DETECTION OF *Salmonella* Anatum ON INSPECTED CUTS OF PORK

(DETECÇÃO DE Salmonella Anatum *EM CORTES DE SUÍNOS INSPECIONADOS)*

R. Z. SABA[1], N. M. NESPOLO[1], D. R. SILVA[1], B. A. KAMIMURA[1], O. D. ROSSI JUNIOR[1]

SUMMARY

Considering that foods of animal origin, such as pork and pork products are important carriers of *Salmonella* sp., the objective of this study was to determine the prevalence of *Salmonella* sp. in pork tongue, tonsils, mandibular and mesenteric lymph nodes, carcass surface and rectum, as well as on the blade surface of knives used on the inspection since the pigs were slaughtered under state inspection. For the isolation, 0.1% peptone water was used for pre-enrichment, while Rappaport-Vassiliadis and selenite cystine broths were used as selective enrichment. The plating was done on MacConkey and brilliant green agar and the presumptive identification on TSI and LIA. The final characterization was performed by serotyping. From the 25 animals sampled, *Salmonella* a*natum* was isolated in the tongue of two animals, corresponding to 8%. The presence *Salmonella* sp. should be treated as a risk factor for food safety, since tongue removal is not a mandatory procedure and may be used as a raw material in some processed foods, such as sausage and bologna. Despite the low prevalence, the possibility of cross-contamination to other raw materials, environment and tools should be taken into consideration.

KEY-WORDS: Food safety. Salmonella. Tonsils. Swine. Lymph nodes. Sausage.

RESUMO

Haja vista que os alimentos de origem animal, entre eles a carne e produtos cárneos de suínos, são considerados importantes transmissores de *Salmonella* sp. para o consumidor, o objetivo do presente estudo foi determinar a prevalência de *Salmonella* sp. em língua, tonsilas, linfonodos submandibulares, linfonodos mesentéricos, superfície de carcaças e de reto de suínos abatidos sob inspeção estadual e também na superfície de lâmina de facas utilizadas na inspeção. Para o isolamento utilizou-se água peptonada a 0,1% no pré- enriquecimento e caldos selenito cistina e Rappaport-Vassiliadis no enriquecimento seletivo. O plaqueamento foi realizado em ágares MacConkey e verde-brilhante e a identificação presuntiva em ágar TSI e LIA. A caracterização final foi realizada através de sorotipagem. Dos 25 animais amostrados, *Salmonella* Anatum foi isolada na língua de 2 animais, correspondendo a 8%. Considerando que a retirada da língua não é um procedimento obrigatório e pode entrar como matéria-prima na produção de alguns alimentos, como mortadela e salsicha, a presença de *Salmonella* sp. representa um fator de risco para a segurança alimentar. Apesar da baixa prevalência, deve-se considerar a possibilidade de contaminação cruzada com outras matérias-primas, ambiente e equipamentos.

PALAVRAS-CHAVE: Segurança alimentar. Salmonella. Tonsilas. Linfonodos. Suínos. Embutidos.

[1]Departamento de Medicina Veterinária Preventiva e Reprodução Animal. FCAV, UNESP.
*Autor para correspondência: Via de Acesso Prof. Paulo Donato Castellane, s/n., Jaboticabal, SP, CEP:14884-900. rachelsaba@hotmail.com

 Submitted: 02/03/2011 Accepted: 07/12/2011

INTRODUCTION

The genus *Salmonella* is considered the major cause of food borne diseases worldwide (WHO, 2009). Although many foods have already been associated with transmission of salmonellosis to consumers, foods of animal origin, especially meat, eggs and milk, have a prominent role as a route of transmission (CARDOSO, 2006).

