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## Prevalence of *Listeria monocytogenes* in pork-meat and other processed products from the Colombian swine industry

### Prevalencia de *Listeria monocytogenes* en carnes y derivados de la industria porcícola colombiana

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#### ABSTRACT

**Objective.** To determine the prevalence of *L. monocytogenes* in pork carcasses, meat cuts, and meat products ("chorizo", sausage and ham). **Materials and methods.** Stratified sampling was implemented in meat-processed products. We analyzed 566 (37%) carcasses, 472 (31%) meat cuts, and 481, (32%) meat-processed products, distributed as follows: 169 (11%) sausage, 163 (11%) ham, and 149 (10%) "chorizo", for a total of 1519 (100%) samples in a period of 18 months. The samples were processed using the ISO-17604, ISO-11290-1 and the USDA/FSIS (MLG-8.03) methods. Genus and species were confirmed by multiplex-PCR. **Results.** We obtained isolates of *L. monocytogenes* from 21 carcasses (10%), 160 (76%) from meat deboning, 10 (5%) from ham, 6 (3%) from "chorizo", and 13 (6%) from sausage. The prevalence found was 3.7% and 33.9% in carcasses and meat deboning respectively. The prevalence in the meat-processed products was 4.03% in "chorizo", 6.13% in ham and 7.69% in sausage. The overall prevalence of *L. monocytogenes* in the study was 13.82%. **Conclusions.** We found *L. monocytogenes* in different products analyzed, with particular interest in ham and sausage since both are consumed without previous heat treatment.

**Key words:** *L. monocytogenes*, porcine, pork-meat, prevalence (Source:DeCS y TDC).

#### RESUMEN

**Objetivo.** Determinar la prevalencia de *L. monocytogenes* en canales de cerdo, cortes de carne y derivados (chorizo, salchicha y jamón). **Materiales y métodos.** Se implementó un muestreo estratificado y se tomaron 566 muestras (37%) de canales, 472 muestras (31%) de cortes de carne y 481 muestras (32%) de derivados cárnicos distribuidos de la siguiente manera: 169 (11%) de salchicha, 163 (11%) de jamón y 149 (10%) de chorizo, para un total de 1519 (100%) muestras en un período de 18 meses. Las muestras se procesaron utilizando las normas ISO-17604 e ISO-11290-

1 y el método USDA/FSIS (MLG-8.03). Posteriormente se confirmaron los aislamientos por PCR-múltiple para identificar el género y la especie. **Resultados.** Se obtuvieron 21 (10%) aislamientos de *L. monocytogenes* en muestras de canales, 160 (76%) en carne de desposte, 10 (5%) en jamón, 6 (3%) en chorizo y 13 (6%) en salchicha. La prevalencia encontrada fue de 3.7% y 33.9% en carne en canal y cortes de carne respectivamente, mientras que la prevalencia en los derivados fue chorizo 4.0%, jamón 6.13% y salchicha 7.69%. La prevalencia general de *L. monocytogenes* en el estudio fue 13.82%. **Conclusiones.** Se encontró *L. monocytogenes* en los diferentes productos analizados, siendo de especial interés el jamón y las salchichas porque ambos son consumidos sin ningún tratamiento térmico.

**Palabras clave:** *L. monocytogenes*, porcino, carne de cerdo, prevalencia (Fuente: DeCS y TDC).

## INTRODUCTION

*L. monocytogenes* is a Gram-positive non-spore forming bacilli that can be found individually, in pairs or as short chains of 3 to 5 microorganisms with a V or Y shape. It lacks a capsule and mobilizes by means of peritrichous flagella with an optimum mobilization temperature between 20 and 25°C, with little or no mobility at 37°C (1). Although they are Gram-positive bacilli, old cultures can express pleomorphism. As a zoonotic and emerging microorganism in the food industry, *L. monocytogenes* causes listeriosis in humans and animals (1,2). This ubiquitous bacterium can contaminate water, concentrates and especially farm silos, resulting in dissemination of *L. monocytogenes* during animal breeding. It is known for swines to excrete the bacteria through their feces, thus acting as reservoirs (3).

