

SPREAD POTENTIAL OF *Salmonella* sp. FROM MESENTERIC LYMPH NODES OF SWINE SLAUGHTERED IN WESTERN PARANA TO WHITE VISCERA, INSPECTION TABLES AND TO STAFF KNIVES AND GLOVES DURING *POST-MORTEM* INSPECTION

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ABSTRACT

The objective of the present study was to evaluate the presence of *Salmonella* sp. serovars in mesenteric lymph nodes of swine as well as the spread potential of the agent during slaughter and inspection activities. Animals were bred in confinement and slaughtered in officially inspected facilities in western Parana, Brazil. The following samples were collected in five replications, at different moments of the slaughter process: 30 chains of mesenteric lymph nodes; 12 swabs of white viscera inspection tables; eight swabs of knives used in lymph nodes inspection; and four swabs of gloves of the

inspection staff. Microbiological analysis of the lymph nodes of 150 animals showed *Salmonella* sp in 17.3% (26/150) of them. The agent was also isolated in 5.0% (2/40) of the knives, and in 28.3% (17/60) of the white viscera inspection tables. None of the gloves was positive (0/20). In conclusion, *Salmonella* serovars from mesenteric lymph nodes and different surfaces that get in contact with slaughtered animals show the agent's spread potential and consequently cross-contamination during the slaughter process.

KEYWORDS: mesenteric lymph nodes; *Salmonella* sp.; swine.

POTENCIAL DE DISSEMINAÇÃO DE *Salmonella* sp. PROVENIENTE DE LINFONODOS MESENTÉRICOS DE SUÍNOS ABATIDOS NA REGIÃO OESTE DO PARANÁ PARA SUPERFÍCIE DE MESA DE INSPEÇÃO DE VÍSCERAS BRANCAS, FACAS E LUVAS DE AUXILIARES DE INSPEÇÃO DURANTE A INSPEÇÃO *POST-MORTEM*

RESUMO

O presente estudo foi realizado com o objetivo de verificar os sorovares de *Salmonella* sp. em linfonodos mesentéricos de suínos, bem como o potencial de disseminação deste agente durante as atividades de abate e inspeção, correlacionando-os com as superfícies amostradas. Esses animais foram criados sob confinamento e abatidos em estabelecimento sob Inspeção Federal localizado na região oeste do Paraná. O experimento foi realizado em cinco repetições sendo que

em cada uma foram coletadas 30 amostras de linfonodos mesentéricos, 12 suabes de mesas de inspeção de vísceras brancas, oito suabes das lâminas de facas utilizadas durante a inspeção dos linfonodos e quatro suabes da superfície das luvas dos auxiliares de inspeção em diversos momentos do abate. A partir da análise microbiológica da cadeia linfática mesentérica de 150 animais, encontrou-se *Salmonella* sp em 17,3% (26/150) dos linfonodos analisados. O agente ainda foi isolado em

5,0% (2/40) das superfícies de lâminas de facas amostradas e em 28,3% (17/60) das mesas de inspeção de vísceras brancas. Nenhuma amostra positiva (0/20) foi encontrada nas luvas dos inspetores. Pode-se concluir que sorovares de *Salmonella* originários de linfonodos

mesentéricos e encontrados em superfícies que entram em contato com o produto demonstram o potencial de disseminação do agente durante o processo de abate com consequente contaminação cruzada.

PALAVRAS-CHAVE: linfonodos mesentéricos; *Salmonella* sp.; suínos.

INTRODUCTION

Foodborne diseases are a great concern for public health authorities and have a profound social and economic impact in different countries all over the world (MOTARJEMI & KÄFERSTEIN, 1999). *Salmonella* is an important causative agent of foodborne disease, and contaminated pork is a frequent vehicle for these bacteria. In industrialized countries, 5 to 30% of all salmonellosis cases may be traced back to pork consumption (; BRYAN, 1980, BRYAN, 1988; BAIRD-PARKER, 1994, BORCH et al., 1996; BERENDS et al., 1997; ALBAN et al., 2002).

In spite of some efforts to prevent foodborne diseases, there has been an increase in the prevalence of *Salmonella* in pork production, slaughter and processing (SWANENBURG et al., 2001a). These microorganisms may enter and spread at any stage of the pork production chain, from the primary phase – by means of contaminated feed and water offered to the animals, or infected people, rodents or animals, or during transportation or housing in the slaughterhouse – to the slaughter process, by means of environmental cross-contamination (VAN DER GAAG et al., 2004). Pigs are recognized as important reservoirs of *Salmonella*, and may act as sources of infection for humans (OOSTEROM et al., 1985; KICH & CARDOSO, 2004).

