by research funding from the Ministry of Agriculture and Forestry, Finland (2849/502/2008), and performed at the Centre of Excellence in Microbial Food Safety Research, Academy of Finland (118602, 141140).

This study was conducted in cooperation with Finnish Animal Breeding Association (FABA). Erika Pitkänen and Anu Seppänen are acknowledged for technical assistance.

REFERENCES

1. **Andersen JK.** 1988. Contamination of freshly slaughtered pig carcasses with human pathogenic *Yersinia enterocolitica*. Int. J. Food Microbiol. **7**:193–202.
2. **Bhaduri S, Wesley IV, Bush EJ.** 2005. Prevalence of pathogenic *Yersinia enterocolitica* strains in pigs in the United States. Appl. Environ. Microbiol. **71**:7117–7121.
3. **Boqvist S, Pettersson H, Svensson A, Andersson Y.** 2009. Sources of sporadic *Yersinia enterocolitica* infection in children in Sweden, 2004: a case-control study. Epidemiol. Infect. **137**:897–905.
4. **Bottone EJ.** 1997. *Yersinia enterocolitica*: the charisma continues. Clin. Microbiol. Rev. **10**:257–276.
5. **Bottone EJ.** 1999. *Yersinia enterocolitica*: overview and epidemiologic correlates. Microbes Infect. **1**:323–333.
6. **EFSA.** 2011. The European Union summary report on trends and sources of zoonoses and zoonotic agents and food-borne outbreaks in 2009. EFSA J. **9**:2090.
7. **Fredriksson-Ahomaa M, Korte T, Korkeala H.** 2000. Contamination of carcasses, offals, and the environment with *yadA*-positive *Yersinia enterocolitica* in a pig slaughterhouse. J. Food Prot. **63**:31–35.
8. **Fredriksson-Ahomaa M, Stolle A, Korkeala H.** 2006. Molecular epidemiology of *Yersinia enterocolitica* infections. FEMS Immunol. Med. Microbiol. **47**:315–329.
9. **Fredriksson-Ahomaa M, Stolle A, Siitonen A, Korkeala H.** 2006. Sporadic human *Yersinia enterocolitica* infections caused by bioserotype 4/O:3 originate mainly from pigs. J. Med. Microbiol. **55**:747–749.
10. **Fredriksson-Ahomaa M, Stolle A, Stephan R.** 2007. Prevalence of pathogenic *Yersinia enterocolitica* in pigs slaughtered at a Swiss abattoir. Int. J. Food Microbiol. **119**:207–212.
11. **Fukushima H, et al.** 1983. Ecological studies of *Yersinia enterocolitica*. I. Dissemination of *Y. enterocolitica* in pigs. Vet. Microbiol. **8**:469–483.
12. **Gierczynski R, Golubov A, Neubauer H, Pham JN, Rakin A.** 2007. Development of multiple-locus variable-number tandem-repeat analysis for *Yersinia enterocolitica* subsp. *palearctica* and its application to bioserogroup 4/O3 subtyping. J. Clin. Microbiol. **45**:2508–2515.
13. **Grahek-Ogden D, Schimmer B, Cudjoe KS, Nygård K, Kapperud G.** 2007. Outbreak of *Yersinia enterocolitica* serogroup O:9 infection and processed pork, Norway. Emerg. Infect. Dis. **13**:754–756.
14. **Gürtler M, Alter T, Kasimir S, Linnebur M, Fehlhaber K.** 2005. Prevalence of *Yersinia enterocolitica* in fattening pigs. J. Food Prot. **68**:850–854.
15. **Huovinen E, et al.** 2010. Symptoms and sources of *Yersinia enterocolitica*-infection: a case-control study. BMC Infect. Dis. **10**:122.
16. **Jones TF.** 2003. From pig to pacifier: chitterling-associated yersiniosis outbreak among black infants. Emerg. Infect. Dis. **9**:1007–1009.
17. **Kaneko S, Ishizaki N, Kokubo Y.** 1995. Detection of pathogenic *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* from pork using the polymerase chain reaction. Contrib. Microbiol. Immunol. **13**:153–155.
18. **Korte T, Fredriksson-Ahomaa M, Niskanen T, Korkeala H.** 2004. Low prevalence of *yadA*-positive *Yersinia enterocolitica* in sows. Foodborne Pathog. Dis. **1**:45–52.
19. **Lanada EB, Morris RS, Jackson R, Fenwick SG.** 2005. A cohort study of *Yersinia* infection in goats. Aust. Vet. J. **83**:567–571.
20. **Laukkanen R, et al.** 2010. Reduction of enteropathogenic *Yersinia* in the pig slaughterhouse by using bagging of the rectum. J. Food Prot. **73**:2161–2168.
21. **Laukkanen R, et al.** 2010. Evaluation of isolation methods for pathogenic *Yersinia enterocolitica* from pig intestinal content. J. Appl. Microbiol. **108**:956.
22. **Laukkanen R, et al.** 2009. Contamination of carcasses with human pathogenic *Yersinia enterocolitica* 4/O:3 originates from pigs infected on farms. Foodborne Pathog. Dis. **6**:681–688.
23. **Long C, et al.** 2010. *Yersinia pseudotuberculosis* and *Y. enterocolitica* infections, FoodNet, 1996–2007. Emerg. Infect. Dis. **16**:566–567.
24. **Nakajima H, et al.** 1992. Detection and identification of *Yersinia pseudotuberculosis* and pathogenic *Yersinia enterocolitica* by an improved polymerase chain reaction method. J. Clin. Microbiol. **30**:2484–2486.
25. **Nesbakken T, Iversen T, Eckner K, Lium B.** 2006. Testing of pathogenic *Yersinia enterocolitica* in pig herds based on the natural dynamic of infection. Int. J. Food Microbiol. **111**:99–104.
26. **Nesbakken T, Nerbrink E, Røtterud OJ, Borch E.** 1994. Reduction of *Yersinia enterocolitica* and *Listeria* spp. on pig carcasses by enclosure of the rectum during slaughter. Int. J. Food Microbiol. **23**:197–208.
27. **Nielsen B, Heisel C, Wingstrand A.** 1996. Time course of the serological response to *Yersinia enterocolitica* O:3 in experimentally infected pigs. Vet. Microbiol. **48**:293–303.

28. **Ranta J, et al.** 2010. Causal hidden variable model of pathogenic contamination from pig to pork. Stat. Modelling **10**:69–87.
29. **Sihvonen LM, et al.** 2011. Multilocus variable-number tandem-repeat analysis, pulsed-field gel electrophoresis, and antimicrobial susceptibility patterns in discrimination of sporadic and outbreak-related strains of *Yersinia enterocolitica.* BMC Microbiol. **11**:42.
30. **Tsubokura M, Fukuda T, Otsuki K, Kubota M, Itagaki K.** 1976. Studies on *Yersinia enterocolitica.* II. Relationship between detection from swine and seasonal incidence, and regional distribution of the organism. Nihon Juigaku Zasshi **38**:1–6.
31. **Wauters G, Kandolo K, Janssens M.** 1987. Revised biogrouping scheme of *Yersinia enterocolitica.* Contrib. Microbiol. Immunol. **9**:14–21.