Different Contamination Patterns of Lineage I and II Strains of Listeria monocytogenes in a Spanish Broiler Abattoir

V. López,* S. Ortiz,* A. Corujo,† P. López,* D. Poza,‡ J. Navas,* R. Moreno,† and J. V. Martínez-Suárez*1

*Departamento de Tecnología de Alimentos, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Carretera de La Coruña km 75, 28010 Madrid, Spain; †Food Research Centre (Nutreco), 45950 Casarrubios del Monte, Toledo, Spain; and ‡SADA p.a. Castilla-Galicia, S. A., 47008 Valladolid, Spain

ABSTRACT The purpose of this study was to determine whether genetically similar or diverse strains of Listeria monocytogenes colonize the environment and carcasses in a single Spanish broiler abattoir over time. The study was composed of 5 surveys over a 1.5-yr period and included the monitoring of cleaning and disinfection procedures. Overall, a total of 212 samples were tested for the presence of L. monocytogenes, and 31% of the samples were found to be positive. Listeria monocytogenes was isolated from carcasses and product contact and noncontact sites in the evisceration and carcass classification areas of the abattoir. A total of 132 L. monocytogenes isolates were characterized by PCR-based serotyping and pulsed-field gel electrophoresis (PFGE) restriction analysis with the endonucleases ApaI and AscI. Molecular serotyping showed that L. monocytogenes isolates were of serotypes 1/2a and 1/2b. Isolates of serotype 1/2b (89.4%) were contaminating carcasses as well as environmental product contact and noncontact sites, whereas isolates of serotype 1/2a (10.6%) were recovered only from environmental product noncontact sites. A relatively low genetic diversity was found in this group of L. monocytogenes isolates from the abattoir; only 14 different PFGE types (A1 to A14) were obtained. Nine pulsotypes belonging to serotype 1/2b (lineage I) were grouped in only one PFGE genetic cluster, whereas 5 pulsotypes belonging to serotype 1/2a (lineage II) were grouped into 4 PFGE genetic clusters. Two genetically related pulsotypes of serotype 1/2b (A1 and A2, 64.4% of the isolates) predominated and persisted in the abattoir. Our study indicated that a few strains of L. monocytogenes lineage I that were genetically very closely related might be specifically adapted to colonizing the evisceration zone of the abattoir and were predominant on carcasses over 1 yr. On the other hand, a genetically diverse group of lineage II strains were present in the abattoir environment, but never contaminated carcasses.

Key words: Listeria monocytogenes, broiler abattoir, molecular typing, genetic lineage, contamination pattern

INTRODUCTION

Listeria monocytogenes is a foodborne human pathogen that has a relatively high incidence in fresh meat and poultry (Farber and Peterkin, 1991; Ryser, 2007). Findings revealed that infection with L. monocytogenes resulted in a significant mortality. In addition, outbreaks occur frequently (ILSI Research Foundation-Risk Science Institute, 2005; Lynch et al., 2006) and several foods, particularly meat and poultry products, have been linked to these outbreaks.

The presence of L. monocytogenes in poultry products in different countries has been well documented (Rorvik et al., 2003; Ryser, 2007; López et al., 2007). The incidence of L. monocytogenes in poultry carcasses is typically high (van Nierop et al., 2005; Lindblad et al., 2006), and as a consequence, the contamination of processing equipment in poultry further-processing plants can be also high. It is well known that the presence of L. monocytogenes in poultry products is primarily caused by contamination during processing (Fenlon et al., 1996; Rorvik et al., 2003). To reduce the contamination of poultry further-processing plants through raw poultry, abattoirs should adhere to specific microbiological control measures, cleaning and disinfection procedures should be strengthened, and raw poultry decontamination procedures should be improved (Lyon et al., 2007).

Some subtypes of L. monocytogenes can persist for months or years in different kinds of food processing plants (Lunden et al., 2003; Berrang et al., 2005). Such
persistent subtypes appear to be unique to each processing unit (Wulff et al., 2006; Pugett et al., 2007). Despite several studies addressing the contamination patterns of poultry abattoirs (Loncarevic et al., 1994; Ojeniyi et al., 1996; Miettinen et al., 2001; Rovvik et al., 2003), it is still not clear how important live animal contamination is and whether particular physiological traits allow some strains to persist over the years (Lawrence and Gilmour, 1995; Ryser, 2007). In this latter case, control procedures targeting such specific strains must be established. Therefore, the ability to precisely track the strains or subtypes of \textit{L. monocytogenes} present in poultry abattoirs is critical for reducing the contamination of carcasses by this foodborne pathogen.