According to BERENDS et al. (1997), there is a positive correlation between the number of animals that are fecal carriers of *Salmonella* and the number of contaminated carcasses at the end of the slaughter line. *Salmonella* carriers have 3 to 4 times more chances of showing *Salmonella* in their carcasses than non-carriers. According to these authors, carriers are responsible for 70% of the contamination of their own carcasses.

On the other hand, as microorganisms may remain in the environment during the whole slaughter process, and stay there for several months, the facility as well as the equipment have an important role in the final level of carcass contamination (HALD et al., 2001). It is estimated that 15 to 30% of the bacterial load on the carcasses

is caused by cross-contamination in the slaughterhouse, by means of contaminated equipment and handling by the butchers and inspectors during the process (BERENDS et al., 1997, SWANENBURG et al., 2001a). All things considered, it may be said that feces and the environment are the main sources of *Salmonella* in slaughterhouses (BORCH et al., 1996).

Lymph nodes are also considered to be important sources of contamination because they are incised during the inspection process. If the animal is a carrier, incision of its lymph nodes may spread *Salmonella* to the utensils and meat (OOSTEROM et al., 1985).

Thus, the objective of the present study was to assess which *Salmonella* serovars are present in mesenteric lymph nodes of swine and to correlate these serovars with the ones found on white viscera inspection tables, and on knives and gloves of the inspection staff during the slaughter process.

MATERIAL AND METHODS

This study was carried out from April to October 2006 in an officially inspected slaughterhouse that can process 1,500 animals / day, is located in western Parana, Brazil, and qualified for exportation. Sample collection was repeated five times, and each replication was identified by a different letter (A, B, C, D and E).

Samples were collected immediately after evisceration in five different moments of the slaughter process, as follows: 150 chains of mesenteric lymph nodes collected when abdominal viscera were placed on the inspection table (30 per replication); 60 smears of the surface of white viscera inspection tables (12 per replication); 40 swabs of the blades of the knives used in lymph node inspection (eight per replication); and 20 swabs of the gloves of the inspection staff (four per replication), in a total of 270 samples.

Mesenteric samples were collected every 10 carcasses, that is, for every chain of mesenteric lymph nodes collected, 10 other carcasses were not sampled. At the end of the sampling procedure,

approximately 300 animals had been slaughtered. Mesenteric and lymph nodes were separated from the intestines and individually placed in adequately labeled plastic bags.

As for the white viscera inspection tables, knives and gloves, samples were collected after every 25, 38 and 75 carcasses, respectively, during the same interval in which 300 animals were slaughtered. For the smears of the inspection tables (60 cm x 80 cm), sponges for surface sampling (NASCO™ – Model NASC-B01245WA) previously hydrated in 10 mL of 0.85% saline solution were used. The whole surface of the moving-top inspection tables was wiped horizontally and vertically with the sponge before tables were washed by a continuous cleaning-in-place system. Tables that were obviously contaminated by bile and/or feces were not used in the sampling procedure. After the sample was collected, the sponge was placed in the sterile plastic bag that comes in the kit.

In the inspection line, samples from the knives used in the inspection of mesenteric lymph nodes were collected from both sides of the blade (Tramontina™ Model 24606/086-6'') with sterile swabs. The same procedure was followed to collect samples from the whole palmar surface of the gloves of the inspection assistants. Immediately after collection, swabs were placed in test tubes containing 10 mL of 1% buffered peptone water. All samples were transported to the laboratory in ice, inside isothermal containers. Collection of the samples during the slaughter process lasted approximately 1 hour and 45 minutes in every replication.

Samples were transported to the Food Microbiology Laboratory in the Palotina Campus of UFPR. Lymph nodes were separated from the mesentery and weighed to an analytical unit equal to 25g. Then, they were diluted in 225 mL of 1% buffered peptone water (BPW), and homogenized in stomacher for 120 seconds. Sponges were mixed with 100mL of BPW and homogenized in stomacher for 30 seconds. Tubes containing swabs were homogenized in vortex for 15 seconds and then analyzed using the method recommended by the United States Department of Agriculture (USDA, 2002). *Salmonella* colonies were tested for seroagglutination using polyvalent flagellar and somatic antiserum (PROBAC™) and submitted to biochemical tests, as follows: urease, indol production, glucose fermentation (VM and VP), motility test and use of citrate and malonate. Positive strains were sent to the Enterobacteria Sector –

Bacteriology Section [*Setor de Enterobactérias – Seção de Bacteriologia*] at *Instituto Adolfo Lutz* for serotyping.