According to the Centers for Disease Control (CDC) salmonellas are also among the most common bacterial infections in the United States. In 2004, from the cases of food borne illnesses registered, 42% were caused by *Salmonella* sp., 37% by *Campylobacter* sp., 15% by *Shigella* sp., 2.6% by *E. coli* O157:H7 and 3.4% by others such as, *Yersinia* sp., *Listeria* sp. and *Vibrio* sp. (USDA, 2009). According to Miller et al. (2005), approximately 100,000 cases of salmonellosis are attributed to the consumption of pork meat products with an annual cost of $80 million dollars in the USA alone.

In the pork meat production chain, salmonella is greatly important from piglet birth, where it can cause clinical symptoms in the animals and even death of the youngest, all the way to slaughtering, when the presence of the bacteria poses a high risk for the safety of food products that are obtained from contaminated raw material.

Once infected, the animal becomes the carrier of *Salmonella* sp., and is able to contaminate the environment through several ways. The bacteria have been isolated in the gastrointestinal and respiratory tracts, lungs, tonsils and lymph nodes (FEDORKA-CRAY et al., 1997). According to Dickson et al. (2008), the intestinal tract and lymph nodes associated with infected animals are a source of bacteria that can spread through the slaughterhouse, contaminating carcasses, environment, equipment and utensils.

Under experimental conditions, Hurd et al. (2001) found that pigs got infected with salmonella after being exposed to a contaminated environment for two hours.

Despite the efforts to control pathogens in the slaughterhouses and pork meat processing industry, a significant number of carcasses and pork products are still contaminated by pathogenic organisms. According to Frenzen et al. (1999) 9% of all swine carcass produced in the USA are contaminated with *Salmonella* sp. In the south of Brazil, Bessa et al. (2004) reported that 55.6% of all slaughtered pigs had *Salmonella* sp. in their lymph nodes and intestinal tract. Another study by Schwarz et al. (2006) reported the bacteria in 71.65% of the mesenteric lymph nodes of pigs slaughtered in southern Brazil.

Despite the fact that lymph nodes, tonsils and tongue are not marketed directly to consumers, they remain on the carcass after slaughtering together with the meat of the head, and are also used to manufacture sausages, bologna and mechanically separated meat (CASTAGNA et al., 2004).

Therefore, the objective of this study is to determine the prevalence of *Salmonella* sp. in the tongue, tonsils, submandibular and mesenteric lymph nodes, carcass surfaces and rectum, as well as on blades of knives used in the inspection, since the pigs were slaughtered under state inspection.

MATERIAL AND METHODS

The samples were collected from a pig slaughterhouse classified as such according to paragraph I of article 9°, law 7.705 of 1992 of the Secretaria da Agricultura do Estado de São Paulo and inspected by the Serviço de Inspeção Estadual (SISP).

From November 2008 to July 2009, during slaughtering routine, samples of tongue, tonsils, submandibular and mesenteric lymph nodes were collected. Also swabs of carcass surfaces, of blade knives used in the inspection and from the rectum of 25 randomly chosen animals were collected. Samples were collected at six different times, stored separately using aseptic plastic bags and transferred inside isothermal box to the Departamento de Medicina Veterinária Preventiva e Reprodução Animal of UNESP, where analyses were performed.

From each tongue, tonsils and submandibular and mesenteric lymph nodes a sample weighing 25 gr was cut and homogenized with 225 mL of 0.1% peptone water; while the swabs of rectum, carcasses and blade knives were diluted in 200 mL of the same diluent. The sets remained at rest for 6 hours at room temperature and were subsequently incubated at 37ºC during 18 hours. For the selective enrichment, two pre-enriched aliquots of 2.0 mL each were inoculated, respectively, in 20 mL of selenite cystine broth followed by 20 mL of Rappaport-Vassiliadis broth, added 0.2 mL of 0,4% novobiocin solution and incubated at 37ºC during 24 hours. Each culture was plated on brilliant green and MacConkey agar, followed by incubation at 37ºC for 24 hours. The characteristic colonies were then inoculated in three sugar iron (TSI) and lysine iron (LIA) agar. The serological testing used the anti-somatic and flagellar salmonella polyvalent sera (APHA, 2001). The isolated strains of *Salmonella* sp. were plated on gelose agar and incubated at 37ºC for 24 hours. After that, the samples were packed and sent for typing to Instituto Adolfo Lutz, in São Paulo.

