Data on distribution and characterization of *Listeria monocytogenes* strains in a pork slaughter and cutting plant in Quebec support an earlier surveillance in the meat production chain.

Fravalo, P.1,2,3*, Larivière-Gauthier, G.1,2,3, Kerouanton, A.3, Quessy, S.1,2,3, Fournaise, S.4, Bekal, S.6 and Letellier, A.1,2,3.

1 NSERC, Industrial Research Chair in Meat Safety, Faculty of veterinary medicine, Université de Montréal, Saint-Hyacinthe, Canada
2 CRIPA, Centre de recherche en infectiologie porcine et avicole, Faculty of veterinary medicine, Université de Montréal, Saint-Hyacinthe, Canada
3 GRESA, Groupe de recherche et enseignement en salubrité alimentaire Faculté de médecine vétérinaire, Université de Montréal
4 Olymel S.E.C.
5 Anses, The French agency for Food, Environmental and Occupational Health Safety-Ploufragan (France)
6 LSPQ Laboratoire de santé publique du Québec -Sainte-Anne-de-Bellevue (Québec).

*corresponding author* : philippe.fravalo@umontreal.ca

*Listeria monocytogenes* is a major public health concern. It has been associated with highly mediatised outbreaks. It was the case in 2008, when an important episode (23 deaths) in Canada showed the need to reinforce the policies to better control *Listeria* in ready-to-eat products. Improved surveillance in the production plants environment increased the detection levels and represents a challenge for both industries and control agencies. In Canada, there is currently no surveillance policy of this microorganism in the steps preceeding ready-to-eat production. The distribution, diversity and characterisation of this microorganism in the slaughter and cutting environments are not well documented either. The aim of the present study is to provide such data, starting with a plant in Quebec. Moreover, recent studies suggested that the ability to produce biofilm and some virulence attributes (presence of SNP in InlA) are linked with “environmental” strains compared to virulent ones. Such characterization will be presented. The plant was sampled at three occasions, after the cleaning and disinfecting procedures, during a two-year period. A total of 874 samples were collected. *Listeria* detection followed the Health Canada standard method, geno-serogrouping was obtained by PCR and isolates were compared by PFGE after *ApaI* and *AscI* restriction. Four serogroups (mainly I/IIb) were found, and 6 out of 21 pulsotypes were regularly or sporadically associated to human clinical strains profiles. We observed an increase in occurence of *Listeria monocytogenes* following the processing steps ($\chi^2 \ p<0.05$). On the contrary, the diversity strongly and repeatedly decrease from step to step : 96.1% of the strains recovered from the cutting room presented the same profile. The “resident strains” have not been represented in clinical cases surveillance. Biofilm formation ability of strains alone could not explain strain transitions. Our results indicate that better knowledge of *Listeria monocytogenes* before food processing could be helpfull to optimise *Listeria monocytogenes* control under a risk analyse approach.

**Introduction :**

*Listeria monocytogenes* is the etiologic agent of listeriosis. The frequently deadly disease has been recognized as a foodborne disease since 1984. Then, numerous outbreaks were identified, associated with consumption of heavily contaminat-ed "ready to eat" products (RTE). Canadian regulation (Health Canada Agency, 2011) requires from RTE food industry, including meat and delicatessen production, the control of *L monocytogenes* both from products and from surfaces in the plant. The introduction of the bacteria in plants, via raw products, is scarcely documented in Canada. Nevertheless, a recent study confirmed raw meat as the main source of L monocytogenes in the meat transformation industry (Choy et al., 2012) and rare previous works, from the end of the previous century, suggested the presence of a single, largely distributed, clone from slaughter (Giovannacci et al., 1999). The present study aimed to describe and follow the evolution of L monocytogenes strains in a pig slaughter/cutting plant in Quebec. Strains were characterised and compared with provincial surveillances data from food and clinical.

**Material and method:**

Sampling: In the lairage, 60 pen floors were sampled (10 X 100cm²) just after emptying. One year later, in the same plant, during 3 successive sampling (one month separated) a total of 274 surface samples were done, after sanitation procedures. Samples consisted in swabbing of 900 cm² after gentle mechanical mobilisation (individual brushes) of the surfaces to be sampled. The slaughter (pre and post evisceration, chilling room) and the cutting zones (surfaces in contact and not with
meat) of the plant were sampled by a total of 92 sites, systematically swabbed per visit.

Detection: *L. monocytogenes* detection followed a procedure derived from MFHPB-30 standard (Health Canada): 2 enrichment steps, respectively 24h at 30°C and 48h at 37°C, in UVM-1 and Fraser broths. Each broth was isolated on chromogenic Aloa agar, incubated 24h at 37°C.

Identification: typical colonies (max. 2 per positive sample) were confirmed and geno-serogrouped by a multiplex PCR protocol (Kérouanton *et al.*, 2010). PFGE profiles (2 enzymes Ascl and ApaI) were obtained according to the CDC Pulsenet standardized protocol (Graves, 2001) for each isolate. Profile comparisons were conducted using BioNumerics® (Applied Maths) software. Strain pulsortype was determined and a new pulsortype was attributed when the profile similarities were strictly fewer than 100%, according to Dice correlation coefficient (1% band position tolerance). Diversity in the strain collections from the different zones of the plant was quantified by Simpson Index calculation.

Characterization: biofilm forming ability of strains was assessed by a biomass producing test: static incubation of strains, 24h in 1/20 TSB-Y e broth in round bottom micro-plates, and staining with crystal violet (Djordjevic *et al.*, 2002). Strains were compared to the collections established during *L monocytogenes* surveillance, food associated and clinical origin, in Quebec from 2001.

