Yersinia enterocolitica contamination of pig feces, carcasses and tonsils in one French slaughterhouse

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Yersinia enterocolitica is the third most important cause of gastro-intestinal diseases transmitted by contaminated foodstuffs consumption in Europe (Efsa, 2011). Pig is considered to be the primary reservoir for the human pathogenic types of Y. enterocolitica; mainly for biotype 4 (serotype O:3). Biotype 2 (serotype O:9) has been isolated from other animal species, such as cattle, sheep and goats. The pigs develop no clinical signs, but carry Y. enterocolitica on the tongue, tonsils and in the lymph nodes and excrete the bacterium in their feces (Nesbakken et al., 2003). Moreover, seasonal trends in the carriage of Y. enterocolitica by pigs have been identified, with winter identified as a risk period in the UK (Milnes et al., 2009) and Germany (Weber & Knapp, 1981). In this study, we assessed the Y. enterocolitica prevalence on carcasses at the end of the slaughtering process, together with the determination of pathogenic and non-pathogenic biotypes, in order to better characterize the importance of the hazard for pork. To this end, parallel samples were made on tonsils, feces and carcass of the same pig in a winter period in order to evaluate the risk of cross-contamination. Unrelated feces and tonsils samples were also collected in summer, as this season is considered to be rather unfavorable to Y. enterocolitica. These data were compared to tonsils and feces results obtained during the cold period.

Material and methods

Sampling: Three campaigns were lead. 121 tonsils and 120 feces were collected from 121 randomly selected pigs during the first campaign (june-july 2009). 114 parallel samples of tonsils, feces and carcasses (23 batches of 5 pigs) were analyzed during campaign 2 (october 2009-march 2010) and 44 parallel samples of feces, tonsils and carcasses (9 batches of 5 pigs) were collected during campaign 3 (november-december 2010). About 10 g of feces were collected on the slaughter line after evisceration, the fore quarter of the corresponding carcasses were swabbed (approx. 500 cm^2), and tonsils were excised before carcass refrigeration.

Enrichment and detection: samples were enriched in ITC broth (Irgasan, Ticarcillin, Potassium chlorate) (48h, 25°C) and streaked onto CIN (Cefsulodin, Irgasan, Novobiocin) agar plates (24h, 30°C). Typical colonies of *Yersinia enterocolitica* were confirmed by using Api 20E strips (Biomérieux).

Pathogenicity determination: Pathogenic and non pathogenic strains biotypes were determined by multiplex PCR combining the method of Thisted-Lambertz and Danielsson-Tham (2005) targeting the three virulence genes *ail*, *vir*F and *rfbC*, with the method of Arnold *et al.*, (2004), which targets the *Yersinia enterocolitica* species specific 16s rRNA gene.

Results

By PCR (figure 1), we showed that 82% and 7% of strains isolated in this study belonged to the pathogenic biotype 4/O:3 and 2/O:9 or 3/O:5,27 respectively, while 11% belonged to the non-pathogenic biotype 1A (amplification of the 16S rRNA coding gene only).

Lanes 1, 14: Ladder VIII (Roche); 2, 3, 6, 9, 10, 11: Y. enterocolitica biotype 4/O:3; 4, 5, 7,8 : Y. enterocolitica biotype 2/O:9 or 3/O:5,27; 12 : Citrobacter freundii, 13: water negative control.

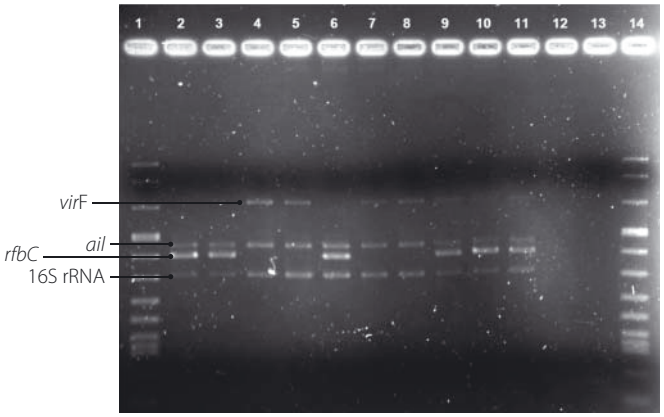

Figure 1: Example of identification of biotypes of strains of *Y. enterocolitica* by multiplex PCR

Detection results on tonsils, feces and carcasses

	positives samples			Positive pigs
	tonsils	feces	carcass	
Campaign 1	0/121	0/120	/	0
Campaign 2	5/114	5/114	0/114	14
Campaign 3	3/44	5/44	0/44	6

- **Campaign 1:** No positive samples.
- **Campaign 2:** 9 pigs were positive for feces and 4 for tonsils. One pig showed both positive tonsils and feces.
- **Campaign 3:** 3 pigs were positive for feces and 1 for tonsils. Two pigs showed both positive tonsils and feces.
- **Campaigns 2 & 3:** no positive carcasses.

Pig and inter batches prevalence estimates

	pig prevalence (%)	Inter batches prevalence (%)
Campaign 1	0	/
Campaign 2	12.3% [7.5 to 19.6%]	34.8% [18.8 to 55.3%]
Campaign 3	13.6% [6.5 to 26.8%]	55.5% [26.2 to 81.3%]

- **Campaign 2:** 8/23 batches were positive with an average of 1.75 positive pigs per batch.
- **Campaign 3:** 5/9 batches were positive with an average of 1.2 positive pigs per batch.

Citations :

• *Arnold et al., 2004. J. Vet. Med., B51, 23-27.* • *Efsa, 2011. The EFSA journal, 9(3):2090, pp. 1-378.* • *Milnes et al., 2008. Epidemiol Infect 136, 739-751.* • *Nesbakken et al., 2003. Int J Food Microbiol., 80, 231-240.*
• *Thisted-Lambertz S.T., Danielsson-Tham L.M., 2005. Appl. Environ. Microbiol., 71, 3674-81.* • *Weber and Knapp, 1981. Zentralbl Bakteriol Mikrobiol Hyg A., 250(1-2):78-83.*

Conclusion and perspective

The frequency of positive tonsils in this study is slightly lower than the 8% [4.5 to 13.6%] observed in winter 2008 in the same slaughterhouse. The detection rate was unexpectedly higher in feces than on tonsils, but despite potential fecal cross contamination during process, no carcass was found to be positive for *Y.enterocolitica*. French usual dressing procedure, with the tongue and tonsils in the unsplit head until refrigeration, could represent a protective factor compared to the slaughtering process of other European countries. With 12.6% [8.3 to 18.7%] of positive pigs in the cold period and 89% of pathogenic strains, this study confirms the importance of *Y. enterocolitica* hazard and its seasonality. At slaughter level, classical tonsils detection of *Y. enterocolitica* should be completed by feces and carcass sampling due to potential cross-contamination.
This study is to be completed by epidemiological analysis of strains isolated.

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