RESULTS AND DISCUSSION

In the present study, *Salmonella* sp. was isolated from 17.3% (26/150) of the mesenteric lymph nodes studied. Individual analysis of replications A, B, C, D and E showed positive results ranging from 0 to 30% these samples, as presented in Table 1. Similarly, BESSA et al. (2004) reported that *Salmonella* prevalence was 17.6% (53/300) in mesenteric lymph nodes of swine slaughtered in three officially inspected slaughterhouses in Rio Grande do Sul. BERSOT (2005) reported 20% positive samples (3/15) in mesenteric lymph nodes of another slaughterhouse in western Parana. CASTAGNA et al. (2004) obtained different results in Rio Grande do Sul, and reported the presence of *Salmonella* in 61% (55/90) of the mesenteric lymph nodes sampled. SWANENBURG et al. (2001a) showed that *Salmonella* prevalence for mesenteric lymph nodes of swine slaughtered in the Netherlands was equal to 9.3%.

These data show a wide variation in detection rates of *Salmonella* in mesenteric lymph nodes of swine at the moment of slaughter. This variation may be caused by differences in the number of carriers due to husbandry practices and regional factors, as well as differences in the methodology used in the studies (LÁZARO et al., 1997; BESSA et al., 2004). The presence of *Salmonella* in mesenteric lymph nodes may be considered an important predictive tool for asymptomatic carriers (BAHNSON et al., 2005). According to BOTTEL DORN et al. (2003), the same *Salmonella* sp. serovars present in the slaughterhouse environment are, most of the times, also found in slaughtered swine. Therefore, the occurrence of *Salmonella* in mesenteric lymph nodes of swine may indicate latent and potential contamination of the slaughterhouse environment.

Salmonella was isolated in 28.3% (17/60) of the samples of the surface of white viscera inspection tables during the slaughter process. BERSOT (2005), in Parana, showed that samples from the surface of evisceration tables showed 26.5% positive results. It should be emphasized that the pathogen was detected by this author after the regular cleaning routine of the facility. Differently, SAMMARCO et al. (1997) isolated *Salmonella* in 6.3% of the evisceration tables in swine slaughterhouses in Italy.

Table 1. Number of *Salmonella* sp. serovars isolated in each replication of positive samples from mesenteric lymph nodes of swine, white viscera inspection tables surface and knives of inspection staff during the slaughter process

Replication*	Samples		
	MLN	Inspection tables	Knives
A	9/30 (30%) Ohio, Panama, Seftenberg	2/12 (16.6%) Panama	ND
B	3/30 (10%) Derby	9/12 (75%) Bredeney, Derby, Typhimurium	ND
C	ND	5/12 (41.6%) Derby, Typhimurium, 1,4,5,12:i-	ND
D	6/30 (20%) Anatum, Ohio, Infantis, Typhimurium,	ND	ND
E	8/30 (26.5%) London, Ohio, Typhimurium	1/12 (8,3%) Bredeney	2/8 (25%) Typhimurium
Total	26/150 (17.3%)	17/60 (28.3%)	2/40 (5%)

* not isolated from gloves MLN = mesenteric lymph nodes; ND = not detected

In a study carried out in the state of Rio de Janeiro, *Salmonella* was recovered in 45.5% of the samples of evisceration tables (LÁZARO et al., 1997). In this case, the company did not have a continuous and automatic cleaning-in-place procedure, and this fact probably influenced the high levels of contamination observed.

Detection of *Salmonella* sp. on the surface of inspection tables corroborates the results of studies that point out pigs as the main sources of contamination in slaughterhouses. Contamination is spread both by the slaughter process (SMELTZER, 1984) and by inspection activities, such as the incision of lymph nodes carried out by inspection assistants in the *post-mortem* examination.

Salmonella was not isolated from any of the samples collected from gloves of handlers, and only 5% of the swabs of knife blades showed the agent (Table 1). SWANENBURG et al. (2001b), in the assessment of *Salmonella* prevalence on the hands of slaughterhouse butchers, reported that 5% of the samples were positive for the agent. Negative results for the gloves of inspection assistants and the low prevalence (2/40) observed in the knives used in lymph node inspection indicate that, as far as handlers are considered, adequate operational hygiene practices were employed in the facility studied. However, positive results in this kind of sample show that the inspection staff should redouble their efforts to achieve impeccable hygienic practices. According to SCHRAFT et al. (1992), *Salmonella* sp. may frequently be identified on hands of workers, work surfaces and equipment, demonstrating the occurrence of cross-contamination between the carcasses and these

surfaces, and underscoring the need for a well-implemented operational hygiene program. The alternate use of two different knives kept in running water over 82.2°C is one of the methods employed to prevent that contamination from the mouths and intestines of the animals is transferred to the carcasses. Therefore, employee training is essential to prevent problems in these stages of the process.