Serotyping is usually the first level of \textit{L. monocytogenes} subtyping. Of the 13 known serotypes of \textit{L. monocytogenes}, only 3 (i.e., 1/2a, 1/2b, and 4b) cause more than 90% of the human cases (Farber and Peterkin, 1991). A multiplex PCR assay has been developed to separate the major serotypes of \textit{L. monocytogenes} strains into 4 distinct groups, 1/2a (1/2a or 3a), 1/2b (1/2b or 3b), 1/2c (1/2c or 3c), and 4b (4b, 4d or 4e; Doumith et al., 2004). This PCR test constitutes a rapid and practical alternative to laborious classical serotyping. \textit{Listeria monocytogenes} also is composed of at least 3 evolutionary lineages; lineage I appeared to be completely clonal, whereas representatives of the other lineages demonstrated evidence of horizontal gene transfer and recombination (Meinersmann et al., 2004). Strains of serotypes 1/2b and 4b belong to lineage I, whereas strains of serotype 1/2a and many other strains isolated from foods belong to lineage II. Lineage I isolates of \textit{L. monocytogenes} are a particular public health concern because they are responsible for most sporadic cases of listeriosis and the vast majority of epidemic outbreaks. Genomic macrorestriction based on rare-cutting endonucleases such as \textit{Ascl} or \textit{ApaI} followed by pulsed-field gel electrophoresis (PFGE; Brosch et al., 1994; Aarnisalo et al., 2003; López et al., 2008) is considered a powerful typing method to conduct effective study of the sets of strains of \textit{L. monocytogenes}.

Virulence of \textit{L. monocytogenes} is also an important issue from a food safety perspective (Kathariou, 2002; López et al., 2006). Studies of infection in animal models (Larsen et al., 2002; ILSI Research Foundation-Risk Science Institute, 2005) and \textit{L. monocytogenes} surface immunodeterminants in clinical versus food strains suggest that \textit{L. monocytogenes} virulence is heterogeneous and demonstrates that the internalin (\textit{InIA}) is an important first step in the pathogenesis of listeriosis (Jacquet et al., 2004; Lecuit et al., 2004).

In this study, characterization of \textit{L. monocytogenes} isolates was achieved using PCR-serotyping, PFGE restriction analysis, and western-blot analysis of the internalin. Our final goal was to compare the contamination patterns of \textit{L. monocytogenes} in a poultry abattoir to determine the similarity or diversity of strains that dominate the environment and contaminate the carcasses over time.

**MATERIALS AND METHODS**

**Surveys and Sampling Procedure**

Sampling was conducted in a broiler abattoir, with a processing capacity of 50,000 animals per day, coming from at least 5 different provinces of Spain. Broiler chickens were the main abattoir product, either as whole raw chickens or as raw pieces. Five surveys were carried out for this study during March (survey 1), April (survey 2), and May (survey 3) in 2004 and May (survey 4), and July (survey 5) in 2005. Samples were taken from carcasses, environmental product noncontact sites, and product contact surfaces (Table 1).

Carcass samples consisted of small pieces of skin that were cut with sterile scissors from 3 to 4 different areas of one carcass surface to produce a 25-g sample (26 samples or carcasses). In 2005, carcasses were also sampled by swabbing at least 10 different animals, using dry sponges (SpongeSicle, Biotrace International, Barcelona, Spain) for each sample (16 samples or 160 carcasses).

Environmental sampling was performed in the different abattoir areas, which included the live receiving (4 samples), scalding and plucking (20 samples), evisceration (88 samples), carcass classification (49 samples) and chilling (5 samples) areas. Sampling locations were chosen to represent those most likely to harbor \textit{L. monocytogenes}. Sampling sites included product contact surfaces (e.g., conveyor belts, working tables, boxes, machines, cutters, hooks) and surfaces away from product (product noncontact surfaces such as walls, trucks, drains, doors, floor handles, hands washer, fire-fighting, rollers, etc). Environmental samples were collected during production and after cleaning and disinfection. Samples were collected during production by using dry sponges (Biotrace), with the exception of the floor drains, scalding water, and wastewater gutters, where 50 mL of liquid was sampled for analysis. Sampling of surface areas after cleaning and disinfection was conducted with sponges premoistened with neutralizing buffer (Biotrace). All samples were collected aseptically, put into sterile containers or bags, kept in a cooler, and analyzed within 24 h.

