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Running title: *Y. enterocolitica* in pigs

Original research paper

Prevalence and dynamics of pathogenic *Yersinia enterocolitica* 4/O:3 among Finnish piglets, fattening pigs, and sows

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ABSTRACT

Pigs are considered the main reservoir of *Yersinia enterocolitica*, hence understanding the ecology of this foodborne pathogen at the farm level is crucial. We calculated Bayesian estimates for the ability of a commercial ELISA diagnostic test kit to detect antibodies against pathogenic *Yersinia* in pigs. The sensitivity and specificity of the test were 75.4% and 98.1%, respectively. We also studied the dynamics of *Y. enterocolitica* infection in three farrow-to-finish pig farms by following the same 30 pens of pigs through their lifetime from farrowing unit to slaughterhouse. Each farm was sampled four times, and 864 fecal and 730 serum samples were collected altogether. Pathogenic *Y. enterocolitica* 4/O:3 was isolated from 31.6% of the fecal samples by culturing, and *Yersinia* antibodies were detected in 38.2% of the serum samples with the commercial ELISA test. The pathogen was not isolated from farrowing units or all-in/all-out weaning units. However, in the weaning and fattening units using continuous management systems, the pathogen was isolated from every pen at some point of the study. After the pigs were transported into slaughterhouse, 150 tonsils were collected and 96.7% were positive by culturing. Among the strains isolated from feces and tonsils, 56 different genotypes of pathogenic *Y. enterocolitica* 4/O:3 were found by multiple-locus variable-number tandem repeat analysis (MLVA). Finally, we collected tonsils of 266 sows from 115 farrowing farms, and *Y. enterocolitica* 4/O:3 was detected in 6.0% of the samples by the culture method, while 77.1% of the tonsils were serologically positive; the estimate for true seroprevalence was 95.8%. In conclusion, sows may not be the main source of *Y. enterocolitica* for piglets, although sows may still play a role in maintaining *Y. enterocolitica* in pig farms. Instead, pigs appear to get this foodborne pathogen mainly during the fattening period, especially if continuous management is applied.

Key words: antibody, ecology, ELISA, enteropathogenic *Yersinia*, foodborne pathogen, MLVA, farm, reservoir, slaughterhouse, swine

Introduction

Yersiniosis is the third most commonly reported bacterial zoonosis in the European Union and is primarily caused by pathogenic *Y. enterocolitica* (EFSA and ECDC, 2018). Yersiniosis manifests as febrile gastrointestinal disease but may also lead to other symptoms and complications such as erythema nodosum or reactive arthritis (Fredriksson-Ahomaa et al., 2010; Bottone et al., 2015). Pigs are considered to be significant reservoirs for *Y. enterocolitica*, and the main source of human infections. *Y. enterocolitica* contaminates carcasses and offal during slaughter processes (Fredriksson-Ahomaa et al., 2000; Laukkanen et al., 2009) and contaminated pork and other food of swine origin have been associated with yersiniosis (Tauxe et al., 1987; Lee et al., 1990; Ostroff et al., 1994; Huovinen et al., 2010). Moreover, genotypically similar strains of *Y. enterocolitica* have been isolated from yersiniosis patients and pigs (Fredriksson-Ahomaa et al., 2001; Fredriksson-Ahomaa et al., 2006; Virtanen et al., 2013).

Pigs are symptomless carriers of enteropathogenic *Yersinia*, especially *Y. enterocolitica*, but the prevalence is variable depending on factors such as age, sampling and detection methodology, farm management, and biosecurity level (reviewed by Laukkanen-Ninios et al., 2014). Despite some geographical variation, bioserotype 4/O:3 is the type most frequently isolated in pigs (Laukkanen-Ninios et al., 2014). Newborn piglets are negative for *Y. enterocolitica*. Piglets start excreting the pathogen in feces around the age of 1–3 months with peak of excretion around the age of 2–5 months, the fecal prevalence starts reducing thereafter, and pigs tend to remain seropositive for longer periods (Fukushima et al., 1983; Nielsen et al., 1996; Gürtler et al., 2005; Nesbakken et al., 2006; Bowman et al., 2007; Wehebrink et al., 2008; Virtanen et al., 2012; Vilar et al., 2013).

