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Full Length Research Paper

Risk factors favoring the presence of *Listeria monocytogenes* in Colombian pork-meat processing plants

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We tracked *Listeria monocytogenes* as a microbiological risk factor in pork processing plants. The five plants analyzed were located in main areas for meat processing in Colombia. Prevalence of *L. monocytogenes* was 11.46% (36/314), represented by serotypes 4b (6/36, 16.7%), 4d/4e (10/36, 27.8%), 4b/4d/4e (4/36; 11.1%), 3a (3/36, 8.3%), 3c (1/36, 2.8) and 1/2c/3c (9/36, 25%); 4/36 isolates (11.1%) were assumed as possible serotypes 4ab or 7. There was no tolerance to per-acetic acid and only 9/36 (25%) surface-isolates displayed tolerance (0.5-2% (v/v)) to alkyl dimethyl benzyl ammonium chloride. The risk factors analysis showed variations for each processing plant and found that surface and equipment had a higher degree of contamination, with the chopper and cutter at the highest risk. The general prevalence of *L. monocytogenes* in pork meat processing plants was 11.46%. All these results demonstrate deficiencies in the implementation and monitoring of cleaning and disinfection programs.

Key words: Risk factors, *L. monocytogenes*, meat products, pork meat processing plants.

INTRODUCTION

Listeria monocytogenes can contaminate a wide variety of foods (Molero et al., 2010; Ramírez Mérida et al., 2010; Ballesteros et al., 2011), with the ready-to-eat food (RTE) as potential transmission sources in particular meat- and dairy-products (Martins and Leal Germano, 2011; Muñoz et al., 2011).

Listeriosis is a foodborne disease caused by *L. monocytogenes* that is acquired in 99% of the cases by consuming contaminated food. The illness can manifest itself as invasive or non-invasive disease. The invasive form of the disease is characterized by spread of a severe- and/or local-infection in the central nervous system (CNS), which can result in death. In addition, it involves complications such as meningitis, and perinatal

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infections (Korkeala and Siitonen, 2003). On the other hand, the non-invasive form is associated with fever and gastrointestinal symptoms with no complications (Aureli et al., 2000; Pichler et al., 2009).

Up to date 13 potentially pathogenic serovars of *L. monocytogenes* have been identified, with 4b (37 - 64%), 1/2b and 1/2a (15 - 25%), and 1/2c (0 - 4%) the most frequent outbreaks and sporadic cases of the disease (Jacquet et al., 1995; Jay, 1996; Miyasaki et al., 2009; Markkula et al., 2011). The population identified at risk for this disease include immunocompromised individuals, pregnant women, infants and adults over 65 years (López et al., 2007; Garmendia et al., 2008; Mammina et al., 2009; Taillefer et al., 2010).

Despite the low incidence of listeriosis and far of surpassing other foodborne pathogens such as Salmonella, listeriosis outbreaks are associated with high mortality (20 and 30%), (Mead et al., 1999; FAO/OMS, 2004). The European Union and the United States of America report an annual incidence of 0.3 per 100,000 and 0.4 per 100,000 inhabitants, respectively (Centers for Disease Control and Prevention Morbidity and Mortality CDC, 2008; EFSA, 2008). Reports from Africa, Asia and South America have been scarce (Rocourt et al., 2003). However, recent reports indicate an increase in the number of cases, possibly associated with an increasing population at risk, in addition to changing trends in food consumption (Taillefer et al., 2010; Scallan et al., 2011).

In Colombia reports on foodborne disease incidence is scarce. The epidemiological data for *L. monocytogenes* shows few cases, probably associated with sub-registration (Vanegas et al., 2009). However, the "Instituto Nacional de Vigilancia de Medicamentos y Alimentos" (INVIMA), the Colombian equivalent of the US FDA, include a surveillance for the pathogen in their programs of food Inspection, Monitoring and Control (IVC). Recent studies have demonstrated the presence of this bacterium in the Colombian pork-industry. Furthermore, studies identified 4b as the most common serovar in contaminated RTE products. However, there is few data on contamination sources or microorganism control measures (Muñoz et al., 2011).

Considering the necessity to provide information on the country's status with respect to *L. monocytogenes* in meat processing plants, our objective was to evaluate and document the risk factors favoring the presence of *L. monocytogenes* in Colombian meat processing plants (pressed ham (sandwich type), standard sausage, and sausage "chorizo").

MATERIALS AND METHODS

Selection of meat products plants

Five meat processing plants were selected based on the following criteria:

1. Surveyed by the "Instituto Nacional de Vigilancia de Medicamentos y Alimentos" (INVIMA) as a registered plant.
2. Located in areas one and two, where the largest number of pork meat processing plants are (Figure 1).
3. Production of at least one of the following meat products: pressed ham (sandwich type), standard sausage, and sausage "chorizo".
4. Use of pork-meat as an ingredient in other processed meat products.

Polls

Each plant was assessed to establish factors that might favor the presence of *L. monocytogenes.* At question were aspects related to: technology level, infrastructure, type of utensils and processing equipment, blanching time and temperature, use of antimicrobials, and frequency of cleaning and disinfection, water quality, raw meat storage temperature, finished product and operator training, among others.

Number of samples

Three hundred and fourteen samples from five plants were selected, distributed as follows: plant 1: 53 samples (16.9%), plant 2: 71 samples (22.6%), plant 3: 61 samples (19.4%), plant 4: 89 samples (28.3%) and plant 5: 40 samples (12.7%). Environmental samples were collected from walls, floors, sewage, desks, equipment such as cutter, grinder, chopper, and raw meat described previously as the most likely sites of contamination (Thévenot et al., 2006).

Sampling numbers

Two samplings were conducted for each of the plants included in this study. However, plant 4 included four samplings due to its complexity.