Overall, a total of 212 samples collected during the 5 surveys of years 2004 and 2005 were tested for the presence of \textit{L. monocytogenes}. This species was only isolated from carcasses and in the evisceration and carcass classification areas.

**Isolation of \textit{L. monocytogenes}**

The USDA procedure for detection of \textit{L. monocytogenes} (USDA, 2004) was followed. Both primary and secondary enrichments were streaked (0.1 mL) onto
both modified Oxford medium and Agar *Listeria* according to Ottaviani and Agosti (ALOA) selective and differential medium for *L. monocytogenes* and non-pathogenic *Listeria* spp. (Jantzen et al., 2006). All media were obtained from Biolife (Milan, Italy), except dehydrated ALOA (Chromogen *Listeria*, Biomedics, Madrid, Spain).

**Table 1.** Distribution based on sample types, total numbers of samples collected and numbers of *Listeria monocytogenes*-positive samples in surveys 1 to 5

<table>
<thead>
<tr>
<th>Survey</th>
<th>Sampling site</th>
<th>No. of samples</th>
<th>No. positive</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>P1 Post C&amp;D2</td>
<td>P Post C&amp;D</td>
<td>P Post C&amp;D</td>
</tr>
<tr>
<td>1</td>
<td>CC, chilling area</td>
<td>8</td>
<td>3</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>PC</td>
<td>10</td>
<td>1 1</td>
<td>10 10</td>
</tr>
<tr>
<td></td>
<td>PNC</td>
<td>10</td>
<td>3 2</td>
<td>30 20</td>
</tr>
<tr>
<td>2</td>
<td>CC, chilling area</td>
<td>9</td>
<td>7</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>PC</td>
<td>11</td>
<td>4 2</td>
<td>36 20</td>
</tr>
<tr>
<td></td>
<td>PNC</td>
<td>11</td>
<td>4 2</td>
<td>20 22</td>
</tr>
<tr>
<td>3</td>
<td>CC, chilling area</td>
<td>9</td>
<td>8</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>PC</td>
<td>11</td>
<td>6 1</td>
<td>55 9</td>
</tr>
<tr>
<td></td>
<td>PNC</td>
<td>13</td>
<td>4 1</td>
<td>9 14</td>
</tr>
<tr>
<td>4</td>
<td>CC, evisceration and postevisceration areas</td>
<td>12</td>
<td>7</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>CC, preevisceration areas</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PC</td>
<td>1</td>
<td>0 1</td>
<td>0 7</td>
</tr>
<tr>
<td></td>
<td>PNC</td>
<td>9</td>
<td>3</td>
<td>33</td>
</tr>
<tr>
<td>5</td>
<td>CC, evisceration and postevisceration areas</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>CC, preevisceration areas</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PC</td>
<td>17</td>
<td>6</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>PNC</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>CC, evisceration and postevisceration areas</td>
<td>42</td>
<td>25</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>CC, preevisceration areas</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PC</td>
<td>50</td>
<td>46</td>
<td>17 5</td>
</tr>
<tr>
<td></td>
<td>PNC</td>
<td>44</td>
<td>26</td>
<td>14 5</td>
</tr>
</tbody>
</table>

1P = production.
2C&D = cleaning and disinfection.
3CC = carcasses.
4PC = product contact surfaces.
5PNC = product noncontact environmental sites away from the product.
6In surveys 4 and 5, each carcass sample was a composite sample obtained by swabbing at least 10 different animals.

Species Confirmation and Main Group Serotype Identification

Confirmation of *L. monocytogenes* was carried out by PCR amplification of the iap gene (Navas et al., 2005) and acid production from rhamnose but not from xylose (USDA, 2004). Isolates were further confirmed and classified in PCR groups of serotypes using a multiplex PCR assay, as described by Doumith et al. (2004).