Based on numerous studies, variable number of slaughter pigs carry enteropathogenic *Y. enterocolitica* in tonsils and feces; 2–93% and 0.5–76%, respectively (reviewed by Laukkanen-Ninios et al. 2014). Usually most pigs are seropositive at slaughter age (Skjerve et al., 1998; Thibodeau et al., 2001; Nesbakken et al., 2006; Virtanen et al., 2012; Vilar et al., 2013, Vilar et al., 2015; Bonardi et al., 2016; Lorencova et al., 2016). Only a few carrier pigs are needed to spread the infection within and between pig

74 farms (Virtanen et al., 2012; Virtanen et al., 2014). In contrast to fattening pigs, the prevalence of
75 *Y. enterocolitica* in sows is less studied. The reported prevalence has been relatively low, varying between
76 0% and 14% (Fukushima et al., 1983; Korte et al., 2004; Bowman et al., 2005; Gürtler et al., 2005; Wehebrink
77 et al., 2008; Farzan et al., 2010; Vilar et al., 2013). However, most sows still appear to be seropositive (Vilar
78 et al., 2013; Vilar et al., 2015) suggesting development of immunity against *Yersinia*. More studies are needed
79 to understand the dynamics of *Y. enterocolitica* at the farm level, especially the role of sows and piglets in
80 *Yersinia* ecology.

81 A commercially available enzyme-linked immunosorbent assay (ELISA) has been used to
82 determine antibodies against pathogenic *Yersinia* in pigs. However, no diagnostic test should be considered
83 fully sensitive and specific. Therefore, we have calculated Bayesian estimations for the sensitivity and
84 specificity of the ELISA test (Vilar et al., 2015). The updated estimates are needed, because the manufacturer
85 has revised the test and increased the recommended cut-off value.

86 The aim of our study was to assess the dynamics of *Y. enterocolitica* infection in three farrow-
87 to-finish pig farms. In addition, we studied the prevalence of pathogenic *Y. enterocolitica* and the
88 seroprevalence of *Yersinia* antibodies in the tonsils of sows collected at slaughterhouses.

Materials and Methods

Experimental plan and sampling

To assess the dynamics of pathogenic *Y. enterocolitica* in pig farms (study I), three Finnish farrow-to-finish farms (A-C) with known *Yersinia* positivity were followed in a longitudinal study. From each farm, pigs from six pens (mean 7.3 pigs per pen) were followed throughout their lifetime (i.e. from farrowing units to weaning and fattening units, and finally the slaughterhouse). In farm B, two subsequent longitudinal follow-ups were performed at a nine-month interval to study the persistence of pathogenic *Y. enterocolitica* strains in the farm; 12 pens were included in the second follow-up. Pigs from different pens were not mixed, except in the fattening unit of farm C, where the pigs were regrouped in two larger pens of approximately 20 pigs each. Conventional straw bedding was used in all the farms and units, except the fattening unit of farm C, which used deep peat bedding. All-in/all-out management systems were used in the weaning units of farms B and C to avoid any contact between pigs from different groups. In contrast, the weaning unit of farm A and the fattening units of all the three farms used continuous management systems.

At the farms, pens were sampled four times, approximately once per month. On average, feces and blood samples were collected from 96% and 88% of the pigs from every pen, respectively. Furthermore, fecal samples were collected from the mother sows (n = 24) during the first sampling at all but farm A. Rectal swabs were used for piglets (first sampling), and also for older pigs if feces could not be collected. Finally, 150 tonsils were collected at the slaughterhouse (last sampling).

To assess the prevalence of pathogenic *Y. enterocolitica* and the seroprevalence of *Yersinia* antibodies in sows, tonsils of 266 sows from 115 farrowing farms were collected from two Finnish slaughterhouses (study II).

