

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

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## **Abstract**

Pig is considered to be the main animal reservoir of human pathogenic *Yersinia enterocolitica* strains. The bacterium can be isolated from its tongue, tonsils, but can also be found in feces and on carcasses. In France, while the main pathogenic biotypes are known for humans (4/O:3, 2/O:9 and 3/O:5,27), few prevalence data are available in the pork chain production and mainly focus on tonsils contamination. The aim of this study was to obtain prevalence data for *Y. enterocolitica* on tonsils, feces and carcasses. In 2009, a prevalence study was initiated in one slaughterhouse located in Brittany (France). Tonsils, feces and carcasses contamination of 279 pigs were followed-up during an 18 months period ranging from June 2009 to December 2010. Three sampling campaigns were lead. Hundred twenty one tonsils and 120 feces (121 randomly selected pigs) were analyzed during the first campaign (June-July 2009). Hundred fourteen feces, tonsils and carcasses (23 batches of 5 pigs) were analyzed during the 2<sup>nd</sup> campaign (October 2009-March 2010) and 44 feces, tonsils and carcasses (9 batches of 5 pigs) for the last campaign (November-December 2010).

Results showed a high variability in the pig *Yersinia enterocolitica* contamination (either positive tonsils or feces): 0%, 12.3% [7.5 to 19.61%] and 13.6% [6.5 to 26.8%] in campaigns 1, 2 and 3 respectively, confirming the reported seasonality of the carriage of *Y. enterocolitica* by pigs. The inter batches prevalence was of 34.8% [18.8 to 55.3%] in campaign 2 and of 55.5% [26.2 to 81.3%] in campaign 3.

On the 20 positive pigs found, 8 (40%) and 15 (75%) were respectively positive only on tonsils or feces, and 3 pigs only (15%) were positive both in tonsils and feces. Despite this unexpected high detection rate on feces, no carcass was found to be positive for *Y. enterocolitica* (swabbing of 500 cm<sup>2</sup>; campaigns 2 and 3). In this study, 82%, 7% and 11% of isolated strains belonged to biotypes 4/O:3, 2/O:9 or 3/O:5,27 and 1A respectively.

In conclusion, 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.

## **Introduction**

In 2007, 8,792 cases of yersiniosis were reported in humans in the European Union, making the zoonotic agent *Yersinia* the third most important cause of enteritis in humans after *Campylobacter* and *Salmonella* (EFSA, 2009). *Yersinia enterocolitica* is the most common species reported in human cases, being isolated from 93.8% of all confirmed cases. Pathogenic *Y. enterocolitica* strains belong to biotypes 1B, 2, 3, 4 and 5, whereas biotype 1A strains are nonpathogenic and widespread in the environment (Bottone, 1999). In France, biotype 4 is the most prevalent pathogenic biotype isolated from humans (69%), followed by biotype 2 (30%) and biotype 3 (1%) (Savin and Carniel, 2008). Infection is most frequently acquired by the ingestion of contaminated food, such as raw or undercooked pig meat in particular. Pigs are considered the principal reservoir for pathogenic *Y. enterocolitica* strains capable of infecting humans, although other animal species, such as cattle, sheep, deer, small rodents, cats and dogs, may also carry pathogenic biotypes (Bottone, 1997; Kapperud and Olsvik, 1982; Bucher et al., 2008). The pigs develop no clinical signs, but carry *Y.*

*enterocolitica* in the oral cavity, particularly on the tongue and tonsils, and in the lymph nodes. They excrete this bacterium in their feces, often with a high prevalence (Thibodeau, 1999, 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 and Knapp, 1981). The incidence of human cases of yersiniosis attributed to pork consumption was recently estimated at 2.826 cases per 100,000 inhabitants per year in Europe. Thus, this bacterium is the second most frequent contaminant of pigs destined for human consumption, after *Salmonella* (3.374), but before *Campylobacter* (2.170) (Fosse *et al.*, 2009).

The aim of this study was to assess 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

Three sampling campaigns were lead in the same slaughterhouse in Brittany. The first campaign of sampling was performed from june to july 2009, where 121 tonsils and 120 feces were collected from 121 randomly selected pigs. Hundred fourteen feces, tonsils and carcasses (23 batches of 5 pigs) were analyzed during the 2<sup>nd</sup> campaign (October 2009-March 2010) and 44 feces, tonsils and carcasses (9 batches of 5 pigs) during the third campaign (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<sup>2</sup>), and tonsils were excised before carcass refrigeration. The microbiological method of detection used included an enrichment in ITC broth (Irgasan, Ticarcillin, Potassium chlorate) (48h, 25°C) and streaking on CIN (Cefsulodin, Irgasan, Novobiocin) agar plates (24h, 30°C). Typical colonies of *Yersinia enterocolitica* were confirmed by using Api 20E strips (Biomérieux). Pathogenic and non pathogenic strains biotypes were determined by multiplex PCR. The PCR method combined the method of Thisted-Lambertz and Danielsson-Tham (2005) targeting the three virulence genes *ail*, *virF* and *rfbC*, with the method of Arnold *et al.*, (2004), which targets the *Yersinia enterocolitica* species specific 16s rRNA gene.