**PFFE Typing**

Plugs were prepared, and PFGE was performed, according to the Centers for Disease Control and Prevention PulseNet standardized procedure for typing *L. monocytogenes* (Graves and Swaminathan, 2001), by using the polygonal contour-clamped homogeneous electric field system (CHEF DR II, Bio-Rad Laboratories, Madrid, Spain). Restriction enzymes Apal and AscI (New England Biolabs, IZASA, Madrid, Spain) were used for cleaving the DNA. The Centers for Disease Control and Prevention standard *L. monocytogenes* strain H2446 was used as a reference strain during all PFGE experiments (Graves and Swaminathan, 2001). The PFGE types (or pulsotypes) were obtained by combining both restriction enzyme profiles and were

<table>
<thead>
<tr>
<th>Survey</th>
<th>Sampling site</th>
<th>Serotype (no. of isolates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CC</td>
<td>1/2b (6)</td>
</tr>
<tr>
<td></td>
<td>PC</td>
<td>1/2b (4)</td>
</tr>
<tr>
<td></td>
<td>PNC</td>
<td>1/2a (4); 1/2b (6)</td>
</tr>
<tr>
<td>2</td>
<td>CC</td>
<td>1/2b (14)</td>
</tr>
<tr>
<td></td>
<td>PC</td>
<td>1/2b (12)</td>
</tr>
<tr>
<td></td>
<td>PNC</td>
<td>1/2a (2); 1/2b (10)</td>
</tr>
<tr>
<td>3</td>
<td>CC</td>
<td>1/2b (16)</td>
</tr>
<tr>
<td></td>
<td>PC</td>
<td>1/2b (14)</td>
</tr>
<tr>
<td></td>
<td>PNC</td>
<td>1/2a (4); 1/2b (6)</td>
</tr>
<tr>
<td>4</td>
<td>CC</td>
<td>1/2b (14)</td>
</tr>
<tr>
<td></td>
<td>PC</td>
<td>1/2b (2)</td>
</tr>
<tr>
<td></td>
<td>PNC</td>
<td>1/2a (4); 1/2b (2)</td>
</tr>
<tr>
<td>5</td>
<td>CC</td>
<td>NF</td>
</tr>
<tr>
<td></td>
<td>PC</td>
<td>1/2b (12)</td>
</tr>
<tr>
<td></td>
<td>PNC</td>
<td>NF</td>
</tr>
</tbody>
</table>

1CC = carcasses.
2PC = product contact surfaces.
3PNC = product noncontact environmental sites away from the product.
4NF = not found.
designated with an A (abattoir) to differentiate them from pulsotypes previously found in the same company and designated with a P (further-processing plant; López et al., 2007). A PFGE profile was considered unique if one or more bands differed from other PFGE profiles (Tenover et al., 1995). A total of 132 isolates of *L. monocytogenes* were analyzed by PCR-based serotyping and PFGE subtyping.

**Western Blot Analysis of Internalin**

Western blot analysis of internalin was carried out as described previously (Olier et al., 2002; Jacquet et al., 2004), with a previously characterized monoclonal antibody directed against InlA (L7.7; Mengaud et al., 1996). Strains of *L. monocytogenes* used as controls were EGD-e (serotype 1/2a; Mengaud et al., 1996) and LO28 (serotype 1/2c; Olier et al., 2002).

**RESULTS AND DISCUSSION**

**Listeria monocytogenes Contamination at the Abattoir**

Out of 212 samples tested, 66 (31%) samples were positive, with 38% taken from carcasses, 29% from environmental product noncontact sites, and 33% from product contact surfaces (Table 1). Table 1 showed high carcass contamination rate in 2004, with the number of positive samples increasing along the different months. The slaughterhouse was less contaminated in March compared with April and May; only 37% of carcasses and 10% of contact sites sampled in March were positive for *L. monocytogenes*, compared with 78 and 36% in April, and 89 and 55% in May, respectively (Table 1). These data suggest the possibility of certain seasonal trends associated with the isolation of *L. monocytogenes* from broiler carcasses.

The relatively high *L. monocytogenes* contamination levels in the processing areas of the abattoir are in agreement with findings of other studies, although the number (or percentage) of positive samples may vary greatly, with the typical level being around 30% (Ojeniyi et al., 1996; Miettinen et al., 2001; Ryser, 2007). We found moderate levels of *L. monocytogenes* contamination in the plant after cleaning and disinfection (Table 1), and because several positive samples were found on product contact and noncontact sites, cleaning and disinfection procedures could be improved.