Isolation and identification of pathogenic Y. enterocolitica

Fecal and tonsil samples from study I and tonsil samples from study II were screened for the presence of pathogenic *Y. enterocolitica* according to the method of the Department of Food Hygiene and Environmental Health (University of Helsinki) (Laukkanen et al. 2010). Briefly, for the fecal samples (study I), a three-step isolation was used before the plating on cefsulodin-irgasan-novobiosin agar (Schiemann, 1979): (i) immediately, and (ii) after one week and (iii) after two weeks of cold enrichment at 4°C. In the case of (iii), an alkali treatment with 0.25% potassium hydroxide solution (Aulisio et al., 1980) was applied after the cold enrichment. For the tonsil samples (studies I and II), a four-step isolation was used, as an additional enrichment in irgasan-ticarcillin-potassium chloride broth (Wauters et al., 1988) at 25°C for two days was applied, but for the sow tonsils (study II) the immediate plating on CIN agar was omitted. After the culturing, presumptive colonies were identified with an urea hydrolysis test, API 20E test (BioMérieux, Marcy l'Etoile, France), biotyping, serotyping and a multiplex PCR targeting the virulence genes *ail* (Nakajima et al., 1992) located on the chromosome, and *virF* (Kaneko et al., 1996) located on the virulence plasmid pYV Laukkanen et al. (2010). To investigate the spread of pathogenic *Y. enterocolitica* among pigs, pathogenic isolates from the three farrow-to-finish farms were genotyped using a multiple-locus variable-number tandem repeat analysis (MLVA) method (Alakurtti et al. 2016).

Enumeration of Yersinia *antibodies and performance of the ELISA test*

The serum samples collected from the farrow-to-finish farms (study I), as well as the tissue fluid samples from the tonsils of sows (study II), were screened for the presence of antibodies against *Yersinia* outer membrane proteins by using a commercial ELISA test (formerly Pigtype Yopscreen, Labor Diagnostik, Leipzig, Germany; currently Pigtype Yersinia Ab, Qiagen, Leipzig, Germany) according to the manufacturer's instructions (short protocol). Samples with activity values (S/P ratios) of 0.3 or higher are considered positive.

Estimates of true prevalence were calculated by using a Bayesian approach as follows: prior distributions of sensitivity and specificity were defined as beta probability distributions using a test with imperfect sensitivity and specificity (Thrusfield, 2007). For the ELISA test, the estimates of sensitivity and

specificity were recalculated from Vilar et al. (2015) using the current recommended cut-off value (S/P ratio) of 0.3 instead of 0.2. These sensitivity and specificity estimations were used to calculate the prior beta probability distributions, Se ~ beta(αSe, βSe), Sp ~ beta(αSp, βSp), thus sensitivity was beta (106.79, 35.52) and the specificity was beta (46.81, 1.89). For the isolation using the culture method, the sensitivity (77.9%) reported by Laukkanen et al. (2010) was used to calculate the prior beta probability distribution beta (39.79, 12.01), whereas a non-informative beta (1,1) was used for the specificity. Models were constructed in OpenBugs 3.2.2 as described by Vilar et al. (2015). Briefly, inferences were based on 50,000 iterations after a burn-in for convergence of 1,000 iterations. For the apparent seroprevalence in the tonsils of sows (study II), exact binomial 95% confidence intervals described by Agresti and Coull (1998) were calculated in Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA).

Statistical analysis

To investigate pathogenic *Y. enterocolitica* in the three farrow-to-finish farms (study I), the pen was the experimental unit and considered positive for *Y. enterocolitica* by culturing, if any fecal sample tested positive, or by serology, if any blood sample tested positive.

The statistical difference between isolation of pathogenic *Y. enterocolitica* and the presence of *Yersinia* antibodies in the tonsils of sows (study II) was assessed by McNemar's test in IBM SPSS Statistics 24.0 (IBM, Armonk, NY).

Results

Performance of the ELISA test

The recalculated estimates for the sensitivity and specificity of the commercial ELISA test were 75.4% and 98.1%, respectively (Table 1).

Transmission of Y. enterocolitica *in farrow-to-finish farms (study I)*

Among the 1014 samples cultured, 418 (41.2%) were positive for pathogenic *Y. enterocolitica* 4/O:3: 273 of 864 (31.6%) of the fecal samples were positive, and 145 of 150 (96.7%) of the tonsil samples. Of the 418 strains genotyped, all were *ail* positive, and 402 were *virF* positive. Antibodies against pathogenic *Yersinia* were found in a total of 38.2% of the blood samples (279 of 730). In the farrowing units (first sampling), no pathogenic *Y. enterocolitica* was detected, although some piglets were seropositive (Table 2). In addition, all the 24 fecal samples taken from sows in the farrowing units were negative. In the weaning units, the pathogen was detected in all of the pens of farm A but not in pigs of farms B and C, which used all-in/all-out management systems (Table 2). In the fattening units using continuous management systems, most of the pens were positive. At slaughter age, 96.3% of the pigs carried pathogenic *Y. enterocolitica* in their tonsils (Table 2).