*L. monocytogenes* has caused several food outbreaks, especially through a group of ready-to-eat-food (REF), where meat- and dairy-products are the most affected (4,5). In several international reports pork galantine and ham, among other contaminated foods, has been declared as an important source of outbreaks in France and Canada (1,6-8).

Presently in Colombia there are no outbreak reports by meat product consumption. However, a recent study conducted by the National Institute of Surveillance and Control of Beverages, Medicines and Food (INVIMA) stated that meat products may be contaminated with this microorganism and could constitute a potential listeriosis' vehicle (9). Recently, the INVIMA in its control and surveillance program reported the withdrawal of a batch of imported ham ("Serrano" type) due to the presence of *L. monocytogenes*. The Colombian legislation does not require searching for *L. monocytogenes* in meat derivatives. However, the Institute of Technical Norms (Icontec), in its standard 1325 (sixth update), establishes the rejection of a product if 25 grams of the sample contain *L. monocytogenes*.

According to the national policy for safety and health regarding the pork chain (10), the Colombian porcine sector has experienced a significant growth with a considerable improvement in productivity. However, there are no reports determining the minimum permitted for this pathogen in the pork chain. One of the objectives stated in this work was to determine the prevalence of *L. monocytogenes* in meat derivatives, to help improve the porcine national chain health status. In Colombia prevalence reports of *L. monocytogenes* in meat derivatives are scarce. This is most likely due to the fact that studies related with this microorganism have been focused on milk derivatives. The few existing reports show prevalence of about 14.3% in meat derivatives (11).

The main objective of this work was to determine the prevalence of *L. monocytogenes* in pork carcasses, pork-meat and pork-meat processed products from the domestic pork industry.

## MATERIALS AND METHODS

**Pork processing plant selection.** Plants were selected according to the following criteria: 1) pork-only abattoirs 2) plants that comply to regulations with Decree 1500, i.e. pay a fee for development 3) plants located in areas determined by Fedegan (10). At the time of this study 42 processing plants were throughout the country, this study included 23.

**Sample size.** Sample size was calculated using the formula of timely estimate of prevalence, taking into account the following criteria: 1.) population size [1.927.273 slaughtered swines/year], 2.) expected prevalence [50%], 3.) expected maximum difference [5%], 4.) error type I [1%], 5.) sample size [566], 6.) number of plants for sampling [23].

**Pork meat selection.** As the inclusion criteria we considered: 1.) markets that sell pork and 2.) points located in the pork-industry areas. The number of samples to study was 472, distributed in 58 lots.

**Selection of meat derivatives.** For the selection of meat-processed products we took into account 1.) use of pork-meat as raw material and 2.) high consumption of pork-meat processed products. The products included were: cooked ham (sandwich type), low-cost sausage (cooked) and pre-cooked "chorizo". The number of included samples were 481, distributed as follows: 169 (35%) sausages, 163 (34%) ham, and 149 (31%) "chorizo".

**Sample collection.** For pork carcasses, samples were collected using the non-destructive method described in standard ISO 17604 (12). Meat cuts were taken directly from market sale-points in different areas of the country, with a sample size of 500 g. Meat-processed products were taken directly from the sale point in commercial presentations with an approximate size of 250 to 500 g.

**Microbiological analysis.** The samples were transported to the Laboratory of Food Microbiology at the Pontificia Universidad Javeriana, under aseptic conditions to be processed immediately. The method used for the processing of pork carcasses was described by the Department of Agriculture of United States (USDA/FSIS) (13). ISO11290-1 method was used for meat cuts and meat-processed products. Briefly, 25 g of sample was taken and homogenized in 225 ml of Fraser-semi broth and incubated at 30°C for 48 hours. One hundred µl suspension was incubated in 10 ml Fraser broth and incubated for 40 hours at 35°C. *L. monocytogenes* was isolated in Ottaviani-Agosti and Palcam agar. Presumptive colonies were purified in TSAYE agar. Catalase test, sugar fermentation (mannitol, Xylose, rhamnose), hemolysis test and Camp test were carried-out. Isolates that biochemically corresponded to *L. monocytogenes* were subjected to molecular identification (genus and species).