Ten different serovars were identified in the study (Table 2). The most frequent ones were Typhimurium (28.2%; 11/39), followed by Derby (17.8%; 7/39) and Bredeney (15.4%; 6/39). Serovar Typhimurium was isolated both in mesenteric lymph nodes and knives in replication E, as shown in Table 1. On the other hand, serovar Panama was detected both in mesenteric lymph nodes and inspection tables in replication A. Serovar Derby was also detected in mesenteric lymph nodes and inspection tables in replication B. The presence of the same serovar in mesenteric lymph nodes and on surfaces in the slaughterhouse environment suggests the spread of the agent during the slaughter process. However, this fact would only be definitively proven by molecular characterization of the cultures.

Serovar Bredeney was isolated only in white viscera inspection tables (35.3% 6/17) and was not detected in mesenteric lymph nodes or other surfaces. This serovar may have come from previous groups of slaughtered animals, and remained in the slaughterhouse environment due to deficient cleaning practices. BERSOT (2005), in Parana, detected *Salmonella* on the surface of inspection tables even after operational hygiene procedures. According to SWANENBURG et al. (2001b), *Salmonella* serovars

may survive in certain niches of the slaughterhouse environment and may become part of the resident microbiota. In fact, contaminated slaughterhouse equipment seem to have a more important role in the final contamination level of carcasses than handlers, once bacterial growth may occur inside or on the surface of equipment throughout the day (HALD et al.,

2001). This observation was corroborated by the results obtained for the hands of the employees (Table 1), demonstrating that frequent hand washing, an important procedure of operational hygiene, is essential to reduce environmental contamination, mainly that caused by the *post-mortem* inspection staff.

Table 2. *Salmonella* serovars isolated from mesenteric lymph nodes of swine, surface of white viscera inspection tables, knives and gloves during pork processing in a slaughterhouse in Parana.

Serovar	Material				Total per serovar Number (%)
	Mesenteric lymph nodes	Inspection tables	Gloves	Knives	
Panama	3	2	0	0	5 (12.8)
Derby	2	5	0	0	7 (17.8)
Typhimurium	6	3	0	2	11 (28.2)
Bredeney	0	6	0	0	6 (15.4)
Ohio	5	0	0	0	5 (12.8)
Infantis	1	0	0	0	1 (2.6)
Anatum	1	0	0	0	1 (2.6)
London	1	0	0	0	1 (2.6)
Seftenberb	1	0	0	0	1 (2.6)
1,4,5,12:i:-	0	1	0	0	1 (2.6)
Total per type of material	20	17	0	2	39 (100)

BAHNSON et al. (2005), in a slaughterhouse located in western USA, detected that serovar Derby, followed by Typhimurium, was the most prevalent in samples of mesenteric lymph nodes of swine. In the Netherlands, a study carried out by SWANENBURG et al. (2001b) showed that Typhimurium was the most frequent serovar isolated both in samples collected from swine and the environment. Similar to the results of the present study, CASTAGNA et al (2004) reported serovars Bredeney (34.8%), Derby (19.6%) and Typhimurium (12.3%) as the most frequent ones in Rio Grande do Sul. Besides *S. Typhimurium*, serovars Bredeney, Derby, Anatum, Enteritidis and Agona have been the most commonly found in carrier pigs in Brazil (BESSA et al., 2004) and abroad (SWANENBURG et al., 2001b).

The present study isolated *Salmonella* serovars that are frequently detected in the swine production chain and have important zoonotic potential, according to the *Salmonella* Annual Summary 2004 of the US Center for Disease Control and Prevention (CDC, 2004). Paraná State is of great importance for the production and slaughter of pigs and reviews on this topic should be frequently conducted to determine the prevalence of *Salmonella* that can assist in the creation and adaptation of control measures.

CONCLUSION

Salmonella serovars isolated from mesenteric lymph nodes were found on surfaces that get in contact with the carcasses, demonstrating that there is a potential for cross-contamination due to the spread of the agent during the slaughter process.

The absence of *Salmonella* in the gloves of inspection staff and the low frequency of the agent in the knives suggest that workers followed adequate operational hygiene procedures, chiefly periodic hand washing and sterilization of utensils, both important tools to prevent the spread of the agent and the occurrence of cross-contamination in the slaughterhouse environment.

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