Impact of the slaughter process on the pork carcasses contamination by *Yersinia entrocolitica*

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Abstract

The aim of the study was to evaluate the impact of the tongue handling practice on the contamination of the pork carcasses: the tongue removed with the pluck set (3 slaughterhouses) *vs* the intact tongue inside the head (3 slaughterhouses). A total of 1920 pigs from 120 different farms were sampled both on their tonsils and carcass surfaces over a one year period. The individual prevalence of *Y. enterocolitica* on tonsils and carcasses was unexpectedly low and estimated respectively to be 5.7% [4.7-6.9] and 0.6% [0.3-1.0] from the pooled samples. The presence of *Y. enterocolitica* on the carcasses was statistically linked to its presence on tonsils. It was nearly five times higher on pigs with positive tonsils, than on pigs with negative tonsils.
Despite the experimental design, we were not able to confirm that the removal of the tongue on the slaughter line had a significant impact on the carcass contamination with *Yersinia enterocolitica*. These results confirm that cross contaminations occur during the slaughtering process and that good hygiene practices are necessary to limit the transfer of *Y. enterocolitca* from the tonsils, or the feces, to the carcasses.

Introduction

Yersinia enterocolitica are psychrotrophic *enterobacteria* responsible for enteric infections in humans, mainly young children's. In 2013, yersiniosis was the third most frequently reported zoonosis in the EU. The confirmed human cases were 6,471 (1.92 cases per 100,000 individuals) (EFSA & ECDC, 2015). *Y. enterocolitica* is classified into six biotypes. Biotypes 1B, 2, 3, 4 and 5 are considered pathogenic to humans while biotype 1A is believed to be non-pathogenic. In Europe, most human-pathogenic strains belong to bioserotypes 4/O:3 and to bioserotypes 2/O:9 and 2/O:5,27 to a lesser extent (EFSA & ECDC, 2015)
Pigs are considered to be the main reservoir of pathogenic strains. Infection is most often acquired by eating contaminated food, particularly raw or undercooked pig meat. Pigs do not develop clinical signs but carry *Y. enterocolitica* in the oral cavity, on the tongue and tonsils, in lymph nodes and they excrete the bacteria in their feces (Thibodeau, 1999, Nesbakken et al., 2003).
A higher prevalence is reported in tonsils, than in the other parts of the carcass (tongue, feces, intestinal content, lymph nodes, offal, or surface of the carcass). In France in 2010-2011, the individual prevalence on tonsils was estimated at 13.7% [10.1-17.3] whereas the inter-batches prevalence was of 74.3% [65-84] (Fondrevez, 2011; 2014). The carcasses and offal may become contaminated during slaughtering process, particularly by fecal contamination from gastrointestinal content during evisceration operations, and more generally by cross contaminations through equipment, personnel and environment of the slaughterhouse (Frederiksson-Ahomaa *et al.*, 2001, Nesbakken *et al.*, 2003). According to the literature, some slaughtering practices and inspection procedures may increase the frequency of contamination of offal and carcasses.
The aim of the study was to evaluate the impact of the tongue handling practice on the contamination of the carcasses by *Y. enterocolitica*: the tongue removed with the pluck set *vs* the intact tongue inside the head. This study has also allowed us to obtain data regarding the frequency of contamination of pig carcasses by *Y. enterocolitica* in France.

Material and Methods

Sampling was performed in six slaughterhouses. Three removed the tongue together with the pluck set on the slaughter line whereas three left the tongue intact inside the head until the end of the cutting process.
A total of 1920 pigs from 120 different farms/batches (60 batches of 16 pigs per type of slaughtering practice) were sampled both on their tonsils and carcass surfaces over a one year period ranging from November 2012 to October 2013. The bottom part of the carcass (sternal region, belly, and throat) and the oral cavity including the tonsils were swabbed. Microbiological analysis were equally shared between Ifip and Aérial laboratories. For each sampling type, the samples were pooled (pool of 4 pigs) and analyzed using 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*, *vir*F and *rfb*C, with the method of Arnold *et al*., (2004), which targets the *Yersinia enterocolitica* species specific 16s rRNA gene. The link between the contamination of tonsils and the contamination of the carcass and the link between the detection on carcasses/tonsils and the type of slaughtering process were assessed using Chi-2 tests. The individual prevalence was estimated using EpiTools (Sergeant, 2014). A logistic regression using SAS (v9.2) was performed to analyze the contamination of the carcasses as a function of either (i) the contamination of tonsils, (ii) the slaughtering process, (iii) the slaughterhouse or (iv) the batch.

Results

The prevalence results on tonsils are depicted in table 1. At the inter-batches level, the overall prevalence was of 48% [38-57]. The overall pooled-samples prevalence was of 21% [17-25], indicating that when a batch is positive, two pooled samples are positive in average. Nearly all slaughterhouses showed a prevalence ranging from 18 to 27,5% at the pooled-samples level. The difference between the tonsils prevalence of both type of slaughtering processes was not significant (p=10% and P=9% respectively) at the inter-batches (40% vs 55%) and pooled-samples (18% vs 24%) level. The overall individual prevalence estimated from the pooled-samples was of 5,7% [4,7-6,9].

