Salmonella sp. in edible offal (liver and tongue) from pigs slaughtered for consumption

Vieira-Pinto, M. [1,*]
Vieira, M. J. [1], Oliveira, M. [2], Fontes, M.C. [1], Themudo, P.[3], Semedo-Lemsaddek, T.[2]

[1] Dept. das Ciências Veterinárias. CECAV. Universidade de Trás-os-Montes e Alto Douro. Vila Real. Portugal.
[2] CIISA/Faculdade Medicina Veterinária. Lisboa . Portugal
[3] Laboratório de Bacteriologia. Unidade de Sanidade Animal. Laboratório Nacional de Investigação Veterinária (INRB, I.P.- LNIV). Lisboa. Portugal

*Author for correspondence: Dept. Ciências Veterinárias. Lab. Inspecção Sanitária (P2).Universidade de Trás-os-Montes e Alto Douro. Apartado 1013. 5001-801Vila Real. Portugal.
Telephone nº +351 259 350510, Fax nº +351 259 350480, E-mail address: mmvpinto@utad.pt

Abstract

During this study, 120 samples from slaughtered pigs (tongue swabs, n=40; liver swabs, n=40; liver parenchyma, n=40) were collected in a slaughterhouse. Salmonella sp. was isolated using conventional microbiological methods and strains were analyzed using serotyping, antimicrobial susceptibility testing and macrorestriction profiling (MRP) by Pulse Field Gel Electrophoresis (PFGE), to identify clonal relationships and potential contamination sources. The highest prevalence of Salmonella sp. was observed in tongue samples (10/40; 25%), followed by liver swab (5/40; 12.5%) and liver parenchyma samples (4/40; 10%). XbaI macrorestriction allowed defining 8 genotypes (MRP) among the 3 analyzed serotypes: S. Rissen (5), S. Typhimurium (2) and S. 4,[5],12:i:- (1). Strains with the same MRP were observed in tongue swab samples collected in different days, suggesting common contamination sources and the persistence of Salmonella sp. clones in the slaughterhouse. Also, the presence of the same MRP in liver parenchyma and in liver and tongue swabs samples seems to indicate that the pig is one of the possible vehicles of Salmonella sp. to those edible products. The results observed in this study underline the significance of Salmonella sp. contamination in pork tongue and liver that will become available for direct or indirect (through meat products) human consumption. They also suggest that measures should be taken in order to improve hygienic conditions to minimize Salmonella sp. contamination during the slaughter process and along liver and tongue processing and selling chain.

Introduction

Salmonella is an important cause of food-borne illness in humans and in recent years the increasing number of studies in this area of research has highlighted the importance of pork as a source of human salmonellosis. However, the information about Salmonella sp. distribution on the two most important pig edible offal (tongue and liver) is very scarce and, to our knowledge, this is the first study performed in Portugal on this subject. In the pig, infection within the intestinal tract may be followed by invasion of the gut cells. Infection is established in the intestinal lymph nodes and, afterwards spread to the liver is observed. Therefore, the presence of Salmonella sp. within the hepatic parenchyma is an incontrovertible evidence of pig infection. In opposite, the presence of Salmonella sp. in the tongue and liver surface mainly reflects the contamination of these offal during slaughtering Contaminated offal, livers and tongues, will become available for human consumption. The major aim of the present survey was to estimate the prevalence of Salmonella–contaminated livers and tongues in pigs slaughtered in Portugal.