Microbiological analysis

Samples were transported to the laboratory under aseptic conditions and processed immediately. The method for processing environmental samples was described by the United States Department of Agriculture, (USDA-FSIS, 2002). For meat cuts, the standard method ISO11290-1 was used (ISO, 1996). Isolates presumptive of *L. monocytogenes* were subjected to molecular identification (genus and species).

Genomic DNA purification and quantification

For genomic DNA extraction 100 µl of each isolate stock was cultivated in BHI supplemented with 0.5% (w/v) glucose during 12 h at 37°C and 250 rpm (Ruiz-Bolivar et al., 2011). One millilitre of culture was taken for DNA purification using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI USA). DNA purity and concentration were determined (NanoDrop 2000c, Thermo Scientific, Waltham, MA, USA) (Sambrook and Russell, 2001).

Molecular identification of *L. monocytogenes*

Two sets of primers were employed: L1/U1 and LF/LR. PCR final

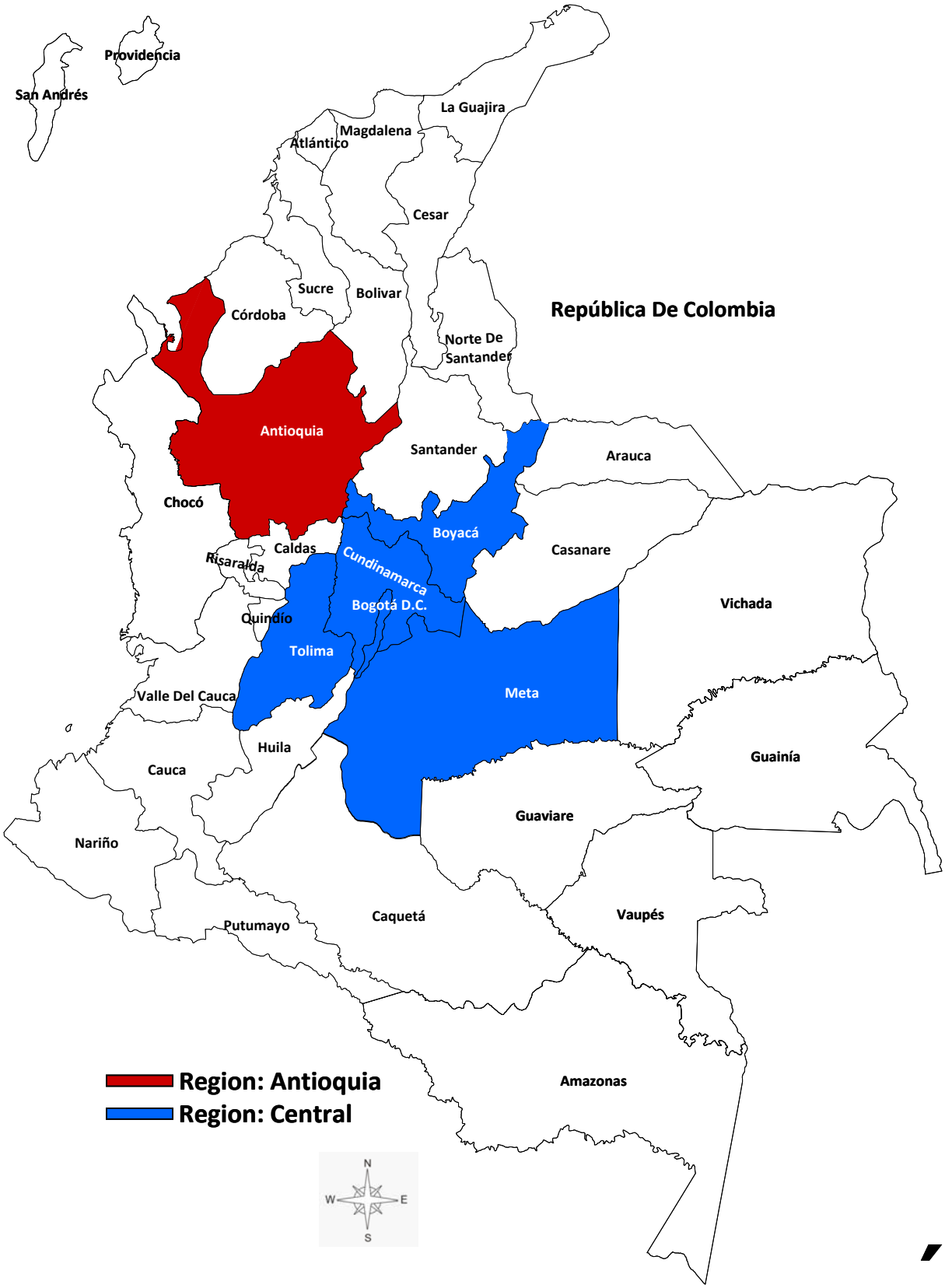

Figure 1. Map of Colombia with the two main areas of pork meat derivate product production.

Table 1. Sets of primers used successively in molecular serotyping *L. monocytogenes*.