The abattoir was especially contaminated in the evisceration and carcass classification areas, and this is in agreement with other studies (Ojeniyi et al., 1996; Rorvik et al., 2003). The number of carcasses sampled in the present study was limited. However, the data indicate that the processing environment, whether at the evisceration area or at the carcass classification area, was the major source of contamination. Nevertheless, Lues et al. (2007) have found high counts of airborne *L. monocytogenes* (and other microorganisms) in the pre-evisceration areas of a chicken abattoir, emphasizing the importance of reducing microbial air contamination before processing.

**Analysis of the Serotypes**

The PCR-based serotyping divided all *L. monocytogenes* isolates into only 2 serotypes, 1/2a (14 isolates, 10.6%) and 1/2b (118 isolates, 89.4%) as shown in Table 2. Serotype 1/2b isolates were present in carcasses and environmental product noncontact and contact sites, whereas serotype 1/2a isolates were only present in environmental product noncontact sites. We mainly found strains belonging to lineage I that might be especially adapted to colonizing the evisceration zone of the abattoir and were predominant on carcasses over a 1.5-yr period, together with a group of lineage II strains that were present in the abattoir environment but never contaminating carcasses (Table 2).

Lineage I and II strains have previously been isolated from different foods. In addition, lineage I has been shown to be common among human listeriosis cases, whereas lineage II strains are more commonly isolated from environmental samples, including foods (Nightingale et al., 2006). The ability of specific strains to multiply in different niches may be determined by the presence or absence of specific genes (Hong et al., 2006), and divergence among *L. monocytogenes* lineages has occurred for many genes that can influence niche-specific fitness (Call et al., 2003; Severino et al., 2007).

**Analysis of the Pulsotypes Obtained**

The PFGE analysis of isolates using *Ascl* and *ApaI* yielded 9 (A-I) and 10 (1 to 10) restriction profiles, respectively. Isolates with one *Ascl* profile (A) resulted in 4 different *ApaI* profiles (1, 2, 5, 6), and other isolates showed the same *ApaI* profile (5) with different *Ascl* profiles (A and D). The profiles obtained with the 2 enzymes were combined, generating 14 pulsotypes (A1 to A14; Table 3). This can be considered a relatively low genetic diversity for this group of *L. monocytogenes* isolates from the abattoir. Isolates with the same PFGE profile always belonged to the same serotype (Table 3), although one PFGE type could represent different serotypes if changes in the genes encoding for cell molecules used for differentiating serotypes occurred.

The dendrograms of the PFGE profiles generated with *ApaI* or *Ascl* are shown in Figure 1. Clustering of the *L. monocytogenes* pulsotypes in 5 groups (CL1 to CL5) at a 0.5 similarity level was observed (Table 3). Nine pulsotypes belonging to serotype 1/2b were grouped in only one genetic PFGE cluster (CL1), and 5 pulsotypes belonging to serotype 1/2a were grouped in 4 genetic PFGE clusters (CL2 to CL5). Clustering of the 14 strains in these 5 groups according to distribution of *L. monocytogenes* serotypes and genetic lineages is in agreement with previous results of Brosch et al. (1994).
The abattoir sites where one or more pulsotypes of *L. monocytogenes* were isolated are listed in Table 4.

**Predominant Pulsotypes**

In 2004 (surveys 1 to 3), the abattoir was dominated by the presence of pulsotypes A1 and A2. In survey 1, pulsotype A1 represented 55% of the isolates, whereas A2 was not detected. In survey 2, pulsotypes A1 and A2 represented 42 and 32% of the isolates, respectively, and in survey 3 these figures were 0 and 60%. Overall, in 2004, pulsotypes A1 and A2 represented 64.3% of the isolates, but in carcasses this figure increased to 88.8%. Pulsotypes A1 and A2 were found both during production and after cleaning and disinfection (Table 3). A different result was obtained with pulsotypes A5, A10, and A11, which were only found after cleaning, and pulsotypes A7, A8, A12, A13, and A14, which were only found during production (Table 3).