From the three farms studied, a total of 56 different MLVA types of pathogenic *Y. enterocolitica* 4/O:3 were found (Table 3, Supplemental figure). During the follow-ups of A, B-I, B-II, and C, a total of 9, 17, 21, and 15 different MLVA types were detected, respectively. The farms had their own MLVA types, except the most frequently isolated type in farm C which was also found at the slaughterhouse in one pig of farm B. Of the MLVA types isolated from farm B at the farm level excluding the slaughterhouse sampling, eight genotypes were detected during only the first follow-up and 10 genotypes during only the second follow-up. Four genotypes were detected during both follow-ups. The highest count of unique genotypes (n = 14) was found in the fattening unit of farm C (Table 3). During the follow-ups of A, B-I, B-II and C, 2, 5, 8 and 1 new MLVA types were found in the slaughterhouse, respectively.

182

183 *Prevalence of* Y. enterocolitica *in the tonsils of sows (study II)*

184 Pathogenic *Y. enterocolitica* was detected in 6.0% (95% CI: 3.5–9.6%) of the tonsil samples of sows. All the 16
185 positive sows carried *Y. enterocolitica* bioserotype 4/O:3 positive for the *ail* gene, whereas 10 strains were
186 positive for the *virF* gene. *Yersinia* antibodies were detected in 77.1% of the sows, and according to our
187 Bayesian model, the true seroprevalence was 95,8% (Table 4). The sows that tested positive by culturing and
188 serology originated from 14 and 104 farms, respectively. Antibodies against *Yersinia* were detected from
189 tonsils significantly more frequently than pathogenic *Y. enterocolitica* was detected by culturing (McNemar's
190 test $p < 0.001$).

191

192 Discussion

193

194 As expected, the modification of the ELISA test's cut-off activity value (S/P ratio) from 0.2 to 0.3 resulted in a
195 decrease in sensitivity (75,4%; previously 79.5%) and increase in specificity (98,1%; previously 96.9%) (Vilar
196 et al., 2015). According to the manufacturer's instructions, the antigens used in the test are produced by
197 pathogenic *Yersinia* strains only, hence no cross reactions to nonpathogenic species or other enterobacteria
198 should exist. However, there are cross reactions to other pathogenic *Yersinia*, especially
199 *Y. pseudotuberculosis*. Nevertheless, the prevalence of *Y. enterocolitica* in pigs is much higher than the
200 prevalence of *Y. pseudotuberculosis* (Laukkanen et al., 2008; Laukkanen et al., 2009, Ortiz Martínez et al.,
201 2009; Ortiz Martínez et al., 2010; Ortiz Martínez et al., 2011).

202 The seropositivity of some piglets already at farrowing units is most likely due to colostral
203 maternal antibodies. Seropositivity levels generally increased later than fecal prevalence, which was
204 expected, since antibodies take time to develop (Nielsen et al., 1996). With the exception of farm C, the
205 seropositivity levels were high by the fourth sampling, which indicates a wide transmission of pathogenic
206 *Y. enterocolitica* in the pig populations. Pigs from farm C had been moved from an all-in/all-out weaning unit

into a continuously filled fattening unit later than pigs from the other farms. This could explain why only some of the pigs were seropositive despite being fecal carriers by the fourth sampling.