Primers set	*Forward sequence*	*Reverse sequence*	*Product Size (bp)*	*Amplification conditions Hot start; (cycles details)* $_{\#\ cycles}$*; final extension*	*Especificity*	*Ref*
L1/U1$^{\nabla}$	CTCCATAAAGGTGACCCT	CAGCMGCCGCGGTAA TWC	938	95°C x 1´; (94°C x 30s, 51°C x 20s, 72°C x 30s) $_{40}$; 72°C x 8´	Genus (16S rADN)	
LF/LR$^{\nabla}$	CAAACGTTAACAACGCAGTA	TCCAGAGTGATCGATGTTAA	750		Specie (*hly*A)	
D1*	CGATATTTTATCTACTTTGTCA	TTGCTCCAAAGCAGGGCAT	214	95°C x 3´; (95°C x 30s; 59°C x 30s; 72°C x 1´) $_{25}$; 72°C x 10´	Divisions I or III	Ruiz-Bolivar et al. (2011)
D2*	GCGGAGAAAGCTATCGCA	TTGTTCAAACATAGGG CTA	140	95°C x 3´; (95°C x 30s; 59°C x 30s; 72°C x 1´) $_{25}$; 72°C x 10´	Division II	
*Fla*A♣	TTACTAGATCAAACTGCTCC	AAGAAAAGCCCCTCGTCC	538	95°C x 3´; (95°C x 30s, 54°C x 30s; 72°C x 1´) $_{25}$; 72°C x 10´	Serotypes ½a y 3a	
GLT♣	AAAGTGAGTTCTTACGAGATTT	AATTAGGAAATCGACCTTCT	483	95°C x 3´; (95°C x 30s, 45°C x 30s; 72°C x 1´) $_{25}$; 72°C x 10´	Serotypes ½b y 3b	
MAMA-C *	CAGTTGCAAGCGCTTGGAGT	GTAAGTCTCCGAGGTTGCAA	268	95°C x 10´; (95°C x 30s, 55°C x 1´, 72°C x 1´) $_{40}$; 72°C x 10´	Serotypes 4a y 4c	
CLM1/CLM2$^{\oplus}$	ACA GCT GGG ATT GCG GT	CCC AGC CAG AGC CGT GGA	1395	95°C x 5´; (95°C x 90s, 54°C x 1´, 72°C x 3´) $_{33}$; 72°C x 7´	Serotypes ½a, ½c, 4b	Comi et al. (1997)

Conditions for electrophoresis.$^{\nabla}$: 1% (w/v) agarose gel in buffer TAE 1X (40mM Tris-acetate, 1mM EDTA pH 8.0 ± 0.2), 120 volts, 1h. *: 2% (w/v) agarose gel in buffer TAE 1X. $^{\bullet}$: 1.2 (w/v) agarose gel in buffer TAE 1X. $^{\bullet}$: 1% (w/v) agarose gel in buffer TAE 1X, a 4 V/cm. $^{\oplus}$: 2.5% (w/v) agarose gel in buffer TAE 1X. Molecular size markers used were 100 bp (Promega, Invitrogen or Axygen). Gels were stained with ethidium bromi de (5 mg/ml).

reaction volume was 35 µl, composed of 1X Green PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 20 pmol of primers and 2U of GoTaq Flexi DNA polymerase (Promega). Five µl of DNA were used for thermal cycling. Cycling temperature was controlled in a C1000TM Thermal Cycler (BioRad, Hercules, CA USA). Amplification cycles and temperatures are listed in Table 1. *L. monocytogenes* (ATCC 19115) was used as PCR positive control (Gamboa-Marín et al., 2013).

Sorting by divisions and molecular serotyping

Isolates of *L. monocytogenes* were sorted-out by divisions using a Multiplex-PCR with two pairs of primers. Pair D1 yields a 214 bp product and classifies isolates into division I (serotypes 1/2b, 3b, 4b, 4d and 4e) or division III (serotypes 4a and 4c). Pair D2 yields a 140 bp product and classifies the isolates into division II (serotypes 1/2a, 1/2c, 3a and 3c). Isolates classified into division II were subtyped using the FlaA primer set to generate a 538 bp product, characteristic of serotypes 1/2a and 3a. The absence of amplification product indicated the presence of serotypes 1/2c or 3c. Isolates grouped into divisions I and III were subtyped with the GLT primer set to obtain a 483 bp product identifying serotypes ½b and 3b. Isolates that did not amplify a 483 bp product were considered serotype 4, and thus further subtyped with primers MAMA-C (LM4/LMB) yielding an amplified product of 268 bp that identifies serotypes 4a and 4c. Consequently, strains that did not amplify a 268 bp fragment were considered of serotype 4b/4d/4e, (Ruiz-Bolivar et al., 2011), (Table 1). The 100 bp ladder (Promega or Invitrogen, Carlsbad, CA USA) was used as molecular marker, and *L. monocytogenes* (ATCC 1915) was used as a positive PCR control.

Serotyping reaction mixtures

Primer sets D1 and D2 were used for classifying into divisions, and primer sets FlaA and GLT were used for PCR subtyping. The reaction mixture consisted of: 25 µl reaction volume, 50 pmol/µl of each primer, 1U of GoTaq Flexi DNA polymerase, 1X of Green PCR Buffer, 0.2 mm of

each dNTP, 2.5 mM $MgCl_2$ and 5 µl of sample DNA. For PCR subtyping of serotype 4 of division III, the primer set MAMA-C was used; the reaction mixture consisted of: reaction volume of 50 µl, 0.5 µmol of each primer, 2U TaqDNApol, 1X PCR buffer, 200 µM of each dNTP, 2.0 mM $MgCl_2$ and 2 µl of sample DNA (Ruiz-Bolivar et al., 2011). Cycles and temperatures of the amplifications are listed in Table 1.

Molecular confirmation of serotypes 1/2a, 1/2c and 4b

Isolates that were classified in serotype groups as 1/2a/3a, 1/2c/3c and 4b/4d/4e were subtyped by a single PCR by using a primer set CLM1/CLM2 which located within the *iap* gen of *L. monocytogenes* with a 1395 bp product. The reaction volume was 50 µl containing 0.1 pmol of each primer, 1.25U Taq DNA polymerase (Vivantis, Oceanside, CA USA distributed by Wacol S.A. Colombia), 1X PCR buffer, 0.125 mM of each dNTP, 1.5 mM $MgCl_2$ and 5 µl (~100 ng) of DNA sample (Table 1). Five microliter of each CLM1/CLM2 PCR-products were digested with *Hind*III restriction enzymes (Vivantis, distributed by Wacol S.A. Colombia), following the manufacturer's instructions (Comi et al., 1997).

