OCURRENCE OF LISTERIA MONOCYTOGENES IN BRAZILIAN SWINE HERDS

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Abstract Listeria monocytogenes is a foodborne pathogen of major concern for public health in industrialized countries. The agent has been detected in pork products such as sausage and hot dogs in Brazil. This fact stimulated the search over the contamination source, since there is no information about it in national literature. A total of 240 fecal samples from growing-finishing pigs raised in 4 swine herds of Sao Paulo State were subjected to isolation of Listeria spp. The herds examined used or not antimicrobials, meat and bone meal, wet or dry feed. Bacteriological results shown 35 samples positives to Listeria spp (14.6%), but none were positive to L. monocytogenes. This result suggests that fecal excretion is not an important contamination source to pork products in Brazilian industries independently of feeding kind; therefore, more studies will be conducted in near future to identify the contamination points at productive chain.

Introduction Listeria monocytogenes is responsible for opportunistic infections especially in vulnerable and immunocompromised subjects, such as new-born infants, pregnant women, cancer or AIDS patients and the elderly. It may cause meningitis encephalitis, sepsis, fetal death, prematurity and death. Listeriosis is a serious illness with a high mortality rate (20-30%) or can cause neurological sequelae due to the tropism of Listeria for the central nervous system (Beloeil et al, 2003). During the 1980s a number of listeriosis outbreaks were linked with the consumption of contaminated foodstuffs. Since then, several outbreaks related to meat products including pork products have been described in Canada, Australia, EUA and Europe (Low and Donachie, 1997).

In Brazil there is an extensive study of with Listeria strains isolated from 1971 to 1997 from different sources (Hofer et al, 2000). However, disease cases related to pork products consumption were not described in the Country. Recently, different authors report the presence of L. monocytogenes and Listeria spp. in hot dogs (Pettinati, 2004) and sausage (Duval et al, 2003; Destro, 1990) of pork origin, but correlations with contamination source were not evaluated.

Considering the data related to pork products contamination with L. monocytogenes reported in Brazil, the reports of fecal excretion of the agent by health pigs, and the role of the animal as primary source of carcass contamination as described by Beloeil et al, 2003, the goal of the present study was evaluate the presence of bacteria of Listeria genera in swine feces in Brazilian herds.

Materials and Methods Two hundred and forty fecal samples collected from growing and finishing pigs raised in four commercial Brazilian swine herds of Sao Paulo State were submitted to bacteriological examination. Each swine herd tested has feed systems with different characteristics. Herd 1–used feed with antimicrobials as grow promoter and for therapeutic treatment, and used meat and bone meal; Herd 2–used feed with antimicrobials as a grow promoter and for therapeutic treatment and did not use meat and bone meal; Herd 3–did not use feed with any antimicrobial treatment for the last four year period, and no meat and bone meal; Herd 4–used wet feed and antimicrobial for therapeutic purposes.

A 1g portion of fecal sample was added in 9ml of Tryptose Phosphate Broth (Difco Laboratories, Detroit, EUA) and kept at 4°C for five days. Following this period 1ml of culture was enriched in 9ml of Half Fraser broth (Oxoid, Basingstoke, UK) incubated at 30°C. After 24 h of enrichment, 1ml of each culture was sub cultured for 24 h at 30°C in Fraser Broth (Oxoid). The Fraser broth was streaked onto PALCAM plates and incubated at 37°C for 72 h with 10% of CO2. The suspected Listeria spp. colonies (15 to 20 colonies for plate) were cultured in BHI (Difco) at 37°C for 24 h. After this period, the cultures were submitted to lyses with lysozime (100mg/ml) and proteinase K (20mg/ml) following DNA extraction protocol described by Boom et al, (1990). An aliquot of each culture was frozen with 30% of glycerol at –86°C for future identification of species and serotype. The characterization as L. monocytogenes or Listeria spp. was done by polymerase chain reaction with primers described by Wesley et al (2002). The amplified products...
were resolved by electrophoresis in 1.5 agarose gel for 1 h, stained with 0.5mg of ethidium bromide and visualized with ImageMaster®VDS.

**Results** *L. monocytogenes* were not found in all 240 fecal samples examined. *Listeria spp.* isolates were obtained in 35 samples (14.58%). Considering the 120 growing pigs fecal samples examined, the frequency of *Listeria spp.* was 10.8%. In the 120 finishing pigs samples collected the frequency was 18.3%. These results are described in table 1. There was no significant association among feed type and presence of *Listeria genera*.

**Discussion** There are few studies about excretion of *L. monocytogenes* in healthy pigs, despite the great potential of agent in public health.

At this trial, from a total of 240 fecal samples processed any *L. monocytogenes* positive animal was found. At consulted literature, the frequency of agent in feces can vary from 0 to 47% (Kanuganti et al, 2002). Beloeil et al (2003), evaluated the presence of *Listeria spp.* in 45 swine herds in France, and described an occurrence of 14% of *L. monocytogenes* isolates and 84% of samples positives to *Listeria spp.* In Brazilian herds, were observed only 14.58% of animals positive to *Listeria spp*. The differences among the results obtained in these studies can be related to study design, climatic conditions, nutritional factor and compounds.

Among different reports there is no concern about the best method of sampling pig feces. Beloeil et al, (2003) analyzed fecal samples collected from three different pigs for pen and from five pens per room. At present, individual fecal samples from rectum from 30 animals from each herd were examined.

The nutritional differences can influence the presence of *Listeria* on feces. Wet feeding is considered a risk factor to the presence of *L. monocytogenes* by Beloeil et al (2003). It’s also been reported that *L. monocytogenes* can be isolated from 2% of the feces on dry feed animals versus 25% to 50% of the feces of wet fed animals. The different contamination can be related to heat treatments employed and the water activity at the feed (Skovgaard and Nørregård, 1989). In relation to wet feed, Beloeil et al (2003) also describes a strong relationship between *L. monocytogenes* presence and the biofilms accumulated on the internal surfaces of pipeline and valves. The alterations of these biofilms by disinfectant and cleaning procedures can facilitate the *Listeria* contamination of the system.

At this study, the herd number four which used wet feed have no isolation of *Listeria spp.*, although one collection is not enough to conclude that it does not interfere with the agent isolation. Maybe the products employed in feed composition or biofilm presented at feed system had protected it from *Listeria* colonization.

The absence of *L. monocytogenes* in swine feces examined suggests that fecal excretion is not the major risk factor involved in carcass or meat contamination. The critical point for pork products contamination in Brazilian slaughter houses must be in another part of the productive chain, such as after the animal death during evisceration, in environment or machines. More studies will be done to elucidate the critical hazard point of *Listeria* contamination.

**Conclusions** The present study did not identify any *L. monocytogenes* in fecal samples analyzed from pigs in Sao Paulo State, Brazil. It suggests that feces are not the most important contamination source for pork products at our conditions.

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**References**


Table 1 - **Listeria spp** and **Listeria monocytogenes** frequency in feces of growing and in finishing pigs.
(Number of positive animals/ collected animals).

<table>
<thead>
<tr>
<th>ORIGIN</th>
<th>PCR of Growing pigs</th>
<th>PCR of Finishing pigs</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>L. spp.</td>
<td>L. monocytogenes</td>
</tr>
<tr>
<td>Herd 1</td>
<td>4/30</td>
<td>0/30</td>
</tr>
<tr>
<td>Herd 2</td>
<td>3/30</td>
<td>0/30</td>
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<tr>
<td>Herd 3</td>
<td>6/30</td>
<td>0/30</td>
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<td>Herd 4</td>
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