**Master Cell Bank (MCB) development.** A colony of each isolate was inoculated in 20 ml of BHI broth, and incubated at 35°C for 24 hours at 120 rpm. The culture was then mixed at a proportion of 1:1 with glycerol at 40% (v/v) and mixed thoroughly. One ml was placed in a cryopreservation vial, and stored at -20°C. Each MCB was periodically studied to verify the microbial purity and viability (14).

**Genomic DNA extraction (gDNA).** For genomic DNA extraction, a frozen vial of each isolate was inoculated in 5 ml BHI broth supplemented with 0.5% (p/v) of glucose. Cells were cultured during 18 hours at 35°C and 120 rpm. One ml culture was centrifuged during 10 minutes at 5000 x g. Cells were washed with 1X TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0±0.2). DNA was extracted by using the "Wizard Genomic DNA Purification Kit" (Promega, Madison WI, USA).

**Genomic DNA (gDNA) quantitation.** DNA purity and concentration were determined (NanoDrop 2000c Thermo Scientific, Waltham, MA, USA) (15).

***L. monocytogenes* molecular identification by Multiplex-PCR.** Primers used for the PCR reaction were L1 (CTCCATAAAGGTGACCCT)/U1(CAGCMGCCGCGGTAA TWC) and LF(CAAACGTTAACAACGCAGTA)/LR (TCCAGAGTGATCGATGTAA) (16). The final volume of the reaction was 35 µl, containing 1X PCR buffer, 2.5 mM MgCl<sub>2</sub>, 0.2 mM each dNTP, 20 pmol each primer and 2U of GoTaqFlexi DNApol (Promega, Madison WI, USA), and 5 µl of gDNA (~120 ng). Reactions were carried out in a Gene Cycletm (BioRad, Philadelphia PA, USA) programmed in the following way: 1 minute at 95°C, followed by 40 cycles (30 s at 94°C, 20 s at 51°C, 30 s at 72°C), and a final extension step of 8 minutes at 72°C. *L. monocytogenes* ATCC 19115 was used as positive control (16).

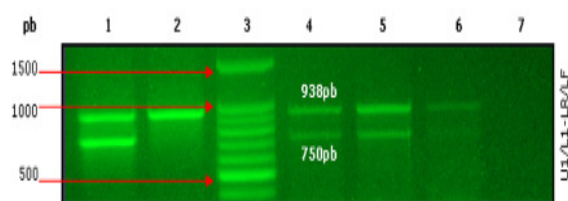
**Prevalence of *L. monocytogenes*.** The following formula was used to calculate prevalence (17).

$$[1] \text{ Prevalence(\%)} = \frac{\text{Positive Samples}}{\text{Total Samples}} \times 100$$

## RESULTS

**Molecular identification of *L. monocytogenes* by Multiplex-PCR.** All the isolates previously identified as *L. monocytogenes* amplified the two expected products of 938 bp and 750 bp, for genus and the species respectively (16). Figure 1 shows representational results.

**Pork carcasses.** Figure 2-top depicts outcomes for each of the analyzed processing plants. *L. monocytogenes* was isolated in (8/23, 34.8%) plants. Plant PB18 presented the greatest number of positive samples (7/20, 35%). *L. monocytogenes* was not isolated in other plants (15/23, 65.2%). The overall prevalence in pork carcasses was 3.7% (21/566).



**Figure 1. Molecular identification of *L. monocytogenes*.** Representative samples of *L. monocytogenes* isolates in 1% (w/v) TAE agarose gel processed in Quantity One V. 4.6.9. (BioRad, Philadelphia PA, USA). The figure shows some of the amplification products obtained by Multiplex-PCR. Lane 1: *L. monocytogenes* ATCC 19115. Lane 2: *Listeria* spp. Lane 3: 100 bp molecular size marker (Promega, Madison WI, USA). Lanes 4, 5, 6: positive isolations for *L. monocytogenes*. Lane 7: Negative control for PCR reagents.