RESULTS AND DISCUSSION

Table 1 shows the rate of contamination with *Salmonella* sp. of pork parts such as, tongue, tonsils, submandibular and mesenteric lymph nodes, as well as the swab of carcass, rectum and knife blades used in the slaughtering house after the evisceration step.

From the six collections, *Sallmonella* sp. was isolated only on samples of the fifth and sixth collections, with infection rate of 20% (1/5) and 50% (1/2), respectively. The infections were detected on tongue samples as shown in Table 1. A total of 175 samples were collected from 25 animals, of which 2 were infected, thus corresponding to a prevalence rate of 8% (2/25). A result similar to that reported by

Swanenburg et al. (2001) who isolated *Salmonella* sp. in 9.3% of tongue samples collected from slaughtered pigs.

Table 1 shows that *Salmonella* sp. was not detected in any of the first four samples. Whereas on the fifth and sixth samplings, one out five and one out of two were infected on the tongue, corresponding to 20% and 50% of the samples, respectively. Prendergast et al. (2008) reported that the prevalence of this bacteria between harvesting days may be related to the fact whether animals of the same lot are carriers or not of *Salmonella* sp., which would have a significant influence on the bacterium isolation.

According to Swanenburg et al. (2001) depending on where the bacteria are isolated, one might be able to establish whether the animal was infected prior to slaughter or during the slaughtering, thus providing information about the sanitary conditions of the procedure. Also Dickson et al. (2008) stated that *Salmonella* sp. found in meat products results either from exposure of live animals to the bacteria or contamination during the slaughtering process.

The fact that *Salmonella* sp. was isolated only on the tongue may imply that the contamination occurred in the slaughtering house, since the tonsils, submandibular and mesenteric lymph nodes were not infected and Dickson et al. (2008) associates the presence of the bacteria in these parts with live animal exposure to the bacteria.

According to Berends et al. (1996) the presence of *Salmonella* sp. represents a high risk of contamination of carcasses and final products, and the carrier animal is the main responsible for the introduction of the bacteria in the slaughtering house and processing. The carcasses from pigs contaminated with salmonella, have a probability 3 to 4 times higher to be positive for *Salmonella* sp. compared to carcasses of salmonella-free pigs (BERENDS et al., 1997).

Table 1 - Number and percentage of samples positive for *Salmonella* sp. in the following pork parts tongue, tonsils, submandibular and mesenteric lymph nodes, as well as the swab of carcass, rectum and knife blades used in the slaughtering house in São Paulo, 2009.

Sample collection	Number of animals	L	T	LS	LM	SC	SR	SF	%
1ª	3	0	0	0	0	0	0	0	0
2ª	5	0	0	0	0	0	0	0	0
3ª	5	0	0	0	0	0	0	0	0
4ª	5	0	0	0	0	0	0	0	0
5ª	5	1	0	0	0	0	0	0	20%
6ª	2	1	0	0	0	0	0	0	50%
Total	25	2	0	0	0	0	0	0	8.00%

L – tongue
T – tonsils
LS – submandibular lymph node
LM – mesenteric lymph node
SR – rectum swab
SC – carcass swab
SF – blade swab

Although the bacteria was not isolated in the submandibular and mesenteric lymph nodes, feces and carcass in the present study, several authors have isolated the bacteria on these same parts. Bessa et al. (2004) reported *Salmonella* sp. in 17.6% and 18.3%, of mesenteric lymph nodes and feces of 26 different serovars, respectively. A similar percentage was reported by Spolaore (2007), who found *Salmonella* sp. in 17.33% of the mesenteric lymph nodes and in 5% of the blades of the sampled knives. Silva (2008) reported that 16.6% (50/300) of pigs were positive at slaughtering, corresponding to 12 (11.54%) tonsil samples and 38 (19.39%) mesenteric lymph nodes.

