

Study on *Salmonella* sp. in the head part of carcasses from slaughtered pigs

Vieira-Pinto, M.M. ^{*(1)}, Morais, L. ⁽¹⁾, Reais, P. ⁽¹⁾, Themudo, P. ⁽²⁾

⁽¹⁾ Dept. Veterinary Science. CECAV. Lab. Food Inspection, University of Trás-os-Montes e Alto Douro. Apartado 1013. 5001 – 911. Vila Real. Portugal

⁽²⁾ INIAV-Animal Production and Health Unit -Dept. Bacteriology. *Salmonella* serotyping (LNR). Estrada de Benfica, 701, 1549-011 Portugal

*corresponding author: mmvpinto@utad.pt

Abstract

To define the importance of the head part of pigs' carcasses as a potential vehicle of *Salmonella*, 105 carcasses were sampled at one abattoir. The results revealed the presence of *Salmonella* sp. in 25 samples (23.8%), which corresponds to a higher value than those previously presented by the same authors in similar studies in pig carcasses (12,9%). By means of serotyping, were identified 5 different serotypes: S. Typhimurium (9, 36%), S. London (6 24%), S. Rissen (6 24%), S. Agona (3, 12%) and S. Goldcoast (1, 4%). This study underlines the importance that the head part of the pigs' carcass can take as a source of *Salmonella* throughout meat chain and a potential vehicle, direct or indirect, to the final consumer. For this reason, the authors suggest that increased hygienic measures should be adopted during head processing and cutting, especially if its meat will be subsequently used for sausage or smoked meat (Figure 1) production that could be consumed without any kind of heat treatment.

Introduction

According to EFSA report (2013), human salmonellosis in Europe, in 2011, still being the second most frequently reported zoonotic disease, accounting for 95,548 cases with a total of 56 fatal cases. Also, according to the same report, *Salmonella* remained the most frequently detected causative agent in foodborne outbreaks reported in the EU.

Recently, BIOHAZ Panel estimated that a rate of around 56.8 % of human salmonellosis cases could be attributable to pigs.

To protect human health against *Salmonella* infections transmissible between animals and humans, EU Regulation (EC) N.º 2160/200313 requires MSs to set up national control programmes for *Salmonella* serovars deemed to be of particular public health significance in animal species including pigs. The purpose of this Regulation is to ensure that proper and effective measures are taken to detect and to control *Salmonella* and other zoonotic agents at all relevant stages of production, processing and distribution, particularly at the level of primary production, including in feed, in order to reduce their prevalence and the risk they pose to public health

The slaughter of *Salmonella*-infected pigs represents a potential risk for contamination of carcasses and other edible products, introducing *Salmonella* into the food chain (Vieira-Pinto *et al.*, 2005).

At the slaughterhouse the swine can carry *Salmonella* on the skin and in several tissues. The tissues mostly referred as affected by *Salmonella* are those from the digestive tract and the corresponding lymphatic tissue such as tonsils and mesenteric- and mandibular lymph nodes (Jung *et al.*, 2001). According to Olsen *et al.* (2001), the spread of *Salmonella* during the slaughter process may occur especially through pharynx (tonsils) and intestinal contents. The (cross-) contamination of carcasses, through contaminated utensils, equipment or handlers, is basically a matter of redistributing the *Salmonella* bacteria from the positive pigs during the various slaughter processes (Lo Fo Wong *et al.*, 2002), even when the slaughter process is performed correctly, the contamination from these reservoirs can be transferred to the carcass (Olsen *et al.*, 2001).

The presence of *Salmonella* sp. in pigs' carcasses has been shown in several international and national research studies. However, no national data was found on the presence of *Salmonella* sp. in the head part of pigs' carcasses, whose muscles are often used for traditional sausages manufacturing or smoked meat products, some of which consumed raw or undercooked. As it was referred Bruun *et al.*, (2009), the ingestion of pig meat not submitted to a deep thermal treatment may enhance health risks for some food borne diseases like Salmonellosis.

Additionally, the presence of *Salmonella* sp. in the head part of pigs' carcasses can constitute a risk, since this tissues can be adversely incised and touched during sanitary inspection (incision of mandibular lymph nodes), during tonsils extraction as well as along some other slaughter procedures. All of these procedures may allow cross-contamination through the hands, knives and other utensils or equipment.

For these reasons, 105 head part of pigs' carcasses were sampled at one abattoir to define its importance as a potential vehicle of *Salmonella* sp.

The results revealed the presence of *Salmonella* sp. in 23.8% of the sampled carcasses and identified five different serotypes: *S. Typhimurium* (9, 36%), *S. London* (6 24%), *S. Rissen* (6 24%), *S. Agona* (3, 12%) and *S. Goldcoast* (1, 4%).

Material and Methods

During this study, 105 carcasses were sampled at one abattoir by means of swabbing the internal face of the head with a cotton sterilised gauze (hydrated in 25 ml of Buffered Peptone Water with 0.1% Tween) (Swanenburg, 2000) (Figure 2).

All the samples were individually packed in a sterile named recipient and transported under refrigerated conditions to the laboratory where *Salmonella* sp. isolation procedures were started on the same day.