**Results:**

*L. monocytogenes* detections form surfaces in the raw meat processing zone were not infrequent after sanitation procedures. Several geno-serogroup including those relevant in a public health perspective: groups IIB and IVB were found. In this study, the more frequent serogroups were different in the different zones: lairage (IIA), slaughter (IIC) and cutting (IIB) (Table 1).

**Table 1: synthesis of the identification of *L monocytogenes* isolates from the lairage to the cutting zones.**

<table>
<thead>
<tr>
<th>Zone (Simpson index)</th>
<th>Geno-serogroup</th>
<th># of isolates</th>
<th>% per zone</th>
<th>Pulsotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lairage</strong> (D=0.01)</td>
<td>IIA</td>
<td>8</td>
<td>73%</td>
<td>2, 3, 11, 12, 19, 22, 23</td>
</tr>
<tr>
<td></td>
<td>IIB</td>
<td>1</td>
<td>9%</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>IVB</td>
<td>2</td>
<td>18%</td>
<td>4, 6</td>
</tr>
<tr>
<td><strong>Slaughter</strong> (D=0.19)</td>
<td>IIA</td>
<td>1</td>
<td>8%</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>IIB</td>
<td>5</td>
<td>38%</td>
<td>1, 8, 10</td>
</tr>
<tr>
<td></td>
<td>IIC</td>
<td>7</td>
<td>54%</td>
<td>16</td>
</tr>
<tr>
<td><strong>Cutting</strong> (D=0.69)</td>
<td>IIA</td>
<td>8</td>
<td>9%</td>
<td>11, 13, 15</td>
</tr>
<tr>
<td></td>
<td>IIB</td>
<td>74</td>
<td>86%</td>
<td>1, 5</td>
</tr>
<tr>
<td></td>
<td>IIC</td>
<td>3</td>
<td>4%</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>IVB</td>
<td>1</td>
<td>1%</td>
<td>6</td>
</tr>
</tbody>
</table>

The increase of the value of Simpson index well described that the diversity (number of different pulsortypes per zone) was high in lairage and low in cutting zone (despite it was easier to detect *L monocytogenes* from surfaces in cutting zone) (Table 1). This suggests a selection, a sorting, of strains between lairage and slaughter and moreover between slaughter and cutting zones (surfaces sampled concomitantly). In slaughter, the pulsortype 16 strains were predominant and detected at each visit. They became sporadic and were replaced by pulsortype 1 and 5 in the cutting zone. Those pulsortypes (1 and 5) were present but much less abundant in slaughter.

This transition could not be explained by a switch during chilling of the carcasses (as it...
could be frequently evoked) as *L monocytogenes* were seldomly detected in the chilling room in our conditions and presented the pulsotype 18, never shown anywhere else during the study.

Some pulsotype present in lairage appeared at some occasions in the cutting place (pulsotype 6 and 11).

Some difference in biofilm forming abilities was shown depending on strain pulsotypes. Pulatypes 1 and 5 strains did not present particular properties in biofilm forming. Pulotype 16, and strains 19 and 22, were the strains that shown the higher values in biomass forming.

**Table 2:** Distribution of the strains in surveillance of food chain/human cases (*Detected in food during outbreak investigations*).

<table>
<thead>
<tr>
<th>Pulsotype as determined in the present study</th>
<th>Not previously detected</th>
<th>Previously detected during food surveillance</th>
<th>Previously detected in human cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>regularly sporadically</td>
<td>regularly sporadically</td>
</tr>
<tr>
<td>2; 3; 4; 8; 10; 11; 12; 13; 16; 18; 23</td>
<td>1; 5; 15; 17</td>
<td>14*</td>
<td>6; 7; 9; 19*; 22</td>
</tr>
</tbody>
</table>

The strains (pulsotype 1 and 5), frequently detected in cutting plant, have never been associated with human cases, nor pulotype 16 and 17 strains frequent in slaughter. But 6 out the 21 strains pulotypes isolated were previously (regularly for pulotype 14 or sporadically) detected in patients (Table 2).

**Discussion:**
In the conditions of this study, isolation of *L monocytogenes* from environment of the plant, after sanitation procedures, described an increasing in homogeneity in the strain collections, from slaughter to cutting zone. The homogeneity of the *L monocytogenes* population in the cutting room is in agreement with previous study Giovannacci *et al.*, 1999). Ability to form biofilm, as assessed in vitro, didn’t appear to be the key to explain persistence.

But we described from lairage and slaughter that numerous strains are susceptible to enter the plant, and some can persist in the slaughter environment. It encourages reconsidering the idea that the primary production is a week source of *L monocytogenes* in meat production (Farzan *et al.*, 2010 and Wesley *et al.*, 2008), two studies that didn’t used chromogenic agar for strain isolation. Consideration of the “entering strains” is relevant (Boscher *et al.*, 2012) as in our study, some of them presented a profile related to clinical strains.

For industrial and public health considerations, better knowledge of the *L monocytogenes* present before the RTE step of meat production chain (identification of the persistent strains in plants and characterization of their virulence properties, e.g. integrity of the InlA gene sequence) should be particularly relevant to support efficient control measures.

**Conclusion:**
Many different *L monocytogenes* strains enter the meat production chain, some sorting exists in subsequent steps. Surveillance earlier in the primary production would permit to adapt the control measures for raw meat production and at the processing step (RTE level).

**References:**


Boscher E, Houard E, Denis M. Prevalence and distribution of Listeria monocytogenes serotypes and pulsotypes in sows and fattening pigs in farrow-to-finish farms (France, 2008). J Food Prot. 2012 May;75(5):889-95

Acknowledgment: This study was supported by the “Programme de soutien à l’innovation en agroalimentaire” by Québec ministry for agriculture food and fisheries.