More recently, Piras (2009) found the bacteria in 30.5% of the mesenteric lymph node samples, in 16.4% of colon content samples and in 14.1% of carcass samples. Castagna et al. (2004) reported a higher yet percentage, 61% (55/90) of mesenteric lymph nodes, 55.5% (50/90) intestinal contents and 36.7% (33/90) tonsils and submandibular lymph nodes were positive for *Salmonella* sp.

Bonardi et al. (2003) found lower percentages of positive samples, 5.3% of tonsils and 6.0% carcasses were positive more commonly to *Salmonella derby* (37.8%), *Salmonella bredeney* (21.6%) and *Salmonella typhimurium* (14.8%). Swanenburg et al. (2001) isolated *Salmonella* sp. in 9.3% of the analyzed mesenteric lymph nodes and in 19.6% of tonsils, the *Salmonella* serotype *typhimurium* was the most prevalent.

Even at low prevalence, the presence of *Salmonella* sp. in carcass, meat cuts, interiors, among others, is

considered as a potential risk factor for cross-contamination of the bacteria to the environment and equipments as well, as stated by Prendergast et al. (2008), who found the bacteria in 3% (24/720) of ham samples and in 12.5% (7/56) of environment swabs.

Considering that the removal of the tongue is not a mandatory procedure and it can be used as a raw material in the production of certain foods such as, bologna and sausages, the presence of *Salmonella* sp. poses a threat for food safety.

Thus, the low prevalence found in the present study does not rule out the risk to public health, especially if the products obtained from this raw material do not undergo a treatment to eliminate the bacteria, also the possibility of cross-contamination between infected samples and other raw materials, the environment and equipments should be taken into account.

REFERENCES

AMERICAN PUBLIC HEALTH ASSOCIATION. **Compendium of methods for the microbiological examination of foods**. 4th ed. Washington, 2001.

BERENDS, B. R.; URLINGS, A. P.; SNIJDERS, J. M. A. Identification and quantification of risk factors in animal management and transport regarding *Salmonella* spp. in pigs. **International Journal of Food Microbiology**, v.30, p.37-53, 1996.

BERENDS, B. R.; VAN KNAPEN, F.; SNIJDERS, J. M. A.; MOSSEL, D.A.A. Identification and quantification of risk factors regarding *Salmonella* spp. on pork carcasses. **International Journal of Food Microbiology**, v.36, p.199-206, 1997.

BESSA, M. C.; COSTA, M.; CARDOSO, M. Prevalência de *Salmonella* sp. em suínos abatidos em frigoríficos do Rio Grande do Sul, Brasil. **Pesquisa Veterinaria Brasileira**, v.24, p.80-84, 2004.

BONARDI, S.; BRINDANI, F.; PIZZIN, G.; LUCIDI, L.; D'INCAU, M.; LIEBANA, E.; MORABITO, S. Detection of *Salmonella* spp., *Yersinia enterocolitica* and verocytotoxin-producing *Escherichia coli* O157 in pigs at slaughter in Italy. **International Journal of Food Microbiology**, v.85, p.101–110, 2003.

CARDOSO, M. Doenças transmitidas por alimentos de origem suína. In: SIMPOSIO UFRGS SOBRE MANEJO, REPRODUÇÃO E SANIDADE SUINA, 1, 2006, Porto Alegre. **Anais....**Porto Alegre: UFRGS, p.92-103. 2006.

CASTAGNA, S. M. F.; SCHWARTZ, P.; CANAL, C. W.; CARDOSO, M. Presença de *Salmonella* sp. no trato gastrintestinal e em tonsilas/linfonodos submandibulares de suínos ao abate. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v.56, n.3. Belo Horizonte. 2004.

DICKSON, J. S.; HURD, H. S.; ROSTAGNO, M. H. *Salmonella* in the Pork Production Chain. Originally published as a National Pork Board/**American Meat Science Association Fact**, Iowa, EUA. 2008.