Salmonella sp. isolation method was performed according to ISO 6579:2002 "Microbiology of food and animal feeding stuffs- Horizontal method for the detection of *Salmonella* spp. Briefly, the diluted samples in BPW were incubated at $37^{\circ}\text{C}\pm 1^{\circ}\text{C}$ for $18\pm 2\text{h}$, afterwards 0,1 ml and 1ml was respectively inoculated in Rappaport-Vassiliadis medium with soya (RVS broth, Oxoid® - 669) and in Muller-Kauffmann tetrathionate/novobiocin broth (MKTn broth, Merk® - 110863). The RVS broth was incubated at $41,5\pm 1^{\circ}\text{C}$ for $24\text{h}\pm 3\text{h}$, and the MKTn broth at $37^{\circ}\text{C}\pm 1^{\circ}\text{C}$ for $24\text{h}\pm 3\text{h}$. After that, one loop of each selective enrichment broth was streaked onto the surface of two selective solid media: Hektoen (Oxoid® - CM419) and XLD (Oxoid® - CM469) agar. Colonies of presumptive *Salmonella* were confirmed by biochemical tests (Oxidase reaction, Triple Sugar Iron Agar (Oxoid® - CM277), Urea broth (Merk® - 1.08483), L-Lysine decarboxylation medium (Oxoid® - CM308S) and serological agglutination with Poli A-I & Vi antiserum (Difco® - 222641).

Suspicious *Salmonella* sp. isolates were serotyped according to the Kauffmann-White scheme in the INIAV (Lisbon, Portugal), the National Reference Laboratory for *Salmonella*.



Figure 1 – Smoked head part of pigs' carcasses

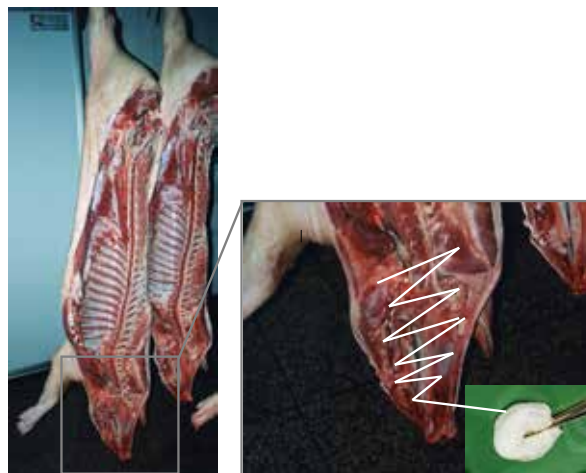


Figure 2 – Swabbing procedure

Results

The results revealed the presence of *Salmonella* sp. in 25 samples (23.8%).

Among these isolates were identified 5 different serotypes: *S. Typhimurium* (9, 36%), *S. London* (6 24%), *S. Rissen* (6 24%), *S. Agona* (3, 12%) and *S. Goldcoast* (1, 4%).

Discussion

The results revealed the presence of *Salmonella* sp. in 25 (23.8%) of swab samples from the internal face of the carcass head, which corresponds to a higher value than those previously presented by the same authors in similar studies (Vieira-Pinto *et al.*, 2005) in pig carcasses (12,9%).

The presence of *Salmonella* sp. in this part of the carcass can be associated to contamination processes during the slaughter and meat inspection procedures and also can be related to oral infection of pigs before slaughter (Swanenburg, 2000) which can be favoured by the typical exploratory and coprophagy behaviour. In fact, the faecal-related contamination of the pig's tonsils has already been described by Vieira-Pinto *et al.* (2006) showing a highly significant association ($p < 0.001$) between the presence of *Salmonella* in the ileum and/or ileocolic lymph nodes and its presence in the corresponding tonsils, as well

as the very high percentage (80%) of positive tonsils having the same *Salmonella* genotype as ileum and/or ileocolic lymph nodes.

According to Swanenburg *et al.* (1999), Hald (2001) and Olsen *et al.* (2001), oral cavity, particularly the tonsils, pharynx and tongue can accommodate large quantities of *Salmonella*, resulting from contamination during transport and at rest in the lairage, by regurgitation of gastric contents or by water scald. From these tissues, *Salmonella* sp. can be transferred to the carcass during removal of the tongue, together with the pluck, while cutting the head or through the incisions made during the meat inspection Olsen *et al.* (2001). These authors have also shown that not removing the tongue of the head intact reduced by 30% the presence of *Salmonella* positive-carcasses.

According to the results found in this research, attention should be paid to the handling procedures of the head part of pigs' carcasses in order to mitigate *Salmonella* sp. contamination to the other edible products.

Also, since many of the national monitoring programmes for *Salmonella* sp. in pig meat are based on sampling at the slaughterhouse (by means of carcass swabbing) and the *Salmonella* criteria laid down by the Regulation (EC) No 1441/2007 prescribe rules for sampling pig carcasses at slaughterhouse, it should be important to understand the influence of this part of the pig carcass on the sensitive sampling method and, consequently, on microbiological results.