Disinfectant tolerance test

All isolates were evaluated "*in vitro*" by using the "Disinfectants Tolerance Test" method, according to Colombian Technical Standard NTC5150, 2003. The disinfectants tested were per-acetic acid (0.5-1.0% v/v) and alkyl dimethyl benzyl ammonium chloride (DCA, 0.5, 2.0 and 3% v/v).

Characterization of the risk factors

Prevalence of *L. monocytogenes* risk characterization in each type of tested surface was analyzed, and the following scale was assigned: 0% insignificant, 1-10% low, 11-30% medium and high with values above 30%. Additionally, contamination of the final product was estimated by using a 2 x 2 matrix following the model proposed by the World Health Organization (WHO). Severity was determined based on the possibility of product contamination with *L. monocytogenes*, by using the following scale.

a) Low: Contaminated surface was not in direct contact with the finished product.
b) Medium: Contaminated equipment or raw meat used before thermal processing.
c) High: Equipment, surfaces or utensils were in direct contact with the finished product after subjected to thermal processing.

RESULTS AND DISCUSSION

For the five plants analyzed our results pointed out the following risks: use of wooden baskets (plant 2), use of equipment with additions made out of iron (plant 2), weekly monitoring instead of a daily inspection of residual chlorine in the water (plant 3), raw meat storage temperatures higher than the country's mandatory temperature (plant 3), and finished product storage temperature higher than 4°C (plants 1 and 5).

The most important extrinsic parameter to control *L. monocytogenes* is temperature, considering its ability to grow in refrigeration temperatures. This aspect is critical for meat products. Even at low initial contamination concentrations, the organism can multiply during storage in the processing plant or the market, reaching levels above the minimum infective dose (1000 CFU/g). It has been shown that these levels can be achieved during the lifetime of the product, when stored at temperatures ranging between 4 - 9°C. From our results we observed that plants 1 and 5 maintain products at storage temperatures within this range, and are therefore at risk (Sergelidis et al., 1997).

A total of 36 isolates of *L. monocytogenes* were molecularly identified and serotyped as follows (Figure 2): plant 1: 13/53 samples were positive (24.5%), plant 2: 10/71 samples were positive (14%); plant 3: 4/61 samples were positive (6.5%), plant 4: 9/89 samples were positive (10%) and plant 5: 0/40 were positive (0%), demonstrating that the presence of this organism due to external factors, for an overall prevalence of 11.46%. Our results agree with studies by Gudbjörnsdóttir et al*.,* (2004) who reported a prevalence ranging between 0 and 15.1% and are inferior to those obtained by Chasseignaux et al., (2002) who reported 23.7%. These differences may be related to the technology level and equipment used. Isolates were recovered from: work surfaces (33%), followed by raw meat (29%) and knives (16.7%). As with other reports the most frequent site of contamination were floors (Thévenot et al., 2006). Non-contact surfaces represented 14% and 10% in processing equipment, demonstrating that any environment within the meat industry may be contaminated with this bacterium.

For many years pork-meat derived products have been associated with foodborne outbreaks around the world (Goulet et al., 1998; CFIA, 2008). When analyzing the causes of outbreaks, it has been determined that contamination in meat processing plants occurs at the post-processing stage or by the presence of contaminated raw meat entering the plants. The latter one are contaminated during the deboning process, because the prevalence of this organism during the benefit has been low 3.7 to 4%, (Gamboa-Marín et al., 2012), 4% (Kanuganti et al., 2002). Several studies indicate that contamination of raw meat occurs mainly in deboning-rooms (Van den Elzen and Snijders, 1993; Nesbakken et al., 1996).

In the present study we found *L. monocytogenes* in four out of five plants, which confirms the ability of this microorganism to enter into meat processing plants, despite established programs for cleaning and disinfection. The presence of *L. monocytogenes* showed a wide spread in meat processing plants, which is consistent with studies by various authors (Chasseignaux et al., 2002; Thévenot et al., 2006). *L. monocytogenes* can survive industrial environments because it can grow