FEDORKA-CRAY, P.; McKEAN, J. C. Ç BERAN, G.W. Prevalence of *Salmonella* in swine and pork: a farm to consumer study. **ISU Swine Reserch Report**, 1997. Disponível em: <http:wextension.iastate.edu/Pages/ansci/swinereports/asl-1507.pdf>. Acesso em: 21 de agosto de 2010.

FRENZEN, P. D.; BUZBY, J. C.; ROBERTS, T. An updated estimate of the economic costs of human illness due to foodborne *Salmonella* in the United States. In: **Proceedings of the 3rd International Symposium on the Epidemiology and Control of Salmonella in Pork**, Washington,DC, p.215-218, 1999.

HURD, H. S.; McKEAN, J. D.; WESLEY, I. V.; KARRIKER, L. A. The effect of lairage on *Salmonella* isolation from market swine. **Journal of Food Protection,** v.64, p.939-944, 2001.

MILLER, G. Y.; LIU, X.; McNAMARA, P. E.; BARBER, D. A. Influence of *Salmonella* in pigs preharvest and during pork processing on human health costs an risks from pork. **Journal Food Protection**, v.68, p.1788-1798. 2005.

PIRAS, F. *Salmonella* spp. in Sardinian slaughter pigs: prevalence, serotypes and genotypic characterization. **UnissResearch.** 2009. Disponível em:<http://eprints.uniss.it/1032/>. Acesso em 24 de Agosto de 2010 às 22:15h.

PRENDERGAST, D. M.; DUGGAN, S. J.; FANNING, S.; CORMICAN, M.; GONZALES-BARRON, U.; BUTLER, F.; DUFFY, G. Prevalence and number of *Salmonella* spp. And Enterobacteriaceae on pork cuts in abbatoirs in the Republic of Ireland. **Journal of Applied Microbiology**, v.105, n.4, p.1209-19, 2008.

SCHWARZ, P. Prevalência sorológica e de isolamento de *Salmonella* enterica em suínos abatidos no sul do Brasil. 2006. 47f. Dissertação (Mestrado Ciências Veterinárias) - Programa de Pós-graduação em Ciências Veterinárias, **Universidade Federal do Rio Grande do Sul**, Porto Alegre.

SILVA, M. C.; FARIA, G. S.; PAULA, D. A. J.; MARTINS, R. P.; CARAMORI JUNIOR, J. G.; KICH, J. D.; COLODEL, E. M.; NAKAZATO, L.; DUTRA, V. Prevalência de *Salmonella* sp. em suínos abatidos no Estado de Mato Grosso. **Ciência Rural,** v.39, n.1, Santa Maria. 2008.

SPOLAORE, A. J. G. Prevalência de *Salmonella* sp. em linfonodos mesentéricos de suínos abatidos na região oeste do paraná e potencial de disseminação em bandejas, facas e luvas de manipuladores durante a

inspeção *postmortem.* Universidade Federal do Paraná**. Dissertação de mestrado.** Curitiba. 2007. 59p. Disponível em:<http://hdl.handle.net/1884/13855>. Acesso em 24 de agosto de 2010 as 15:30hs.

SWANENBURG, M.; URLINGS, H. A. P.; SNIJDERS, J. M. A.; KEUZENKAMP, D. A.; van KNAPEN, F. *Salmonella* in slaughter pigs: prevalence, serotypes and critical control points during slaughter in two slaughterhouses. **International Journal of Food Microbiology**, v.70, n.3, p.243-254, 2001.

USDA. Salmonella **Questions and Answers. Disponível em:** <http://199.140.94.5/Fact_Sheets/Salmonella_Questions & Answers/index.asp> Acesso em: 24 de junho de 2009 às 10:10h.

WHO. World Health Organization. **Health topics: Salmonella**. 2009. Disponível em:<http://www.who.int/topics/salmonella/en/>. Acesso em: 22 de junho de 2009 às 16:30h.