Abstract

*Listeria monocytogenes* is a pathogenic bacterium found in the environment and food. It causes Listeriosis, a serious disease that presents largest fatality rate among foodborne illness. The persistence of these bacteria in food processing plants, favoured by biofilm formation is a major problem both for industry and public health. The aim of the study was to compare strains isolated from three sectors in slaughterhouses for their ability to form biofilms. The strains were isolated from holding pens, slaughtering, and cutting in 4 slaughterhouses and for 3 different periods of the year. Biofilm formation analyses were performed for 186 strains under static conditions at 30 °C for 48 hours and 7 °C for 15 days, frequent temperature in meat processing plants. Biomass production in BHI is measured in microplates after staining with crystal violet and measuring the absorbance at 595 nm, the data is normalized to obtain inter strain comparability in 96 well plate. Results show significant difference in biofilm formation between strains according to the period of sampling and origin of strains (slaughterhouse). Biofilm formation by strains belonging to the sampling of period 2 (February to May 2014) were greater than in summer 2015 at 30 °C ($p<0,0001$) and at 7°C ($p=0,019$). Strains isolated from plants 1 and 2 produced more biomass than those collected from plant 4 at 30°C ($p=0,0015$) and 7°C, biofilm formation in isolates from plants 1and 2 were greater than in strains from plants 3 and 4 ($p<0,0001$). No difference in biofilm formation ability was revealed depending on the place sampled in the plant (holding pens, slaughter or cutting rooms), whatever the temperature tested. Among strains studied here, strong differences in biofilm forming ability were identified; these results are the beginning of our investigation aiming to answer the question of strain persistence.

Introduction

*Listeria monocytogenes* is a foodborne pathogen that causes listeriosis. Persistence of this pathogen in food industries is critical for food safety. Some of strains of this bacteria can persist for a long time in the slaughterhouses (Ortiz et al., 2010). The origine of the persistance of some strains in food plants is still questionned, some authors explained these recurrent presence by the ability of these strains to form biofilms (Ortiz et al., 2010) while other concluded that only cleaning and disinfection procedures, in particular in inaccessible places for cleaning, are involved (Carpentier et al., 2011). In our study we aimed to provide response to the persistance of some strains of *Listeria monocytogenes* by differences in biofilm formation abilities and we tried to show here if there is differences in biofilm formation depending on the season that the strains are isolated. Our main result show differences in biofilm formation ability according to the season and origin of strains (slaughterhouse).

Material and Methods

*Listeria monocytogenes* strains (N=186) were studied for their capacity to form biofilm at two temperatures: 30°C and 7°C. These strains were isolated, after sanitation procedures, from environmental sampling of 4 slaughterhouses, 3 sectors: holding pens, slaughtering and cutting areas and at 3 different periods of the year (table 1).
One isolate per positive sample was retained for this study. Biofilm formation was performed in flat bottom 96 well plates under static conditions and biomass production was measured by OD at 595 nm after staining with crystal violet 0.1% (p/v) and destaining with ethanol 95%.

3-way Analysis of variance was used to determine the differences in biofilm produced by these strains according to the period and origin of sampling (slaughterhouse and sector). Data were Log transformed to normalise the distributions.

**Table 1: Number of strains isolated per period and per sectors of the 4 slaughterhouses**

<table>
<thead>
<tr>
<th>Sector</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Holding pens</td>
<td>1</td>
<td>8</td>
<td>24</td>
</tr>
<tr>
<td>Slaughtering</td>
<td>10</td>
<td>10</td>
<td>32</td>
</tr>
<tr>
<td>Cutting</td>
<td>32</td>
<td>49</td>
<td>25</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>98</td>
<td>79</td>
</tr>
</tbody>
</table>


**Results**

The results show three different groups of strains in the two temperatures: strains with low biofilm production, normal production and a high biofilm production. Strains did not behave in the same way at 30 °C than at 7 °C as strains which form the 3 groups at 30 °C are not the same at 7 °C.

Biofilm formation abilities by strains isolated during period 2 was greater than those from period 1 and 3 at 30 °C, (Fig 2, A) and the period 2 and 3 when tested at 7 °C (Fig 2, B). No difference was shown between strains from the period 1 and 3 at 7°C.

At 30°C, strains isolated from plants 1 and 2 produced more biofilm than those collected from plant 4 and no difference was observed between plant 3 and the rest of the facilities (Fig 3, A). At 7°C, biofilm formation by isolates from plants 1 and 2 was higher than in strains from plants 3 and 4.

Our results showed no significant differences in biofilm formation between strains isolated from all sectors: holding pens, slaughtering and cutting areas and at whatever the two temperatures tested.

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**Discussion**

The main of this study was to compare *Listeria monocytogenes* strains isolated from samples collected during three seasons and from different sectors of four slaughterhouses. This is the first study in our knowledge where the differences of biofilm formation of *Listeria monocytogenes* strains are made according to the different periods of sampling. Our results demonstrated differences in biofilm formation depending to the period of sampling and to the slaughterhouses at the two temperatures tested (7°C, 30°C). Previous studies showed differences in biofilm formation by *Listeria monocytogenes* serotypes or lineages, according to the temperatures (Di Bonaventura et al. 2008) and origin of strains (Harvey et al. 2007). In our study and at this time, we didn’t have more information about characterisation of these strains yet but we can make the following hypothesis: Strains isolated from period 2 are different from those isolated from period 1 and 3; this will be confirmed after characterisation. In the other hand sampling is performed in two seasons different: winter for periods 1 and 2, summer for period 3, differences observed in these periods can lead us to suppose that there is seasonal effect on biofilm formation but more study is necessary to affirm this hypothesis. The same hypothesis could be done for differences observed in plant 1 and 2 and at the two temperatures.
One isolate per positive sample was retained for this study. Biofilm formation was performed in flat bottom 96 well plates under static conditions and biomass production was measured by OD at 595nm after staining with crystal violet 0.1% (p/v) and destaining with ethanol 95%.

3-way Analysis of variance was used to determine the differences in biofilm produced by these strains according to the period and origin of sampling (slaughterhouse and sector). Data were Log transformed to normalise the distributions.

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<thead>
<tr>
<th>Sector</th>
<th>Period 1</th>
<th>Period 2</th>
<th>Period 3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Holding pens</td>
<td>7</td>
<td>8</td>
<td>24</td>
<td>39</td>
</tr>
<tr>
<td>Slaughtering</td>
<td>19</td>
<td>18</td>
<td>32</td>
<td>69</td>
</tr>
<tr>
<td>Cutting</td>
<td>32</td>
<td>60</td>
<td>25</td>
<td>117</td>
</tr>
<tr>
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<td>48</td>
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One health approach under a concept of farm to fork

Conclusion
Our results showed a difference in biofilm production between strains depending on the period, that represented different seasons in the year. Biofilm formation, whatever the temperature considered, was greater for strains picked up in winter. Such an association need to be confirmed.

The biofilm formation ability appears different depending on the slaughterhouse considered. But the strains didn’t show differences in ability for biofilm formation according to the sectors tested, despite the known different characteristics of these steps of the production (in terms of temperatures, intensity of cleaning and disinfection...)

For further studies: Different strains with different biofilm formation ability will be studied for their differential genetic expression of genes involved in biofilm formation.

Acknowledgements
Thanks to Dr Philippe Fravalo, supervisor of this project and to Dr. Guy Beauchamps for statistic analysis.

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03. Impact of the slaughter process on the pork carcasses contamination by Yersinia enterocolitica

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. enterocolitica from the tonsils, or the faces, 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] (Fondreziz, 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-Ahoma 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.