	Batches (4 pools)		**Pooled samples (4 pigs)**		**Individual**
	Number of positive samples	**Prevalence**	**Number of positive samples**	**Prévalence**	**Estimated prevalence**
Overall	57/120	48% [38-57]	101/480	21% [17-25]	5,7% [4,7-6,9]
Without tongue withdrawal	33/60	55% [42-68]	58/240	24% [19-30]	6,7% [5,1-8,5]
With tongue withdrawal	24/60	40% [28-54]	43/240	18% [13-23]	4,8% [3,5-6,4]

Table 1: Prevalence results of *Y. enterocolitica* on tonsils according to the slaughtering process

The prevalence results on carcasses are depicted in table 2. The overall inter-batches and pooled samples prevalence were of 7,5% [3,4-13,8] and 2,3% [1,1-4,1] respectively. The carcasses contamination was not statistically different between slaughterhouses removing the tongue on the slaughter line *vs* leaving the tongue intact inside the head, either at the inter-batches (p=12%) and pooled-samples level (p=9%). The overall individual prevalence estimated from the pooled-samples was of 0,6 % [0,3-1,0].

The presence of *Y. enterocolitica* on the carcasses was statistically linked to its presence on tonsils (p=1,8%). It was nearly five times higher on pigs with positive tonsils (5,9%), than on pigs with negative tonsils (1,3%). The number of pigs having positive carcasses and tonsils was close between

slaughterhouses removing the tongue on the slaughter line (7%) *vs* leaving the tongue intact inside the head (5,2%). The number of pigs having positive carcasses and negative tonsils was nearly the same regardless of the type of slaughtering process (1,5% *vs* 1,1%).

	Batches (4 pools)		**Pooled samples (4 pigs)**		**Individual**
	Number of positive samples	**Prevalence**	**Number of positive samples**	**Prevalence**	**Estimated prevalence**
Overall	9/120	7,5% [3,4-13,8]	11/480	2,3% [1,1-4,1]	0,6% [0,3-1,0]
Without tongue withdrawal	4/60	6,7% [1,8-16,2]	5/240	2,1% [1,3-9,5]	0,5% [0,2-1,2]
With tongue withdrawal	5/60	8,3% [2,7-18,4]	6/240	2,5% [1,8-10,6]	0,6% [0,2-1,4]

Table 2: Prevalence results of *Y. enterocolitica* on carcasses according to the slaughtering process

The statistical analysis showed that the tonsils contamination was the only factor that significantly influenced the carcasses contamination (p=1,2%). The tongue withdrawal did not significantly impact the carcasses contamination (p=60%).
By PCR, we showed that 93,2% and 6,5% of strains (352) isolated in this study belonged to the pathogenic biotype 4/O:3 and 2/O:9 or 3/O:5,27 respectively, while 0,3% belonged to the non-pathogenic biotype 1A.

Discussion

The overall inter-batches tonsils prevalence was lower than the 74,3% [65-84] observed in France in 2011 (Fondrevez et al., 2014). The pooled-samples and individual tonsils prevalence were also lower than expected. These results could be explained by: (i) the detection method used, (ii) the impact of the pooling on the sensitivity of the detection method, and (iii) an under estimation of the prevalence for slaughterhouses removing the tongue on the slaughter line due to the partial withdrawal of the tonsils, thus reducing the surface to swab. Aerial and Ifip analyses of the same samples by the same method but in two different labs showed a strong consistency of the results (kappa: 0,66).
The overall estimated individual carcasses prevalence (<1%) was low as we expected. Our results were consistent with those of Bonardi et al. who identified a pork carcasses prevalence at slaughterhouse of 2,4% (Bonardi et al., 2013). However, they were much lower than the 15% prevalence observed in Belgium (Van Damme et al., 2013).
The aim of the project was to evaluate whether the withdrawal of the tongue on the slaughter line had an impact on the carcasses contamination. The sampling protocol was designed to highlight differences in prevalence higher to 4% at the pooled samples level. However, the low tonsils and carcasses prevalence observed did not allow us to evaluate the impact of the tongue handling practice on the contamination of the carcasses by *Y. enterocolitica*, even when taking into account the initial contamination of the tonsils. In our study, the tonsils contamination was the only factor that influenced significantly the carcasses contamination of the corresponding pig.
The prevalence of the bioserotypes identified was consistent with published data (Feurer et al., 2011, Bonardi et al., 2013, Laukkanen-Ninios, 2014).

Conclusion

The carcasses contamination was not statistically different between slaughterhouses removing the tongue on the slaughter line compared to the ones leaving the tongue intact inside the head.
Thus, despite the experimental design, we were not able to confirm that the removal of the tongue on the slaughter line had a significant impact on the carcass contamination with *Yersinia enterocolitica*. However, these results confirmed that the carcasses contamination is linked to the initial contamination of the corresponding tonsils. Cross contaminations appeared to be low but existed and

good hygiene practices remain necessary to limit the transfer of *Y. enterocolitca* from the tonsils, or the feces, to the carcasses.

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