Y. enterocolitica prevalence, on fresh pork, poultry and beef meat at retail level, in France.

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Abstract
Y. enterocolitica is a zoonotic agent, and the third bacterial cause of human enteritis in Europe. The objective of this study was to assess consumer exposure to the pathogen Y. enterocolitica through meat consumption over a one-year period, in France. In this context, the prevalence of Y. enterocolitica was established on samples of fresh pork, beef and poultry collected at retail level in France. Of the 649 samples, 5.1% (34) were positive for Y. enterocolitica. No significant difference in prevalence between the categories of fresh meat was observed: the prevalence was 5.2% for pork, 5.2% for beef and 5.9% for poultry meat. However, tongues of pork were highly contaminated by Y. enterocolitica (12.5%) compared to other type of meat.

Although the isolation methods of Y. enterocolitica was done by a method promoting the detection of pathogenic biotypes 1B, 2, 3, 4 and 5 (enrichment ITC and streaking on CIN), only strains carrying biotype 1A were isolated. Strains of biotype 1A are considered non-pathogenic for humans. However, recent studies indicate that biotype 1A strains isolated from clinical cases have in their genomes genes known to play a role during disease. The presence of the three virulent genes inv, myfA and ystB were tested; the gene ystB being strongly related to the clinical biotype 1A. The three genes were detected for some of our strains and 71% of our isolates carried the ystB gene. Thus, these strains can be potentially pathogenic for humans.

Introduction
Y. enterocolitica is an important food-borne enteropathogen, known to cause a wide variety of clinical symptoms ranging from mild gastroenteritis to invasive syndromes like terminal ileitis (Bottone, 1999). Y. enterocolitica is the third bacterial cause of human enteritis in Europe (EFSA and ecdc, 2012).

This pathogen is of particular concern for consumers’ safety because it is able to growth in food stored at refrigeration temperatures without apparent signs of spoilage.

The species Y. enterocolitica is divided into six biotypes. The biotype 1A generally regarded as nonpathogenic and the pathogenic biotypes 1B, 2, 3, 4, 5 (Wauters et al., 1988). In France and most other countries worldwide, biotype 4 is the most prevalent biotype isolated from humans (69%), followed by biotype 2 (30%) and biotype 3 (Savin and Carniel, 2008). Genes involved in virulence have been characterized. Among them, the gene inv for invasive gene mediates cell invasion, the myf-gene encodes the production of fibrillae Myf and the yst-gene which encodes enterotoxin.

Y. enterocolitica has frequently been isolated from animals, food and environment (Falcao et al., 2006). Pigs are considered the principal reservoir for human pathogenic strain of Y. enterocolitica. Pigs do not develop clinical signs, but they do carry Y. enterocolitica in their oral cavity, on tongues and tonsils, and in lymph nodes, and excrete this bacterium in their feces (Thibodeau et al., 1999).

It is assumed that the main sources of infection in humans are pig and pork products (Bottone, 1999; Gousia et al., 2011), however Y. enterocolitica have been found on other type of meat (Bonardi et al., 2010).

A one year survey, done on pig tonsils at slaughterhouse in 2010-2011 by our laboratory, estimated that 13.7% of the pigs carried Y. enterocolitica and that 74.3% of the pig batches contained at least one positive pig (Fondrevez, 2012). Most of the strains were of biotype 4. In this context we investigate the occurrence of Y. enterocolitica in the major meat species, pork, beef and poultry at retail level in France. The isolated stains were then screened for the presence of virulence genes.

Material and Methods
Food samples
A total of 649 raw meat samples was collected at retail level in France during the year 2012. Samples were consisted in raw meat of pork (n=237), beef (n=210) and chicken (n=202). All the samples were kept at 4°C during transport and during
storage before analysis. The analysis was done within 4 days after purchase.

**Microbiological analysis**

The presence of *Y. enterocolitica* in meat samples was assessed as follow: 25g of meat were diluted 1:10 in peptone salt broth (AES chemunex) and homogenized in stomacher for 90s. One ml of this suspension was then placed in a tube containing 9 ml irgasan–ticarcillin–potassium chlorate (ITC) broth (Bio-Rad, Marnes La Coquette, France).

The ITC enrichment broth was incubated for 48h at 25°C. Streaking was done on cefsulodin–irgasan–novobiocin (CIN) agar plates (*Yersinia* Selective Agar Base and *Yersinia* Selective Supplement, Oxoid, Basingstoke, UK).

After 24h at 30°C, we checked for the presence of typical colonies on CIN plates. We then streaked a maximum of four characteristic colonies on each plate containing the *Y. enterocolitica* chromogenic medium (YeCM) (prepared in the laboratory as described by Weagant, 2008), for the presumptive selection of *Y. enterocolitica* isolates carrying pathogenic biotypes (red “bull’s-eye” colonies) and of *Y. enterocolitica* isolates carrying the biotype 1A (bleu-purple colonies). Each isolate was then subcultured on Plate Count Agar (PCA) plates (AES, Bruz, France) and incubated at 30°C for 24h for biochemical assays. The ability of isolate to degrade urea was used to confirm that the isolates belong to *Yersinia*. Strains were stored in peptone glycerol broth, at −80°C.