In the fattening unit of farm C 14 different MLVA types were found. The use of deep peat bedding that can provide a reservoir for pathogenic *Y. enterocolitica* or the high number of pigs per pen might explain the highest amount of detected MLVA types. The pigs were regrouped from six smaller pens into two larger pens, which possibly enhanced the spread of the pathogen. A similar amount (*n* = 13) of MLVA types were found in the fattening unit and slaughterhouse during the second follow-up of farm B (B-II). However, 12 pens (instead of six, as in the other farms) were studied, which may explain this result. MLVA has a relatively high discriminatory power and the results should be interpreted carefully (Virtanen et al., 2013; Alakurtti et al., 2016). The strains from a single farm often had multiple different VNTR loci, and even in the least discriminatory VNTR loci V4 and V9 (Virtanen et al., 2013; Alakurtti et al., 2016) variation was observed between the strains from different farms (Table 3, Supplemental figure), indicating that there were several different strains. Our subsequent studies of farm B reveal that several genotypes might persist – and perhaps dominate – on farms. New MLVA types were found at the slaughterhouse, which could indicate mutations, or more likely cross-contamination during transportation and slaughter processes.

Management system is a key factor in controlling the spread of pathogenic *Y. enterocolitica* in pig farms, as highlighted in our study. Once pigs were moved to units where all-in/all-out systems were not used, the infection vigorously spread among the pig population. Earlier studies support our findings, as mixing pigs from different groups has been identified as a risk factor for the spread of pathogenic *Y. enterocolitica* (Virtanen et al., 2012; Virtanen et al., 2014). Other factors such as pig movement, biosecurity level, water source, feed and bedding, have also been associated with the prevalence of pathogenic *Yersinia* in pigs (Skjerve et al., 1998; Nowak et al., 2006; Laukkanen et al., 2009; Virtanen et al., 2011; von Altrock et al., 2011; Novoslavskij et al., 2013; Vilar et al., 2013, Vanantwerpen et al., 2017).

According to our prevalence study of tonsil fluid in sows, pathogenic *Y. enterocolitica* is widely spread among sows. However, almost all sows appear to be seropositive only, and just a few sows seem to carry the pathogen in their tonsils – at least to the extent that the pathogen can be isolated by culturing.

Sows may have developed immunity against pathogenic *Yersinia*, as also suggested in earlier studies. Vilar et al. (2013) found that only 5% of sows excreted pathogenic *Y. enterocolitica* in feces, while as many as 67% were serologically positive. The true prevalence of *Yersinia* antibodies in serum of Finnish sows was 74% (Vilar et al., 2015).

Sows may not be the main source of *Y. enterocolitica* for piglets, because pigs appear to get infected in weaning and fattening units, especially if all-in/all-out management systems are not used. However, sows may still be an important reservoir for pathogenic *Y. enterocolitica*. The high prevalence of antibodies indicates regular contact with the pathogen. Albeit seldom carriers, sows may still sporadically transfer *Y. enterocolitica* to some piglets that later start excreting the pathogen in feces. When piglets get older, levels of maternal antibodies appear to decrease, as indicated in our study. Although pigs primarily get *Y. enterocolitica* in weaning and fattening units, this main reservoir may partly be maintained by sows. Pathogenic *Y. enterocolitica* is rarely isolated from the pig farm environment (Laukkanen-Ninios et al., 2014, Vilar et al., 2013), indicating that the main route of spread is most likely between pigs, specifically their secretions and snout contacts. Nevertheless, it has been possible to maintain pig herds free from *Y. enterocolitica* in closed, specific pathogen-free herds (Nesbakken et al., 2007). To control this foodborne pathogen at the farm level, comprehensive prevention strategies are needed, for example purchasing uninfected animals only, employing all-in/all-out management systems, and avoiding mixing pig groups.

Conclusions

Pathogenic *Y. enterocolitica* is widely spread in fattening pigs, and several genotypes are found on each farm. The main reservoir for pathogenic *Y. enterocolitica* appears to be fattening pigs, especially if all-in/all-out management systems are not used. Based on the antibodies present in tonsils, essentially all sows have been in contact with this pathogen. Although pathogenic *Y. enterocolitica* is rarely isolated from tonsils of sows, they may nevertheless be an important reservoir for the pathogen, by maintaining it in pig farms.

Comprehensive prevention, especially all-in/all-out management systems, are needed to control this foodborne pathogen at the farm level.

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Disclosure Statement

There are no competing financial interests to declare that are related to this publication.

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Table 1. Estimates[a] for the sensitivity and specificity of the diagnostic test (Pigtype Yopscreen[b], Labor Diagnostik, Leipzig, Germany) used for the detection of *Yersinia* antibodies.

