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

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## Introduction

*In 2013, yersiniosis was the third most frequently reported zoonosis in Europe with 6,471 confirmed human cases (EFSA & ECDC, 2015). Pig is considered to be the primary reservoir for the human pathogenic types of Y. enterocolitica; mainly for biotype 4 (serotype O:3) and biotype 2 (serotype O:9 and O:5,27) to a lesser extent. 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 (Nesbakken et al., 2003). The carcasses and offal may become contaminated during slaughtering process (fecal contamination from gastrointestinal content during evisceration, 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 different handling practices of the tongue on the contamination of the carcasses by Y. enterocolitica on the slaughter line: tongue removed with the pluck set vs tongue left intact inside the head. This study has also allowed us to gather data regarding the contamination of pig carcasses by Y. enterocolitica in France.*

## Material and methods

**Sampling:** Six slaughterhouses: 3 removing the tongue with the pluck set and 3 leaving the tongue intact inside the head. 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 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. For each sampling type, the samples were pooled (pool of 4 pigs) in order to be able to conclude on the impact of the slaughtering practices

**Enrichment and detection:** Pooled 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 *rfb*C, with the method of Arnold *et al.*, (2004), which targets the *Yersinia enterocolitica* species specific 16s rRNA gene.

## Results

*Prevalence results on tonsils according to the slaughtering process*

| | 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] |

- **No significant difference of the tonsils contamination** between slaughtering processes : - At the inter-batches level (p=10%)
  - At the pooled samples level (p= 9%)
- **A low overall individual prevalence** estimated from the pooled-samples

*Prevalence results on tonsils according to the slaughtering process*

| | 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] |

- **No significant difference of the carcasses contamination** between slaughtering processes : - At the inter-batches level (p=12%)
  - At the pooled samples level (p= 9%)
- **A very low overall individual prevalence** estimated from the pooled-samples

*Prevalence results according to the sampling site and slaughtering process*

| | Tonsils (Pool) | Carcasses (Pool) | |
|---|---|---|---|
| | | Negative | Positive |
| Overall | Negative | 374 | 5 |
| | Positive | 95 | 6 |
| Without tongue withdrawal | Negative | 180 | 2 |
| | Positive | 55 | 3 |
| With tongue withdrawal | Negative | 194 | 3 |
| | Positive | 40 | 3 |

- The presence of *Y. enterocolitica* on the carcasses is statistically linked to its presence on tonsils (p=1,2%). Carcass contamination is 4,7 times higher on pigs with positive tonsils, but cross-contaminations accounted for about 50% of the presence.
- The tongue handling practice does not significantly impact the carcasses contamination (p=60%).
- 93,2% of the 352 isolated strains belong to the pathogenic biotype 4/O:3

## Conclusions

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 and that cross contaminations occur during the slaughtering process. Our results in six slaughterhouses with different processes indicate that the contamination of the carcass with *Y. enterocolitica* was very low and good hygiene practices remain necessary to limit the transfer of *Y. enterocolitca* from the tonsils, or the feces, onto the carcasses.

**Citations :** *Arnold et al., 2004. J. Vet. Med., B51, 23-27; EFSA & ECDC, 2015. The EFSA journal, 13 (1):3991. Fredriksson-Ahomaa et al., 2001. Syst Appl Microbiol 24, 457-463. 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.*

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