The differences found between pulsotype A1 and A2 by PFGE using the 2 enzymes *Ascl* and *ApaI* were only in one band in the *ApaI* profile (data not shown), and it might have been caused by genomic mutation. Furthermore, sequencing of the variable region of the *actA* gene (Zhou et al., 2005; López et al., 2007) revealed an identical sequence for these 2 pulsotypes (data not shown). This implies that there might be a common clonal origin of the 2 predominant *L. monocytogenes*

### Table 3. Description of the *Listeria monocytogenes* pulsotypes

<table>
<thead>
<tr>
<th>Pulsotype</th>
<th>No. of isolates</th>
<th>Serotype</th>
<th>PFGE profiles</th>
<th>Dendrogram cluster (PFGE groups)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>24</td>
<td>1/2b</td>
<td>1A</td>
<td>CL1 (AS3-AP4)</td>
</tr>
<tr>
<td>A2</td>
<td>54</td>
<td>1/2b</td>
<td>2A</td>
<td>CL1 (AS3-AP4)</td>
</tr>
<tr>
<td>A3</td>
<td>4</td>
<td>1/2a</td>
<td>3B</td>
<td>CL2 (AS1-AP3)</td>
</tr>
<tr>
<td>A4</td>
<td>2</td>
<td>1/2b</td>
<td>4C</td>
<td>CL1 (AS3-AP4)</td>
</tr>
<tr>
<td>A5</td>
<td>0</td>
<td>1/2b</td>
<td>5D</td>
<td>CL1 (AS3-AP4)</td>
</tr>
<tr>
<td>A6</td>
<td>2</td>
<td>1/2a</td>
<td>5A</td>
<td>CL1 (AS3-AP4)</td>
</tr>
<tr>
<td>A7</td>
<td>2</td>
<td>1/2a</td>
<td>5E</td>
<td>CL2 (AS1-AP3)</td>
</tr>
<tr>
<td>A8</td>
<td>8</td>
<td>1/2b</td>
<td>6C</td>
<td>CL1 (AS3-AP4)</td>
</tr>
<tr>
<td>A9</td>
<td>8</td>
<td>1/2b</td>
<td>7F</td>
<td>CL3 (AS2-AP3)</td>
</tr>
<tr>
<td>A10</td>
<td>0</td>
<td>1/2a</td>
<td>1H</td>
<td>CL1 (AS3-AP4)</td>
</tr>
<tr>
<td>A11</td>
<td>0</td>
<td>1/2a</td>
<td>6A</td>
<td>CL1 (AS3-AP4)</td>
</tr>
<tr>
<td>A12</td>
<td>4</td>
<td>1/2a</td>
<td>9F</td>
<td>CL4 (AS1-AP2)</td>
</tr>
<tr>
<td>A13</td>
<td>2</td>
<td>1/2a</td>
<td>10G</td>
<td>CL5 (AS1-AP1)</td>
</tr>
</tbody>
</table>

1^P = production.

2^C&D = cleaning and disinfection.

3^According to Figure 1.
pulsotypes. The discriminatory ability of PFGE using these 2 endonucleases is very high (Fugett et al., 2007), but detection of small genetic differences that may not be epidemiologically significant could be considered a disadvantage (Tenover et al., 1995). In our case, PFGE subdivided the serotype 1/2b isolates into different pulsotypes, but they seem to be very closely related on a genetic level (Figure 1). Similar minor differences in PFGE profiles of lineage I isolates obtained at different sampling times from the same plant have been previously observed (Eifert et al., 2005). These findings suggest genomic diversification of L. monocytogenes lineage I isolates.

**Persistent Pulsotypes**

In 2005 (May and July, surveys 4 and 5) we sampled the abattoir to determine whether the predominant pulsotype A2 was also a persistent strain. Listeria monocytogenes-positive samples were found at each sampling, except for the carcasses which were sampled ahead of the evisceration (Table 1). A total of 63 samples were taken in 2005 (Table 1). Listeria monocytogenes was detected in 17 of these, and in 11 of these, pulsotype A2 was isolated. At some sampling sites, this was the only pulsotype found. The 2 samplings in 2005 were focused on the preevisceration and evisceration areas of the abattoir. Carcasses were found contaminated with only pulsotype A2, but in the preevisceration area and at the beginning of the evisceration area, L. monocytogenes was not isolated from carcasses (Table 1). After cleaning and disinfection, product contact sites were negative, but during production pulsotypes A2, A8, and A9 were found contaminating the evisceration equipment, as occurred in 2004.