## Results

The results on tonsils, feces and carcasses are depicted in table 1. During the summer period (campaign 1), none of the tonsils and feces samples were positive for the presence of *Y. enterocolitica* , but 12.6% [8.3 to 18.7%] of pigs were found positive in the cold period (campaign 2 and 3). During campaign 2, 13 pigs were positive for either tonsils or feces, with 9 positive feces and 4 positive tonsils, and one pig only was both positive on feces and tonsils. During campaign 3, 1 pig showed positive tonsils, 3 showed positive feces, and two pigs showed both positive tonsils and feces. No carcass was found to be positive.

	Positives samples			Positive pigs
	tonsils	feces	carcass	
<b>Campaign 1</b>	0/121	0/120	/	0
<b>Campaign 2</b>	5/114	10/114	0/114	14
<b>Campaign 3</b>	3/44	5/44	0/44	6

Table 1: Detection results on tonsils, feces and carcass for the three sampling campaigns

The inter batches prevalence is depicted in table 2. During campaign 2, 8/23 batches were positive for the presence of *Y. enterocolitica* with an average of 1.75 positive pigs per batch. During campaign 3, 5/9 batches were positive for the presence of *Y. enterocolitica* with an average of 1.2 positive pigs per batch.

	<b>Pig prevalence (%)</b>	<b>Inter batches prevalence (%)</b>
<b>Campaign 1</b>	0	/
<b>Campaign 2</b>	12.3% [7.5 to 19.61%]	34.8% [18.8 to 55.3%]
<b>Campaign 3</b>	13.6% [6.5 to 26.8%]	55.5% [26.2 to 81.3%]

Table 2: pig prevalence and inter batches prevalence results

By PCR, we showed that 89% 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.

## Discussion

The frequency of positive tonsils in this study is slightly lower than the 8% [4,5-13,6] observed in winter 2008 in the same slaughterhouse, and lower than the prevalence estimated in France (Feurer et al, 2010). According to literature, the prevalence reported in tonsils is generally higher than that in other parts of the carcass (tongues, feces, intestinal content, lymph nodes, offal or the surface of the carcass). Contrary to Nesbakken et al. (2003), Frederiksson-Ahomaa et al. (2007) and Gürtler et al. (2005) who reported the frequency of pathogenic *Yersinia* isolated from tonsils to be significantly higher than that from feces at slaughter, we found more positive samples in feces than on tonsils.

At the end of the slaughter line no positive carcass could be found, which is in accordance with other published results: Gürtler et al. (2005) isolated virulent *Y. enterocolitica* from 38.4% of tonsils and 0.3% of carcass surfaces before chilling.

Carcasses and offal may become contaminated during the slaughtering process, particularly due to fecal contamination during evisceration operations and, more generally, by cross-contamination mediated by slaughterhouse and inspection equipment, slaughterhouse staff and the environment (Nesbakken, 1988; Frederiksson-Ahomaa et al., 2000, 2001; Nesbakken et al., 2003). During the process, the carcass and the pluck set may readily become contaminated with bacteria from the tonsils (Frederiksson-Ahomaa et al., 2001) and cross-contamination seems to play a greater role in pluck set contamination than in carcass contamination (Laukkanen et al., 2009). In France the tonsils remain generally intact in the unsplit head attached to the carcass until chilling, whereas in many other European countries the tongue is removed with the tonsils attached, together with pluck set (trachea, lungs, liver and heart), and the carcass is split to the end of the head. This alternative dressing procedure had been proposed for avoiding or limiting contamination by Christensen and Luthje in 1994. Together with good hygiene practices to avoid fecal cross contamination, this dressing procedure could explain the absence of positive carcasses in our study.

## Conclusion

The frequency of positive carcasses for pathogenic *Y. enterocolitica*, either positive on tonsils, feces or carcass surface, observed in this study at the end of the slaughter line is rather low compared to other published results, but it confirms the importance of the hazard in pork. The seasonal trend in the carriage of *Y. enterocolitica* by pigs has been confirmed, with no positive samples during summer. 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. Epidemiological analysis of strains isolated in this study could allow us to identify the diversity of circulating strains of *Y. enterocolitica* and whether they are specific to a batch in the course of time.

Meat from deboned head has already been identified as a risk factor for *Y. enterocolitica* infection in humans. Nevertheless, little is known about its frequency of contamination and it has to be further investigated to better assess risk regarding meat products produced with this raw material, especially when tonsils are present at the beginning of the deboning process.

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