**Meat cuts.** Fifty eight lots of fresh pork meat were analyzed and *L. monocytogenes* was isolated in 40 lots (69%). Out of the 40 lots thirteen samples were contaminated with *L. monocytogenes* (22.4%). The bacterium was not isolated in the remaining 31% (18/58) of pork meat samples. There was an overall high prevalence (33.9%, 160/472), (Figure 2-middle).

#### Pork meat processed products.

**Ham.** *L. monocytogenes* was isolated in 10 ham samples, with 6.13% prevalence (Figure 2-bottom).

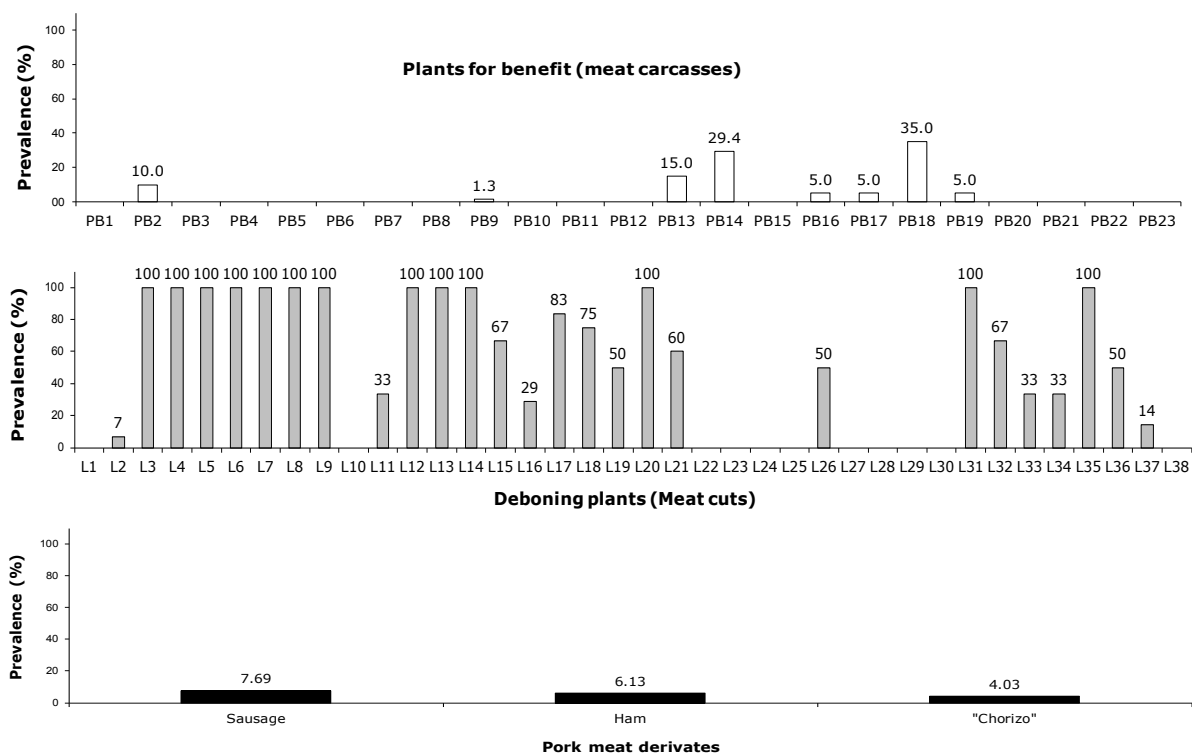
**Sausage.** Out of 169 samples analyzed, *L. monocytogenes* was isolated in thirteen. The prevalence was 7.69% (Figure 2-bottom).

**"Chorizo".** Six isolates of *L. monocytogenes* were obtained for a prevalence of 4.03% (Figure 2-bottom).

## DISCUSSION

*L. monocytogenes* is a food-transmitted pathogen with implications in the food industry due to its ubiquitous nature. This feature allows the bacterium to be disseminated in the environment, facilitating accession to meat-processing plants, in particular pork-meat processing product plants, favoring food contamination (18-20).