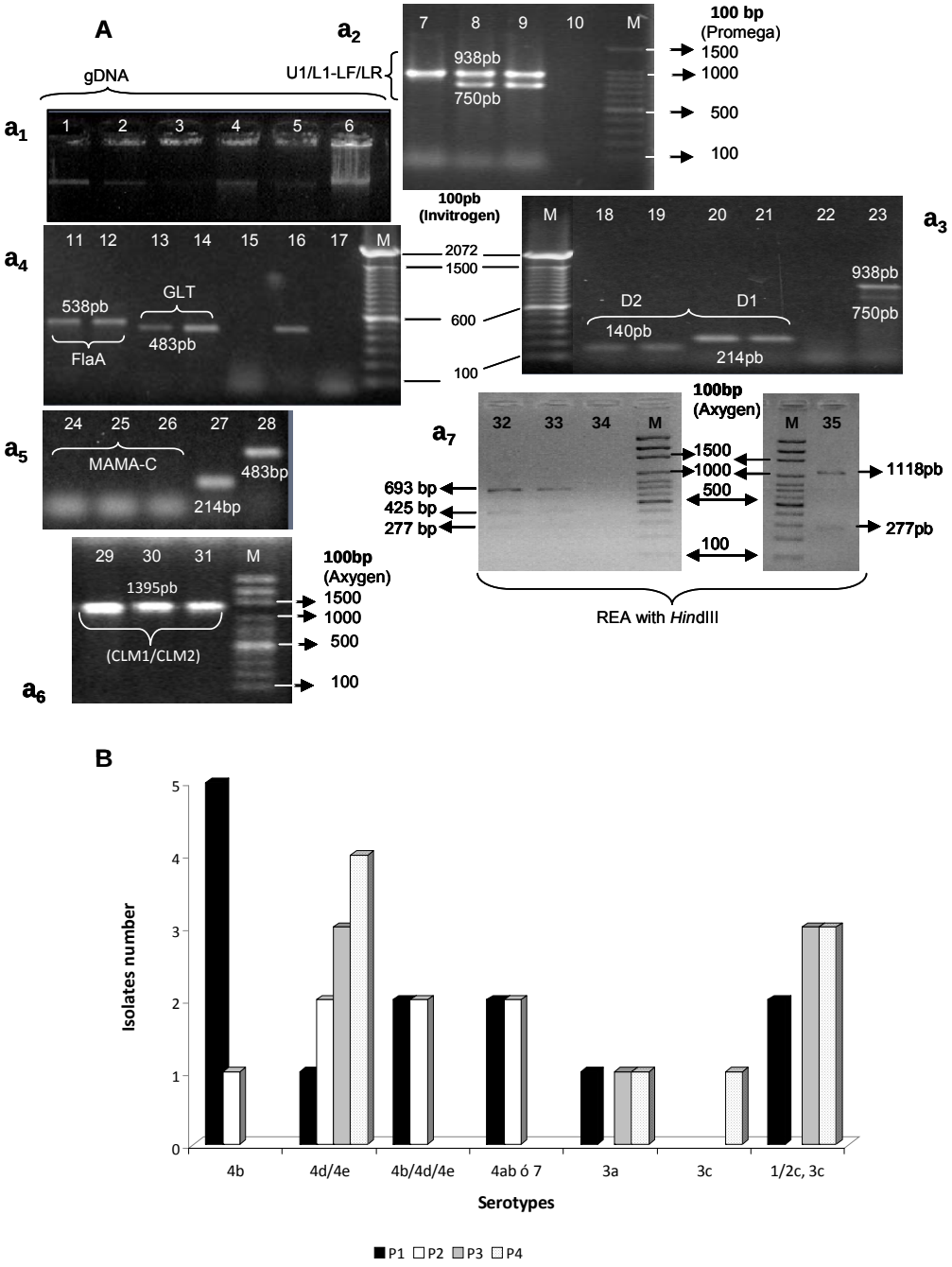

Figure 2. **A**: DNA isolation, molecular identification and serotyping of isolates of *L. monocytogenes*. (**a1**) 1 - 6. DNA extractions. (**a2**) Amplification with U1/L1-LF/LR for genus and species Lane 7: *Listeria* spp. isolate, Lane 8: *L. monocytogenes* ATCC 19115, Lane 9: *L. monytogenes* isolate (present study), Lane 10: PCR reagent control. (**a3**) Amplification with D1/D2 to clasify in Division I or Division II and III. Lanes 18 and 19: *L. monytogenes* isolates (present study), Lane 20: *L. monocytogenes* ATCC 19115, Lane 21: *L. monytogenes* isolate (present study), Lane 22: PCR reagent control, Lane 23: *L. monocytogenes* isolate (present study) amplified with U1/L1-LF/LR for genus and species. (**a4**) Lanes 11 and 12: Amplification with FlaA for individuals in the Division II, Lane 13: *L. monocytogenes* ATCC 19115 amplified with GLT serotype 1/2b, Lanes 14 and 16: amplification with GLT of 1/2b or 3b isolates, Lane 17: PCR reagent control. (**a5**) Lanes 24 - 26: isolates of serotype 4 that were not amplified with MAMA-C, therefore classified as serotypes 4b/4d/4e, Lane27: the same DNA as in lane 20, Lane 28: the same DNA as in lane 14. (**a6**) amplification with CLM1/CLM2, Lane 29: *L. monocytogenes* ATCC 19115, Lanes 30 and 31: serotypes 4b/4d/4e isolates). (**a7**) Restriction Enzyme Analysis of amplification products obtained with CLM1/CLM2, Lane 32: same DNA as in lane 30, Lane 33 same DNA as in lane 31, Lane 34: isolate of serotype 1/2a, 3a showing the expected restriction pattern with *Hin*dIII, therefore serotype 3a, Lane 35: isolate of serotype 4b/4d/4e, showing the expected restriction pattern with *Hin*dIII, therefore serotype 4b. **B**: Distribution of serovars of *L. monocytogenes* in meat processing plants.

at low temperatures, adheres easily to surfaces, and some strains are tolerant to disinfectants (Ruiz-Bolivar et al., 2011). According to Kathariou (2002) the main source of food contamination by *L. monocytogenes* before arriving to the final consumer seems to be the processing environment. The RTE food can become contaminated by contact with raw meat or ingredients, equipment and utensils, or post-processing contamination. This issue was confirmed by our results as we demonstrated *L. monocytogenes* on meat choppers.

Gandhi and Chikindas (2007), emphasize that food contamination can occur at any stage of processing, and the most important source of re-contamination are raw products. In the present study two plants were found to be contaminated with raw meat. Other authors indicate that *L. monocytogenes* can survive in different types of surfaces, including stainless-steel (material commonly used in the meat industry for equipment, tools and work tables). Previous studies have demonstrated *L. monocytogenes'* ability to persist and form biofilms (Mattos de Oliveira et al., 2010). All these risk factors favor the cross-contamination between the environment and all processing equipment (Jemmi et al., 2002).

Figure 2A shows the identification and molecular characterization of isolates and Figure 2B depicts the serovars isolated. Serovar 4d/4e was detected in all plants positive for *L. monocytogenes.* Serovar 4b was isolated from plants 1 and 2; in this latter one it was isolated from the chopper. Serovar 3a was recovered in samples from three different plants. Some of the isolates only amplified bands for genus (938 bp) and species (750 pb), but no amplification products were generated with primers used for serotyping. Thus they were grouped as members of serotypes 4ab or 7, these results remain to be confirmed experimentally (Figure 2B).

Although Orsi et al., (2011), indicated that serotypes 1/2 a, 1/2 c, 3a and 3c are often found in food processing plants because of their greater ability to adapt to the environment, this study also identified isolates of serotype 4. We assert the finding of serovar 4b, as it has been associated to primary causes of foodborne outbreaks.

