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 0.5 mg of ethidium bro-
mide and visualized with ImageMaster®VDS.

Results L. monocytogenes were not found in all 240 fecal samples examined. Listeria spp. iso-
lates 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 associ-
ation 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 ani-
mal 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 posi-
tive 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 con-
sidered 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 Nørregaard, 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 alter-
ations of these biofilms by disinfectant and cleaning procedures can facilitate the Listeria contami-
nation 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 isola-
tion. 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 to 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 stud-
ies 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

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Destro, M.T., 1990. Isolamento de _Listeria spp_ e estudos de sua ocorrência em carnes, leites e derivados. *Dissertação de Mestrado* 73p


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<th>ORIGIN</th>
<th>PCR of Growing pigs</th>
<th>PCR of Finishing pigs</th>
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<tr>
<td></td>
<td>L. spp.</td>
<td>L. monocytogenes</td>
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Table 1 - _Listeria spp_ and _Listeria monocytogenes_ frequency in feces of growing and in finishing pigs. (Number of positive animals/ collected animals).