In the present study the prevalence of *L. monocytogenes* in meat carcasses was 3.7%. These values are within the range of those published by other investigations: 2.0% in Finland (21), 4.1% in United States (22). Our results are lower than those presented by Gil and Jones who detected 8.3% in a Canadian plant. However, the Gil and Jones study only had 24 samples, which is not representative. Prevalence data from this research are consistent with several authors who have pointed out that processing plants are not a significant source of *L. monocytogenes* contamination. (23-27).



**Figure 2.** Prevalence of *L. monocytogenes* in carcasses, meat cuts and meat products from the Colombian swine industry.



The presence of the pathogen in swines is associated to the fact that these animals are carriers of *L. monocytogenes*. For example it has been shown that pork lodge this bacterium in tonsils and intestine (3, 21, 22, 28). For this reason if during evisceration there is organ rupture, the carcass can be contaminated with *L. monocytogenes*. Although it is frequent for *L. monocytogenes* to contaminate carcasses and processing plants, subsequent washing and disinfecting procedures should get rid of the contamination. Pathogen-free product depends on the effectiveness of these processes (21).

In this study meat cuts had a prevalence of 33.9%. This percentage was the highest among all samples assayed. Our results agree with those reported by authors in different countries. Ochiai et al. reported an incidence of 35.7% in Tokyo (20). In contrast, other research shows lower prevalence and incidence: 0.15 - 1.2% in the United States (29), 4.0% in Finland (21), 5.0% in Bulgaria (30), and 24.0% in Canada (8). The low prevalence in these countries can be associated to control measures implementation during the stages of cleaning and disinfection. It has been determined during deboning there might be cross-contamination with utensils. In particular if this process is carried out at room temperature below 10°C. This favors the presence of *L. monocytogenes* due to its psychrotrophic nature (20, 28). The conditions of contact surfaces and equipment affect the persistence of *L. monocytogenes*. Stainless steel surfaces are used in the food industry to prevent contamination (26).

In the present study prevalence of *L. monocytogenes* in sausage was 7.69%, similar to that found by Vera et al (11) in Bogotá in 2004 (document published in 2006). Vera et al (11) reported a prevalence of 8.7% with 21 positive samples of 239 evaluated sausages. Similar findings were reported by Karakolev et al (30) in 2009. They analyzed and determined prevalence in crude dried sausage (11.3%) and raw smoked sausage (8.6%) in Bulgaria (30). Other investigations have presented prevalence of 42.0% (31), 28.2% in fresh and fermented sausages (32), and 16.9% in dry cured sausage (33). These studies show that there is variability in the prevalence of *L. monocytogenes* in sausage, which may be related to preparation process and storage (29). It is noteworthy that *L. monocytogenes*' prevalence is dependent on the country and the nature of the product-processing. As a case in point, raw fermented sausage or products without proper packaging can have a higher prevalence. In contrast, the samples evaluated in this study were sausages cooked and vacuum packed at the production plant.

Sausages are regional products that vary depending on the country with regards to ingredients and production processes. In some cases they are considered a type of fermented sausage, making it difficult to compare pork-meat processed products from different origins, in particular with the international literature.

The prevalence of *L. monocytogenes* in "chorizo" was 4.0%, less than what was stated by Vera et al (11). They reported in Bogotá (2004) a prevalence of 18.1% with two positive samples out of 11 assessed. Their results might be due to a low sampling number and sampling of pre-cooked sausage.

In the case of the ham the prevalence in the present research was 6.13%. This prevalence was higher than what was reported by Martins and Leal (0.8%) (20), but lower than that reported by Cabedo et al (33) (12.5%). Vera et al (11) also reported higher prevalence (16.8%) with 67 positive samples out of 398 analyzed. Ham contamination may occur during slicing and/or packaging probably due to the presence of the pathogen in equipment, when hygiene conditions are not appropriate (20,26).

Chasseignaux et al. pointed-out to contamination of meat products with *L. monocytogenes* associated to cross-contamination with raw meat, equipment, processing areas (34), cooling areas, or even staff of the establishment. Therefore the incorporation of the microorganism in REF occurs after having subjected the product to thermal processes.

Lastly, it is important to note that *L. monocytogenes* was found in different samples analyzed. This demonstrates the importance of pork meat products as a transmission vehicle for this bacterium.

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