Parameter	Median (95% probability interval)
Sensitivity	75.4 (68.9–81.4)
Specificity	98.1 (90.8–99.9)

[a] Calculated from the data originally published by Vilar et al. (2015), but using a revised cut-off activity value (S/P ratio) of 0.3 (instead of 0.2 as used earlier) for the ELISA test according to the instructions by the manufacturer (short protocol).

[b] Currently known as Pigtype Yersinia Ab (Qiagen, Leipzig, Germany).

Table 2. Prevalence of *Yersinia enterocolitica* 4/O:3 in feces of pigs collected at farrow-to-finish farms and tonsils of pigs collected at abattoirs; prevalence of *Yersinia* antibodies in serum of pigs collected at farrow-to-finish farms.

Farm and method	No. pens	No. pigs per pen ± SD[a]	No. pigs sampled per pen ± SD[b]	Sampling time at farm												Abattoir
				3-6 w			8-10 w			12-15 w			16-20 w			21-26 w
				No. positive pens	True prevalence of positive pigs (95% PI)[c]	Unit[d]	No. positive pens	True prevalence of positive pigs (95% PI)[c]	Unit[d]	No. positive pens	True prevalence of positive pigs (95% PI)[c]	Unit[d]	No. positive pens	True prevalence of positive pigs (95% PI)[c]	Unit[d]	No. positive pigs (true prevalence, 95% PI)[c]
Culturing																
A	6	7.3 ± 1.7	6.7 ± 1.8	0	1.5 (0.1–7.9)	Farr.	6	96.1 (87.8–99.4)	Wean.	6	77.4 (62.7–88.6)	Fatt.	5	16.5 (7.3–29.9)	Fatt.	31 (89.6%, 77.1–96.8)
B-I	6	7.3 ± 1.8	7.2 ± 1.8	0	1.5 (0.1–7.9)	Farr.	0	1.6 (0.1–8.1)	Wean.[e]	6	98.4 (91.9–99.9)	Fatt.	6	96.2 (88.0–99.5)	Fatt.	22 (93.2%, 78.9–99.0)
B-II	12	7.3 ± 0.9	7.0 ± 1.0	0	0.8 (0.0–4.1)	Farr.	0	0.8 (0.0–4.0)	Wean.[e]	12	67.2 (56.6–76.7)	Fatt.	9	22.9 (14.8–32.8)	Fatt.	74 (97.8%, 92.9–99.7)
C	6	7.3 ± 0.7	7.0 ± 0.8	0	1.5 (0.1–7.9)	Farr.	0	1.6 (0.1–8.2)	Wean.[e]	0	1.6 (0.1–8.2)	Wean.[e]	2[f]	100 (100–100)	Fatt.	18 (96.4%, 82.3–99.9)
Total	30	7.3 ± 1.3	7.0 ± 1.4	0	0.3 (0.0–1.7)		6	19.3 (14.4–24.8)		24	62.3 (55.5–68.8)		26	52.2 (45.4–59.0)		145 (96.3%, 92.4–98.5)
Serology																
A	6	7.3 ± 1.7	6.2 ± 1.8	0	1.7 (0.1–8.4)	Farr.	5	32.3 (19.6–47.0)	Wean.	6	98.2 (90.5–99.9)	Fatt.	6	97.8 (88.9–99.9)	Fatt.	
B-I	6	7.3 ± 1.8	6.6 ± 1.6	4	31.5 (19.1–46.1)	Farr.	1	6.6 (1.6–16.9)	Wean.[e]	4	22.6 (11.5–37.3)	Fatt.	no data	no data	Fatt.	
B-II	12	7.3 ± 0.9	6.7 ± 1.2	9	57.8 (47.5–67.8)	Farr.	0	0.8 (0.0–4.4)	Wean.[e]	8	53.3 (42.1–64.3)	Fatt.	12	94.1 (87.5–97.9)	Fatt.	
C	6	7.3 ± 0.7	5.9 ± 1.8	2	8.1 (2.5–18.2)	Farr.	0	1.8 (0.1–9.0)	Wean.[e]	1	4.8 (0.7–15.0)	Wean.[e]	2[f]	31.7 (16.5–50.2)	Fatt.	
Total	30	7.3 ± 1.3	6.4 ± 1.6	15	31.3 (25.3–37.7)		6	7.8 (4.6–12.1)		19	46.7 (39.6–53.9)		23	83.2 (76.4–88.9)		

[a] Average number of pigs per pen at the beginning of the study ± standard deviation.