Were identified 5 different serotypes: *S. Typhimurium* (9, 36%), *S. London* (6 24%), *S. Rissen* (6 24%), *S. Agona* (3, 12%) and *S. Goldcoast* (1, 4%).

The most prevalent serotype was *S. Typhimurium*. This serotype has been described as the main serotype identified in swine in several studies as well as in the baseline studies based on the EU Regulation 2160/2003, in Europe and in Portugal (EFSA, 2007). This serotype should be also under special attention due to its virulence to humans and animals and to its high resistance rate to antibiotics (Botteldoorn *et al.*, 2004). According to EFSA (2013), *S. Typhimurium* human cases are mostly associated with the consumption of contaminated pig, poultry and bovine meat.

Respecting to *Salmonella* Rissen, scarce references were found on its occurrence in swine, and in these references no particular attention to this serotype were found. But, in the fifth European Unionwide baseline survey carried out at farm level to determine the prevalence of *Salmonella* in pig breeding holdings (between January 2008 and December 2008), *S. Rissen* appeared to occur only in a few countries, but was one of the most common serotype in Portugal and Spain (EFSA, 2007). Nevertheless, this serotype doesn't seem to have implication in the number of Human salmonellosis in both countries.

Conclusion

This study underlines the importance that the head part of the pigs' carcass can take as a source of *Salmonella* sp. throughout meat chain and a potential vehicle, direct or indirect, to the final consumer. For this reason, the authors suggest that increased hygienic measures should be adopted during head processing and cutting, especially if its meat will be subsequently used for sausage production that is to be consumed without any kind of heat treatment.

References

Botteldoorn, N., Herman, L., Rijpens, N. & Heyndrickx, M. (2004). Phenotypic and molecular typing of *Salmonella* strains reveals different contamination sources in two commercial pig slaughterhouse. *Applied and Environmental Microbiology*, 70, 5305-5314.

Bruun, T., Sørensen, G., Forshell, L. P., Jensen, T., Nygard, K., Kapperud, G., Lindstedt, B. A., Berglund, T., Wingstrand, A., Petersen, R. F., Müller, L., Kjelsø, C., Ivarsson, S., Hjertqvist, M., Löfdahl, S. & Ethelberg, S. (2009). An outbreak of *Salmonella* Typhimurium infections in Denmark, Norway and Sweden. *Euro Surveillance*, 14 (10), 1-6.

EFSA (2013). The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2010; *EFSA Journal* 2012; 10(3):2597.

EFSA, 2007. Opinion Report of the Task Force on Zoonoses Data Collection on a proposal for technical specifications for a baseline survey on the prevalence of *Salmonella* in breeding pigs. *EFSA Journal* 2007, 99, 1-28.

Grimont, P. and Weill, F-X. (2007) Antigenic formulae of the *Salmonella* serovars, 9th edition. WHO Collaborating Centre for Reference and Research on *Salmonella*. Institute Pasteur, Paris.

Hald, T. (2001). *Salmonella* in pork. Epidemiology, control and the public health impact. Ph. D. Thesis. Royal Veterinary and Agricultural University. Copenhagen. Denmark, 310 Pp.

Jung, B. Y, Lee, W. W., Lee, H. S., Kim, O. K. & Kim, B. H. (2001). Prevalence of *Salmonella* in slaughter pigs in South Korea. Proceedings of the 4th International Symposium on the Epidemiology and Control of *Salmonella* and other food borne pathogens in Pork. Leipzig. Germany, 202-204.

Lo Fo Wong, D.M.A., Hald, T., van der Wolf, P.J. & Swanenburg, M. (2002). Epidemiology and control measures for *Salmonella* in pigs and pork. Livestock Production Science, 76, 215–222.

Olsen, A-M, Jensen, T., Dahl, J. & Christensen, H. (2001). Reduction in level of *Salmonella* on swine carcasses after slaughter without splitting the head. Proceedings of the 4th International Symposium on the Epidemiology and Control of *Salmonella* and other food borne pathogens in Pork. Leipzig. Germany, 124-126.

Swanenburg, M. (2000). *Salmonella* in the pork production chain: Sources of Salmonella on pork. PhD Thesis.161. Netherlands

Swanenburg, M., Urlings, H. A. P., Keuzenkamp, D. A. & Snijders, J. M. A. (1999). Tonsils of slaughtered pigs as marker sample for *Salmonella* positive pork. Proceedings of the 3rd International Symposium on the Epidemiology and Control of *Salmonella* in Pork. Washington, 264-265.

Vieira-Pinto, M., Tenreiro, R.; Martins, C. (2006). Unveiling contamination sources and dissemination routes of *Salmonella* sp. in pigs at a Portuguese slaughterhouse through macrorestriction profiling by Pulsed-Field Gel Electrophoresis. *International Journal of Food Microbiology*.110: 77-84.

Vieira-Pinto, M. M., Temudo, P. & Martins, C. (2005). Occurrence of *Salmonella* in the ileum, ileocolic lymph nodes, tonsils, mandibular lymph nodes and carcasses of pigs slaughtered for consumption. Journal of Veterinary Medicine Series B (Infectious Diseases and Veterinary Public Health), 52, 476-481. 264-265.