**DNA extraction and Real-Time PCR for detection of virulence genes**

Real Time PCR was used to evaluate the presence of virulence genes *inv*, *myfA* and *ystB*. Strains were sub-cultured on PCA at 30°C for 24h. DNA was extracted from some colonies with QIAamp DNA mini kit (Qiagen, USA) following the manufacturer's instructions. The PCRs were performed on a CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, California), in a final volume of 25μl with the Sybr* Green JumpstartTM Taq ReadyMix TM (Sigma-Aldrich, Saint Louis, Missouri) at 1X. The genes *inv* (Rasmussen *et al.*, 1994), *myfA* (Kot and Trafny, 2004) and *ystB* were detected with specific primers as indicated in Table 1. The final concentration of primers in the PCR reaction was 0.3 μM. The amplification conditions for each gene are detailed in Table 1.

**Table 1: Primer sequences and PCR conditions for detection of virulence genes by Real-Time PCR**

<table>
<thead>
<tr>
<th>Genes</th>
<th>Primer sequence</th>
<th>First step</th>
<th>Cycle of amplification</th>
<th>Melt Curve</th>
<th>Size in bp</th>
<th>Expected Tm</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>inv</em></td>
<td>F-CTG TGG GGA GAG TGG GGA AGT TGG G R-GAA CTG CTG GAA TCC CTG AAA ACC G</td>
<td>94°C 2 min</td>
<td>34 cycles of Denaturation 94°C 60 sec Annealing 61°C 60 sec Extension 72°C 30 sec</td>
<td>56°C -&gt; 95°C, increment of 0.5°C for 5 sec</td>
<td>570</td>
<td>87.5°C</td>
</tr>
<tr>
<td><em>myfA</em></td>
<td>F - CAG ATA CAC CTG CCT TCC ATC T R- CTC GAC ATA TTT CTC AAC ACG C</td>
<td>94°C 2 min</td>
<td>35 cycles of Denaturation 94°C 60 sec Annealing 58°C 90 sec Extension 72°C 90 sec</td>
<td>56°C -&gt; 95°C, increment of 0.5°C for 5 sec</td>
<td>272</td>
<td>84.5°C</td>
</tr>
<tr>
<td><em>ystB</em></td>
<td>F-AAA GCG TGC GAT ACT CAG AC R-CAG CAT ACC TCA CAA CAC CA</td>
<td>95°C 5 min</td>
<td>34 cycles of Denaturation 94°C 30 sec Annealing 55°C 30 sec Extension 72°C 30 sec</td>
<td>56°C -&gt; 95°C, increment of 0.5°C for 5 sec</td>
<td>68</td>
<td>79°C</td>
</tr>
</tbody>
</table>

**Results**

A total of 649 samples, including raw meat of pork, beef and chicken were obtained from different supermarkets in France. Of the 649 samples, 5.1% (32) were positive for *Y. enterocolitica*. Although the isolation methods of *Y. enterocolitica* was done by a method promoting the detection of pathogenic biotypes 1B, 2, 3, 4 and 5 (enrichment ITC and streaking on CIN), only strains carrying biotype 1A were isolated. The prevalence of this biotype in the different categories of meat was 5.2% for pork, 5.2% for beef and 5.9% for poultry meat (Table 2).
No significant difference in prevalence of Y. enterocolitica was observed between the three categories of fresh meat. Among pork meat sample, the tongues showed the highest prevalence (12.5%) followed by the minced meat (6.9%) (Table 3). The other pork meats like pork chop, fillet and roast were very little contaminated (2.1%). The occurrence of Y. enterocolitica in tongue was significantly higher than that of other pork meats ($\chi^2; p=0.05$) but not of that of minced meat ($\chi^2; p=0.42$).

Because recent studies indicate that biotype 1A strains may have in their genomes genes known to play a role during disease, the strain were screened for the presence of virulence genes inv, myfA and ystB. The incidence of virulence genes detected in the three categories of meat is presented in table 4.

Among the strains tested, only six lacked the virulence genes tested. The inv, myfA and ystB genes were detected with an incidence of 65%, 12% and 71%, respectively. The inv, and ystB virulent genes were detected in the various categories of meat. Only two isolates from pork and two isolates from chicken carried the gene myfA. This gene is not detected in isolates obtained from beef meat.

### Discussion

In the present work, Y. enterocolitica were detected in the three main category of meat. The prevalence was about 5% to 6% for raw pork, poultry and beef meat. This prevalence was lower than that observed in Italy by Bonardi et al. (2010) which detected a contamination rate of 15.2% for raw pork meat and 32.5% for chicken meat. In our study, tongues of pork were highly contaminated by Y. enterocolitica (12.5%) compared to other type of meat. This is coherent with the carriage of Y. enterocolitica by pig for which Y. enterocolitica is particularly present in their oral cavity (Thibodeau et al., 1999). The great majority of Y. enterocolitica isolates from food product belongs to biotype 1A (Bonardi et al., 2010), which is in agreement with our results. Indeed, none Y. enterocolititia carrying enteropathogenic biotype were detected in the present study although the isolation methods used promote the detection of pathogenic biotypes. Like previous studies (Bonardi et al., 2010; Falcao et al., 2006; Grant et al., 1998), the predominant genotype for virulent genes was inv+ and ystB+.

The presence of ystB is strongly related to the clinical biotype 1A (Grant et al., 1998) and is found in 71% of our isolates. The presence of the three genes ystB, inv and myfA for some of our strains reveals that these strains can be potentially pathogenic for humans.

### Conclusion

Our study indicates that consumption of meat from pork, beef or poultry presents a low risk of Y. enterocolititia for humans. Indeed, the prevalence of Y. enterocolititia is low and strains carried the biotype 1A considered as non-pathogenic for humans. However, some of these strains have several genes associated with pathogenicity especially the ystB gene often associated with clinical cases with this biotype. This risk should not be ignored.

### Acknowledgements

The authors gratefully acknowledge Aurore Fablet for technical help.

### References