[b] Average number of pigs sampled per pen during the study ± standard deviation.

[c] Estimate for the true prevalence of positive pigs ± 95% probability interval using Bayesian methods as described by Vilar et al. (2015). Note that revised sensitivity and specificity values for the commercial ELISA test were used (Table 1).

[d] Unit where the pigs were sampled: Farr. = farrowing unit; Wean. = weaning unit; Fatt. = fattening unit.

[e] All-in/all-out management system was used.

[f] In the fattening unit of farm C, the pigs were regrouped into two larger pens of approximately 20 pigs.

Table 3. Genotypic distribution of *Yersinia enterocolitica* 4/O:3 strains isolated from feces and tonsils of pigs in farrow-to-finish farms and abattoirs, based on multiple-locus variable-number tandem repeat analysis (MLVA).

Farm	Sampling time at farm												Abattoir	
	8–10 w				12–15 w				16–20 w				21–26 w	
	MLVA type	No. (%)	Pens[a]	Unit[b]	MLVA type	No. (%)	Pens[a]	Unit[b]	MLVA type	No. (%)	Pens[a]	Unit[b]	MLVA type	No. (%)
A	5-2-6-9-6-3	22 (53.7)	4	Wean.	5-2-6-9-6-3	13 (44.8)	4	Fatt.	5-2-6-9-6-3	3 (50.0)	3	Fatt.	5-2-6-10-7-3	11 (35.5)
	5-2-6-10-7-3	13 (31.7)	3	Wean.	5-2-6-9-7-3	11 (37.9)	4	Fatt.	5-2-6-9-7-3	1 (16.7)	1	Fatt.	5-2-6-9-6-3	9 (29.0)
	5-2-6-9-7-3	5 (12.2)	3	Wean.	5-2-6-10-7-3	3 (10.3)	2	Fatt.	5-2-6-10-7-3	1 (16.7)	1	Fatt.	6-2-6-9-6-3	5 (16.1)
	5-2-6-10-6-3	1 (2.4)	1	Wean.	5-2-6-11-7-3	1 (3.4)	1	Fatt.	5-2-6-10-9-3	1 (16.7)	1	Fatt.	5-2-6-9-7-3	4 (12.9)
					5-2-7-9-6-3	1 (3.4)	1	Fatt.					6-2-6-8-6-3	1 (3.2)
													5-2-6-10-9-3	1 (3.2)
B-I	SN[e]			Wean.[c]	13-2-7-7-6-3	25 (58.1)	4	Fatt.	13-2-7-7-6-3	25 (59.5)	5	Fatt.	13-2-7-7-6-3	14 (63.6)
					11-2-7-8-6-3	11 (25.6)	2	Fatt.	11-2-7-8-6-3	11 (26.2)	3	Fatt.	3-6-7-11-13-5	1 (4.5)
					13-2-5-7-6-3	2 (4.7)	2	Fatt.	13-2-7-7-22-3	2 (4.8)	2	Fatt.	11-2-7-7-6-3	1 (4.5)
					13-2-8-7-6-3	2 (4.7)	2	Fatt.	11-2-7-8-20-3	1 (2.4)	1	Fatt.	11-2-7-8-6-3	1 (4.5)
					11-2-7-7-6-3	1 (2.3)	1	Fatt.	12-2-7-7-6-3	1 (2.4)	1	Fatt.	11-2-7-9-6-3	1 (4.5)
					11-15-7-8-6-3	1 (2.3)	1	Fatt.	12-2-7-8-6-3	1 (2.4)	1	Fatt.	13-2-7-7-14-3	1 (4.5)
					13-3-7-7-6-3	1 (2.3)	1	Fatt.	13-2-7-7-5-3	1 (2.4)	1	Fatt.	13-2-8-7-6-3	1 (4.5)
													13-2-7-9-6-3	1 (4.5)
													13-2-7-9-22-3	1 (4.5)
B-II	SN[e]		Wean.[c]	11-2-7-7-6-3	30 (55.6)	9	Fatt.	11-2-7-7-6-3	13 (72.2)	5	Fatt.	11-2-7-7-6-3	38 (51.4)	