Overall, none of the pulsotypes was present in all samplings, but most surveys showed 1 or 2 of the dominating pulsotypes (A1 and A2). Pulsotype A2 was especially common and was found in 4 of 5 surveys. Pulsotype A2 persisted in the abattoir, where it was reisolated after a year and clearly was the dominant strain. Of the 132 isolates typed by PFGE, 58 (44%) were A2 (Table 3), but if A1 and A2 were considered together, this figure increased to 64.4%.

Only 2 other pulsotypes (A6 and A8) were found contaminating carcasses. Of these, one (A8) was found contaminating the evisceration area 1.5 yr later together with the main pulsotype A2. However, contamination of carcasses with pulsotypes A6 and A8 was not significant (Table 3). Pulsotypes A3, A8, and A9 were found in product contact and noncontact sites in different surveys, and these strains probably enter continuously into the abattoir. Pulsotypes A4, A7, A10, A11, A12, A13, and A14 were found in the environment only in one survey, probably because they were eliminated by cleaning and disinfection procedures. Of these, A7, A10, A13, and A14 belonged to serotype 1/2a. Pulsotype A3 was the only 1/2a strain recovered from several samplings (the 3 samplings in 2004).

Each processing plant usually has its own molecular subtypes of L. monocytogenes, and most of the plants have persistent in-house L. monocytogenes subtypes (Miettinen et al., 2001; Rorvik et al., 2003). Our study clearly demonstrates that this abattoir harbors its own population of L. monocytogenes pulsotypes. The serotype 1/2b strain A2 could be an environmental contaminant specific to this plant. Strain A2 was predominant in spite of the diverse geographical origins of the animals received at the abattoir.

However, other studies have revealed that some subtypes of L. monocytogenes can be found in several processing plants, especially when they have an inter-trade relationship (Miettinen et al., 2001; Markkula et al., 2005). These subtypes can also be isolated from different geographical locations (Fugett et al., 2007). In our case, one of the persistent pulsotypes (A2) was previously found in a poultry further-processing plant of the same company, where it was designated P10 and represented 3.9% of the isolates studied in that survey (López et al., 2007). That further-processing plant is

Table 4. Broiler abattoir sites where Listeria monocytogenes was isolated and pulsotypes that were obtained

<table>
<thead>
<tr>
<th>Isolation site</th>
<th>Pulsotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC¹</td>
<td>Evisceration area</td>
</tr>
<tr>
<td></td>
<td>Evisceration hook</td>
</tr>
<tr>
<td></td>
<td>Gutter behind cloaca cutter</td>
</tr>
<tr>
<td></td>
<td>Neck cutting machine</td>
</tr>
<tr>
<td></td>
<td>Evisceration machine</td>
</tr>
<tr>
<td></td>
<td>Evisceration checking machine</td>
</tr>
<tr>
<td>PC²</td>
<td>Carcass classification area</td>
</tr>
<tr>
<td></td>
<td>Carcass classification box</td>
</tr>
<tr>
<td></td>
<td>Floor</td>
</tr>
<tr>
<td></td>
<td>Drain</td>
</tr>
<tr>
<td></td>
<td>Wall piping</td>
</tr>
<tr>
<td></td>
<td>Labeling machine</td>
</tr>
<tr>
<td></td>
<td>Roller under boxes</td>
</tr>
<tr>
<td></td>
<td>Drain</td>
</tr>
<tr>
<td></td>
<td>Water dripping from a metal plate</td>
</tr>
<tr>
<td>PNC³</td>
<td>Carcass classification area</td>
</tr>
<tr>
<td></td>
<td>Evisceration area</td>
</tr>
<tr>
<td></td>
<td>Drain</td>
</tr>
<tr>
<td></td>
<td>Wall piping</td>
</tr>
<tr>
<td></td>
<td>Roller under boxes</td>
</tr>
<tr>
<td></td>
<td>Tagging machine</td>
</tr>
<tr>
<td></td>
<td>Water dripping from a metal plate</td>
</tr>
</tbody>
</table>

¹CC = carcasses.
²PC = product contact surfaces.
³PNC = product noncontact environmental sites away from the product.
located 260 km away from the abattoir, but broiler carcasses are regularly received from the abattoir.