Regarding disinfectants evaluation, no isolates were tolerant to per-acetic acid at the concentrations studied. Nine isolates (25%) from: floors (3), walls (3) and sewage (3) displayed tolerance to ACDA at concentrations of 0.5 and 2.0% (v/v). When analyzing the isolates' tolerance to disinfectants, it was found that those isolated from sewage, floors and walls showed certain tolerance degree to ACDA, which suggests that contact with organic matter and high humidity, promotes the formation of a biofilm, increasing the tolerance to disinfectants (Denyerae and Stewart, 1998). Additionally, *L. monocytogenes'* presence in sewage received sublethal concentrations of disinfectant, allowing it to gradually adapt to these substances, favoring the persistence of some strains (Mattos de Oliveira et al., 2010).

Our results are consistent with other studies that demonstrated an increase in the tolerance of *L. monocytogenes* to quaternary ammonium (Mereghetti et al., 2000), traditionally used disinfectants in the food industry. This data suggest the possible presence of efflux pumps as an adaptation mechanism in sensitive strains (To et al., 2002).

Risk factors classification for each meat processing plant is described in Table 2. As depicted in this table risk varied among plants. None the less, some surfaces and equipment had increased contamination, with chopper and cutter at the highest risk. Furthermore, we found this organism in raw meat.

Traditionally studies to assess the risk factors of *L. monocytogenes* in the industry are established through the sampling of different sites where the organism may be present and correlated with environmental factors (Giovannacci et al., 1999; Chasseignaux et al., 2002). However, this appraisal cannot determine the impact that contamination could have on the final product. In this study we propose to qualify the risk factors. This model proposes to estimate the probability level of contaminated foods leaving the factory. This tool allowed to demonstrate that the contamination vary depending on the processing plant, agreeing with several authors (Chasseignaux et al., 2002; Thévenot et al., 2006). Moreover, this tool allowed establishing that the number of contaminated sites along the production process is proportional to the risk of final product contamination. It was also demonstrated that the critical risk on plant 3 was the chopper. If this equipment is contaminated with *L. monocytogenes*, it increases the possibility of releasing contaminated product to the customer. Additionally it demonstrated the low risk that sewage represents to the final product. However, it remains an issue to be considered. Last, this model allowed determining which and how areas within the plant should be treated to reduce contamination of the final product.

Conclusions

Finally, one of the most important practices for the control of *L. monocytogenes* in processing plants should be to implement a cleaning and disinfection program under strict monitoring. As part of this program it should be included cleaning of sewage. It is decisive during this cleaning to avoid the formation of aerosols and the use of high pressure systems.

The general prevalence of *L. monocytogenes* in pork meat processing plants was 11.46%. The main risk factors were: raw meat, cutter, mixer, sewage and choppers. The best strategy for reducing *L. monocytogenes* in meat processing plants is the implementation and monitoring of cleaning and disinfection programs.

Table 2. Risk factors that favor the presence of *L. monocytogenes* in Colombian pork meat processing plants.

Sample origin	Number of Samples taken	Positive Samples	Prevalence	Frequence	Severity	Risk
Plant 1						
Floors	3	0	0	Insignificant	Low	Insignificant
Walls	4	0	0	Insignificant	Low	Insignificant
Sewage	5	2	40	High	Low	Low
Desks	2	1	50	High	Medium	High
Knives	2	0	0	Insignificant	Medium	Insignificant
Mill	6	3	50	High	Medium	High
Mixer	4	2	50	High	Medium	High
Stuffer	9	1	11	Medium	Medium	High
Cutter	6	3	50	High	Medium	High
Chopper	6	0	0	Insignificant	High	Insignificant
Baskets	2	0	0	Insignificant	High	Insignificant
Raw meat	2	1	50	High	Medium	High
Plant 2						
Floors	4	0	0	Insignificant	Low	Insignificant
Walls	4	0	0	Insignificant	Low	Insignificant
Sewage	6	0	0	Insignificant	Low	Insignificant
Desks	4	1	25	Medium	Medium	High
Knives	2	1	50	High	Medium	High
Mill	6	0	0	Insignificant	Low	Insignificant
Mixer	6	0	0	Insignificant	Low	Insignificant
Stuffer	15	0	0	Insignificant	Low	Insignificant
Cutter	6	1	17	Medium	Medium	High
Oven	2	1	50	High	Medium	High
Chopper	9	5	56	High	High	Critical
Baskets	3	0	0	Insignificant	Low	Insignificant
Emulsifier	2	1	50	High	Medium	High
Raw meat	2	1	50	High	Medium	High
Plant 3						
Floors	2	0	0	Insignificant	Low	Insignificant
Walls	4	3	75	High	Low	Insignificant
Sewage	11	0	0	Insignificant	Low	Insignificant
Mill	5	0	0	Insignificant	Low	Insignificant
Mixer	8	0	0	Insignificant	Low	Insignificant
Stuffer	17	0	0	Insignificant	Low	Insignificant
Chopper	6	1	17	Medium	High	High
Emulsifier	1	0	0	Insignificant	Low	Insignificant
Raw meat	1	0	0	Insignificant	Low	Insignificant
Plant 4						
Floors	7	1	14	Medium	Low	Low
Walls	5	0	0	Insignificant	Low	Insignificant
Sewage	14	5	35	High	Low	Insignificant
Mill	18	2	11	Medium	Medium	Higher
Mixer	12	0	0	Insignificant	Low	Insignificant
Stuffer	14	0	0	Insignificant	Low	Insignificant
Oven	2	0	0	Insignificant	Medium	Insignificant
Baskets	3	0	0	Insignificant	Low	Insignificant
Emulsifier	1	0	0	Insignificant	Low	Insignificant
Raw meat	1	0	0	Insignificant	Low	Insignificant

Conflict of Interests

The author(s) have not declared any conflict of interests.