				9-2-7-7-6-3	9 (16.7)	4	Fatt.	12-2-7-7-6-3	4 (22.2)	3	Fatt.	9-2-7-7-6-3	12 (16.2)	
				12-2-7-7-6-3	3 (5.6)	1	Fatt.	9-2-7-7-6-3	1 (5.6)	1	Fatt.	12-2-7-7-6-3	12 (16.2)	
				10-2-7-7-6-3	2 (3.7)	2	Fatt.					13-2-7-7-6-3	3 (4.1)	
				13-2-7-7-6-3	2 (3.7)	1	Fatt.					9-2-7-7-18-3	1 (1.4)	
				9-2-5-7-6-3	1 (1.9)	1	Fatt.					9-2-7-9-6-3	1 (1.4)	
				9-2-7-7-8-3	1 (1.9)	1	Fatt.					9-4-17-12-12-3	1 (1.4)	
				9-2-7-9-6-3	1 (1.9)	1	Fatt.					11-2-7-8-6-3	1 (1.4)	
				9-2-7-15-8-3	1 (1.9)	1	Fatt.					12-2-7-7-21-3	1 (1.4)	
				9-12-9-4-6-3	1 (1.9)	1	Fatt.					12-2-8-7-6-3	1 (1.4)	
				11-2-3-7-6-3	1 (1.9)	1	Fatt.					12-2-12-23-8-2	1 (1.4)	
				11-2-7-9-6-3	1 (1.9)	1	Fatt.					12-2-12-23-21-2	1 (1.4)	
				11-2-9-7-6-3	1 (1.9)	1	Fatt.					13-2-6-7-6-3	1 (1.4)	
C	SN[e]		Wean.[c]	SN[e]			Wean.[c]	3-6-7-11-13-5	11 (27.5)	2[d]	Fatt.	3-6-7-11-13-5	9 (50.0)	
								3-6-7-11-12-5	9 (22.5)	2	Fatt.	3-6-7-9-8-5	2 (11.1)	
								3-6-8-11-16-5	4 (10.0)	1	Fatt.	3-6-7-9-12-5	2 (11.1)	
								3-6-8-12-16-5	3 (7.5)	2	Fatt.	3-6-7-11-12-5	2 (11.1)	
								3-6-8-11-12-5	2 (5.0)	2	Fatt.	3-6-8-12-16-5	2 (11.1)	
								3-6-8-12-12-5	2 (5.0)	1	Fatt.	3-6-7-9-13-5	1 (5.6)	
								3-6-9-12-16-5	2 (5.0)	1	Fatt.			
								3-6-7-4-2-5	1 (2.5)	1	Fatt.			
								3-6-7-9-12-5	1 (2.5)	1	Fatt.			
								3-6-7-9-13-5	1 (2.5)	1	Fatt.			
								3-6-7-9-18-5	1 (2.5)	1	Fatt.			
								3-6-8-10-12-5	1 (2.5)	1	Fatt.			
								3-6-8-12-2-5	1 (2.5)	1	Fatt.			
								3-6-9-12-12-5	1 (2.5)	1	Fatt.			

[a] Number of pens from which the MLVA type was found.

[b] Unit where the pigs were sampled: Farr. = farrowing unit; Wean. = weaning unit; Fatt. = fattening unit.

[c] All-in/all-out management system was used.

[d] In the fattening unit of farm C, the pigs were regrouped into two larger pens of approximately 20 pigs.

[e] Samples negative for pathogenic *Y. enterocolitica*.

Table 4. Presence of *Yersinia* antibodies and isolation of *Yersinia enterocolitica* 4/O:3 in tonsils *(n* = 266*)* collected from sows at abattoirs.

	Isolation by culturing			
Serology	No. isolated	No. not isolated	Apparent seroprevalence (95% CI)	True seroprevalence (95% PI)[a]
No. positive	11	194	77.1% (71.7–81.7)	95.8% (87.4–99.7)
No. negative	5	56		

[a] Estimate for the true prevalence of positive pigs using Bayesian methods as described by Vilar et al. (2015). Note that revised sensitivity and specificity values for the commercial ELISA test were used (Table 1).