Autio et al. (2003) noted that strains of *L. monocytogenes* that were characterized as persistent within a plant were genetically distinct from those that were isolated only sporadically. However, their results were clearly different from ours because in their study persistent strains did not form any specific cluster and there was no difference between the 2 main lineages of *L. monocytogenes*.

Persistent strain A2 may be adapted to the environment of the abattoir, being able to survive and grow there. There exist different possibilities to explain this adaptation (Wulff et al., 2006): (a) strain A2 might be more common than other strains or might colonize the processing environment first (Miettinen et al., 2001). (b) Strain A2 might be a better colonizer [e.g., more efficient to adhere to surfaces (Berrang et al., 2005)]. Cutters and other processing machines provide sites at which *L. monocytogenes* may become established (Lunden et al., 2003; López et al., 2008). (c) Strain A2 might be more resistant to disinfectants (Aase et al., 2000), although resistance to sanitizing agents may be due to attributes of the biofilm substances and not to an intrinsic attribute of the cells (Pan et al., 2006). The presence of a persistent strain may also indicate the need for more thorough cleaning of potential niches in the evisceration environment. (d) Finally, strain A2 might be able to outcompete other strains on the processing equipment or during the enrichment procedure (Navas et al., 2007). Nevertheless, pulsotype A2 belongs to *L. monocytogenes* lineage I, and other studies indicate a competitive advantage for lineage II as compared with lineage I strains (Borucki et al., 2003; Bruhn et al., 2005).

**Expression of Internalin**

Western-blot analysis of the 14 pulsotypes showed that only pulsotypes A13 and A14 within serotype 1/2a did not express full-size InlA (data not shown). Recently, truncated, nonfunctional forms of InlA in several *L. monocytogenes* strains belonging to serotypes 1/2a and 1/2c (Jacquet et al., 2004), and to a lesser extent 1/2b (Nightingale et al., 2005), have been shown to be related to attenuation or loss of virulence. These results could be related to previous findings showing that certain pulsotypes of *L. monocytogenes* commonly found in food were less invasive than others in cell cultures (Larsen et al., 2002). We have shown here that strains of *L. monocytogenes* found in food processing plant environments may also differ in their pathogenic potential (Kathariou, 2002; López et al., 2006). Furthermore, we found that the only strains with a truncated InlA belonged to lineage II.

We can conclude that a few strains of *L. monocytogenes* of lineage I that were genetically very closely related might be particularly adapted to colonizing the evisceration zone of the abattoir and were predominant on carcasses over 1 yr. In addition, a genetically diverse group of lineage II strains were present in the abattoir environment, but without ever contaminating carcasses. Further studies specific to strain A2 are necessary to determine why it appears so readily in this poultry abattoir.

**ACKNOWLEDGMENTS**

This work was supported in part by Nutreco Servicios, S. A., the Spanish Ministry of Education grants CAL03-027-C2-1, PTR1995-0789-OP, RTA2005-00202-C02-01, and TRT2006-00048-00-00 (INIA-FEDER), and a fellowship of INIA from Spain (JNF). We are grateful to P. Cossart (Institute Pasteur, Paris, France) for kindly providing antibody L7.7 directed against InlA, and to C. Jacquet (Institut Pasteur, Paris, France), J. C. Pérez-Díaz (Hospital Ramón y Cajal, Madrid, Spain), and M. Suárez (Universidad Complutense de Madrid, Spain), for respectively providing strains H2446, EGD-e, and LO28. We also thank L. M. Graves (Centers for Disease Control and Prevention, Atlanta, GA) for providing typing protocols.

**REFERENCES**


Navas, J. S., S. Ortiz, P. López, V. López, and J. V. Martínez-Suárez. 2007. Different enrichment procedures for recovery of Listeria monocytogenes from raw chicken samples can affect the results of detection (by chromogenic plating or real-time PCR) and lineage or strain identification. J. Food Prot. 70:2851–2854.


Ojenyi, B., H. C. Wegener, N. E. Jensen, and M. Bisgaard. 1996. Listeria monocytogenes in poultry and poultry prod-