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REFERENCES

Aureli P, Fiorucci GC, Caroli D, Marchiaro G, Novara O, Leone L, Salmaso S (2000). An outbreak of febrile gastroenteritis associated with corn contaminated with *Listeria monocytogenes*. New Eng. J. Med. 342:1236-1241.

Ballesteros L, Moreno Y, Cuesta G, Rodrigo A, Tomás D, Hernández M, Ferrús MA, García Henández J (2011). Persistence of *Listeria monocytogenes* strains in a frozen vegetables processing plant determined by serotyping and REP-PCR. Int. J. Food. Sci. Technol. 46:1109-1112.

Centers for Disease Control and Prevention Morbidity and Mortality CDC (2008). Outbreakof *Listeria monocytogenes* infections associated with pasteurized milk from a local dairy—Massachusetts, 2007 MMWR. 57:1097-1100.

CFIA (2008). Agence de la santé publique du Canada. *Listeria monocytogenes* outbreak.

Chasseignaux E, Gérault P, Toquin MT, Salvat G, Colin P, Ermel G (2002). Ecology of *Listeria monocytogenes* in the environment of raw poultry meat and raw pork meat processing plants. FEMS Microbiol. Lett. 210:271-275.

Comi G, Cocolin L, Cantoni C, Manzano M (1997). A RE-PCR method to distinguish Listeria monocytogenes serovars. FEMS Imnunol. Med. Microbiol. 18:99-104.

Denyerae SP, Stewart GSAB (1998). Mechanisms of action of desinfectants. Int. Biodet. Biodeg. 41:261-268.

EFSA (2008). The community summary report on trends and sources of zoonoses, Zoonotic agents and food-borne outbreaks in the european union in 2008. 8:1-358.

FAO/OMS (2004). Evaluación de riesgos de *Listeria monocytogenes* en alimentos listos para el consumo. 1-54.

Gamboa-Marín A, Buitrago SM, Pérez-Pérez KI, Mercado R. M, Poutou-Piñales RA, Carrascal-Camacho AK (2012). Prevalence of *Listeria monocytogenes* in pork-meat and other processed products from the Colombian swine industry. Rev. MVZ-Córdoba. 17:2827-2833.

Gamboa-Marín A, Mejía-Wagner DC, Moreno-Ocampo PA, Buitrago SM, Pérez-Pérez KI, Ruiz-Bolivar Z, Poutou-Piñales RA, Carrascal-Camacho AK, Velasco-Briceño A, Ocampo-Guerrero ML (2013). Antimicrobial susceptibility of *Listeria moncytogenes*, *Listeria ivanovii,* and *Listeria* species isolated from swine processing facilities in Colombia. J. Swine Health Prod. 21:10-21.

Gandhi M, Chikindas ML (2007). Listeria a foodborne pathogen that knows how to survive. Int. J. Food Microbiol. 113:1-15.

Garmendia Y, Vergara V, Morales MR, Benítez E, López R, Rodríguez Y, González D (2008). Listeriosis en embarazada: reporte de un caso. Rev. Soc. Ven. Microbiol. 28:145-149.

Giovannacci I, Ragimbeau C, Queguiner S, Salvat G, Vendeuvre J-L, Carlier V, Ermel G (1999). *Listeria monocytogenes* in pork slaughtering and cutting plants use of RAPD, PFGE and PCR-REA for tracing and molecular epidemiology. Int. J. Food Microbiol. 53:127-140.

Goulet V, Rocourt J, Rebiere I, Jacquet C, Moyse C, Dehaumont P, Salvat G, Veit P (1998). Listeriosis outbreak associated whit the consumption of rilletes in France in 1993. J. Infect. Dis. 177:155-160.

Gudbjörnsdóttir B, Suihko ML, Gustavsson P, Thorkelsson G, Salo S, Sjöberg AM, Niclasend O, Bredholt S (2004). The incidence of *Listeria monocytogenes* in meat, poultry and seafood plants in the nordic countries. Food Microbiol. 21:217-225.

ISO (1996). International Standardizartion Organization. Microbiology of food and animals feeding stuffs: horizontal method for the detection and enumeration of *Listeria monocytogenes* I. Detection method (ISO-11290).

Jacquet C, Catimel B, Goulet V, Lepoutre A, Veit P, Dehaumont P, Rocourt J (1995). Typing of *Listeria monocytogenes* during epidemiological investigations of the french listeriosis outbreaks in 1992, 1993 and 1995. In: Proceedings of the 12th International Symposium on Problems of Listeriosis. Promaco Conventions. Perth, Western Australia.

Jay JM (1996). Prevalence of *Listeria spp*. in meat and poultry products. Food Control 7:209-214.

Jemmi T, Pak SI, Salman MD (2002). Prevalence and risk factors for contamination with *Listeria monocytogenes* of imported and exported meat and fish products in Switzerland, 1992-2000. Prev. Vet. Med. 54:25-36.

Kanuganti SR, Wesley IV, Reddy PG, Mckean J, Hurd HS (2002). Detection of *Listeria monocytogenes* in pigs and pork. J. Food Prot. 65:1470-1474.

Kathariou S (2002). *Listeria monocytogenes* virulence and pathogenicity, a food safety perspective. J. Food Prot. 65:1811-1829.

Korkeala H, Siitonen A (2003). *Listeria monocytogenes*. Isolates from invasive infections: variation of sero-and genotypes during an 11 year period in Finland. J. Clin. Microbiol. 41:1694-1700.

López L, Gaztelurrutia L, Martínez I, Aguirrebengoa K, Valls A, Hernández JL, Alkorta M, Barrón J (2007). Listeriosis. Estudio de 16 años en un hospital terciario en España. Rev. Panam. Infect. 9:30-37.

Mammina C, Aleo A, Romani C, Pellissier N, Nicoletti P, Pecile P, Nastasi A, Pontello MM (2009). Characterization of *Listeria monocytogenes* isolates from human listeriosis cases in Italy. J. Clin. Microbiol. 47:2925-2930.