Material and Methods

During this study, samples from 40 slaughtered pigs were collected in one slaughterhouse along 8 sampling days during 5 months (5 pigs each visit). From each pig the following samples were collected after removal of the pluck (evisceration) and before chilling: liver swabs, tongue swabs and liver parenchyma. The liver and the tongue swabs were performed according to Swanenburg et al. (2001) and a piece of parênquima of approximately 30g was collected with aseptically material. A total of 120 samples were collected. All the samples were individually packed in a sterile labelled recipient and transported under refrigerated conditions to the laboratory where Salmonella sp. isolation was performed on the same day, according to ISO 6579:2002. Subsequently, presumptive Salmonella sp. isolates were serotyped according to the Kauffmann-White scheme in the "Laboratório Nacional de Investigação Veterinária" (Lisbon, Portugal), the Portuguese Reference Laboratory for Salmonella. Antimicrobial susceptibility testing was performed as recommended by

the Clinical and Laboratory Standards Institute (CLSI, 2008). All Salmonella sp. isolates were tested against a total of 13 antimicrobial agents: ampicillin (10 µg), amoxicillin-clavulanic acid (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), cefepime (30 µg), gentamicin (10 µg), kanamycin (30 µg), streptomycin (10 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), tetracycline (30 µg), chloramphenicol (30 µg) and trimethoprim-sulfamethoxazole (25 µg).
PFGE genotyping, was performed as described before by Vieira-Pinto et al. (2006). After PFGE, the gel was stained with ethidium bromide, photographed under UV transilumination with ImageMaster VDS DE 230 VAC (Pharmacia Biotech). The BioNumerics software (version 4.61, Applied Maths, Kortrijk, Belgium) was used to register the macrorestriction profiles, normalize densitometric traces, calculate Pearson product-moment correlation coefficient -r- and perform cluster analysis by the UPGMA algorithm.

Results

A total of 19 Salmonella-positive samples were identified. The highest Salmonella sp. prevalence was observed in tongue swab samples (10/40; 25%), followed by the liver swabs (5/40; 12.5%) and the liver parenchyma samples (4/40; 10%). A total of 7 (17.5%) livers were positive for Salmonella. In 54.6% of the sampled pigs, Salmonella sp. was observed at more than one sampling site.
Following isolation and presumptive identification, Salmonella sp. strains were analyzed by serotyping, antimicrobial testing and macrorestriction profiling, aiming to identify clonal relationships and assess for the correlation between the presence of Salmonella sp. in different pig samples, in order to unravel routes of dissemination and potential contamination sources.
Among the positive samples, 3 serotype were identified, namely S. Rissen (15/19; 79%), S. Typhimurium (3/19; 16%) and S.4,[5],12:i:- (1/19; 5%).
Table 1 presents the distribution of the Salmonella sp. serotype and antibiotic resistance profile (ARP) among the positive samples, and additional epidemiological data, regarding slaughter date and farm. XbaI macrorestriction allowed defining 8 MRP among the 3 analyzed serotypes. The results of Salmonella sp. isolation from the different samples, combined with the serological, antimicrobial resistance and MRP results, may allow us to disclose hypothetical relationships between Salmonella- positive samples:

- S. Typhimurium isolated from the liver parenchyma and tongue swab of the same pig harbour the same MRP/ ARP (Fig. 1–A), suggesting a common source of Salmonella sp. in life (before slaughter) and/or tongue contamination by the pig itself during slaughter process.
- A strain of S. Rissen isolated from the liver parenchyma of one infected pig presented the same MRP/ARP identified in the liver swabs from pigs slaughtered in the same day at the same farm (Fig. 1–B); this scenario points to the pig as the possible source of liver contamination with Salmonella sp.
- Strains of S. Rissen with the same MRP/ARP were also observed in tongue swabs from different days (Fig. 1–C), suggesting common contamination sources and the persistence of Salmonella sp. clones along the slaughter process or in the lairage.

Table 1 - Serotype and antibiotic resistance profile (ARP) of the Salmonella sp. isolates and additional epidemiological data related to the slaughter date and the farm of origin.