Markkula A, Lindström M, Korkeala H (2011). *Listeria monocytogenes* serotypes 1/2c and 3c possess *inl*H. Foodborne Pathog. Dis. 8:1125-1129.

Martins EA, Leal Germano PM (2011). *Listeria monocytogenes* in ready-to-eat, sliced, cooked ham and salami products, marketed in the city of São Paulo, Brazil: Occurrence, quantification, and serotyping. Food Control 22:297-302.

Mattos de Oliveira MM, Brugnera DF, Cardoso MdG, Alves E, Hilsdorf-Piccoli R (2010). Disinfectant action of *Cymbopogon* sp. essential oils in different phases of biofilm formation by *Listeria monocytogenes* on stainless steel surface Food Control 21:549-553.

Mead PS, Slutsker L, Dietz V, McCaig L, Bresee JS, Shapiro C, Griffin PM, R.V. T (1999). Food-related illness and death in the United States. Emerg. Infect. Dis. 5:607-625.

Mereghetti L, Quentin R, Marquet-Van Der Mee N, Audurier A (2000). Low sensitivity of *Listeria monocytogenes* to quaternary ammonium compounds. Appl. Environ. Microbiol. 66:5083-5086.

Miyasaki KN, Chiarini E, Sant Ana AD, Destro MT, Landgraf M, Franco BD (2009). High prevalence, low counts and uncommon serotypes of *Listeria monocytogenes* in linguica, a Brazilian fresh pork sausage. Meat Sci. 83:523-527.

Molero G, Tarradas C, Ramírez I, Gallardo F, Montiel M (2010). Aislamiento e identificación de *Listeria monocytogenes* durante el

proceso de beneficio de pollos en plantas beneficiadoras en el estado Zulia, Venezuela. Ciencia 18:108-114.
Muñoz AI, Vargas M, Otero L, Díaz G, Guzmán V (2011). Presencia de *Listeria monocytogenes* en alimentos listos para el consumo, procedentes de plazas de mercado y delicatessen de supermercados de cadena en Bogotá D.C, 2002-2008. Biomédica 31:428-439.
Nesbakken T, Kapperud G, Caugant D (1996). Pathways of *Listeria monocytogenes* contamination in the meat processing industry. Int. J. Food Microbiol. 31:161-171.
Orsi RH, den Bakker HC, Wiedmann M (2011). *Listeria monocytogenes* lineages: Genomics, evolution, ecology, and phenotypic characteristics. Int. J. Med. Microbiol. 301:79-96.
Pichler J, Much P, Kasper S, Fretz R, Auer B, Kathan J, Mann M, Huhulescu S, Ruppitsch W, Pietzka A, Silberbauer K, Neumann C, Gschiel E, de Martin A, Schuetz A, Gindl J, Neugschwandtner E, Allerberger F (2009). An outbreak of febrile gastroenteritis associated with jellied pork contaminated with *Listeria monocytogenes*. WKW. 121:149-156.
Ramírez Mérida LG, Morón de Salim A, Alfieri Graterol AY, Gamboa O (2010). Detección de *Listeria monocytogenes* en queso blanco criollo, mediante la reacción en cadena de la polimerasa (PCR). Arch. Lat. Nut. 60:254-260.
Rocourt J, BenEmbarek P, Toyofuku H, Schlundt J (2003). Quantitative risk assessment of *Listeria monocytogenes* in ready-to-eat Foods: the FAO/WHO Approach. FEMS Immunol. Med Microbiol. 35:263-267.
Ruiz-Bolivar Z, Carrascal-Camacho AK, Neuque-Rico MC, Gutiérrez-Triviño C, Rodríguez-Bocanegra MX, Poutou-Piñales RA, Mattar S (2011). Enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR) fingerprinting reveals intra-serotype variations among circulating *Listeria monocytogenes* strains. Afr. J. Microbiol. Res. 5:1586-1598.
Sambrook J, Russell DW (2001). Molecular cloning: A laboratory manual, Cold Spring Harbor Laboratory Press, New York.
Scallan E, Griffin PM, Angulo FJ, Tauxe RV, Hoekstra RM (2011). Foodborne illness acquired in the United States-unspecified agents. Emerg. Infect. Dis. 17:16-22.
Sergelidis D, Abrahim A, Sarimvei A, Panoulis C, Karaiannoglou P, Genigeorgis C (1997). Temperature distribution and prevalence of *Listeria spp*. in domestic, retail and industrial refrigerators in Greece. Int. J. Food Microbiol. 34:171-177.
Taillefer C, Boucher M, Laferrière C, Morin L (2010). Perinatal listeriosis: Canada's 2008 outbreaks. J. Obstet. Gynaecol. Can. 32:45-48.
Thévenot D, Dernburg A, Vernozy-Rozand C (2006). An updated review of *Listeria monocytogenes* in the pork meat industry and its products. J. Appl. Microbiol. 101:7-17.
To MS, Favrin S, Romanova N, Griffiths MW (2002). Postadaptational resistance to benzalkonium chloride and subsequent physicochemical modifications of *Listeria monocytogenes*. Appl. Environ. Microbiol. 68:5258-5264.
USDA-FSIS (2002). Isolation and Identification of *Listeria monocytogenes* from red meat, poultry, egg and environmental samples. MLG 8.03. In: USDA/FSIS Microbiology Laboratory Guidebook. United States Department of Agriculture. Food Safety and Inspection Service, Washington, D.C.
Van den Elzen AM, Snijders JM (1993). Critical points in meat production lines regarding the introduction of *Listeria monocytogenes*. Vet. Q. 15:143-145.
Vanegas MC, Vasquez E, Martinez AJ, Rueda AM (2009). Detection of *Listeria monocytogenes* in raw whole milk for human consumption in Colombia by real-time PCR. Food Control 20:430-432.