			Liver parenchyma		Liver Swab		Tongue swab	
Date	Pig	Farm	Serotype	ARP	Serotype	ARP	Serotype	ARP
20 Jan	1-2	F	-	-	-	-	-	-
	3-5	A	-	-	-	-	-	-
27 Jan	6-10	C	-	-	-	-	-	-
03 Feb	11-15	E	-	-	-	-	-	-
10 Feb	16 -20	F	-	-	-	-	-	-
24 March	21	G	-	-	-	-	S. Typhimurium	AML, S, TE
	22-25	G	-	-	-	-	-	-
	26	G	S. Rissen	TE	-	-	S. 4,[5],12:i:-	S, C, TE
	27	G	S. Rissen	TE	S. Rissen	TE	S. Rissen	TE
28 March	28	G	-	-	-	-	S. Rissen	TE
	29	G	-	-	S. Rissen	TE	-	-
	30	G	-	-	S. Rissen	TE	S. Rissen	TE
	31	B	-	-	-	-	S. Rissen	TE
	32	B	-	-	-	-	S. Rissen	TE
05 May	33	B	S. Rissen	AML, SXT, TE	-	-	S. Rissen	TE
	34	B	S. Typhimurium	AML, NA, S, C, TE	S. Rissen	TE	S. Typhimurium	AML, NA, S, C, TE
	35	B	-	-	S. Rissen	AML,SXT,TE	S. Rissen	TE
19 May	36 - 40	D	-	-	-	-	-	-
TOTAL	40	7	4/40 (10%)		5/40 (12,5%)		10/40 (25%)	

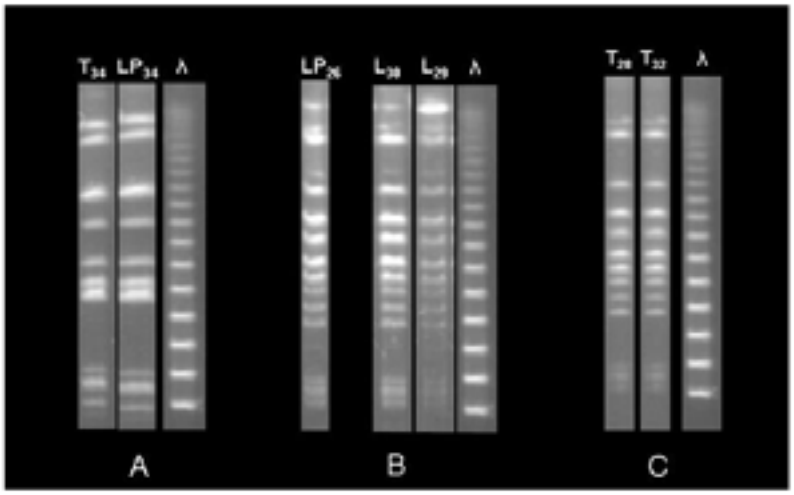

Figure 1 – Examples of MRP patterns observed in Salmonella sp. isolated from different samples (T – Tongue swab; LP – Liver parenchyma; L – Liver swab)

Discussion

In this study, the highest Salmonella sp. prevalence was observed in the tongue swab samples (10/40; 25%). This value was higher than previously observed by Swanenburg et al. (2001) who reported a prevalence of 9.3%. The presence of Salmonella sp. in the surface of the tongue may indicate not only a potential contamination by ingestion of contaminated material before slaughtering but may also suggest a contamination during the slaughtering process, for instance during evisceration, tonsils extraction or during meat inspection. Strains of Salmonella Rissen with the same MRP/ARP were obtained from tongue swabs of different days (Fig.1–C), suggesting common contamination sources and the persistence of Salmonella sp. clones along the slaughter process or in the lairage. Thus, pointing to a possible presence of a residential Salmonella **f**ora in the slaughterhouse (EFSA, 2008a). In order to avoid that, a regular accurate cleaning and disinfecting of all equipment and installation should be carried out (Swanenburg et al., 2001).

As previously reported by Peterson et al. (2002), the oral cavity of pig's head is frequently contaminated with pathogenic bacteria. According to our results, the tongue also seems to have an important level of Salmonella sp. contamination, underlining that additional hygienic measures should be adopted during the evisceration process that involves the removal of the tongue together with the pluck. According to Olsen et al. (2001), the presence of Salmonella sp. in the pig's oral cavity, including the tongue, may in**f**uence the carcass contamination. About this subject, Peterson et al. (2002) adviced that the head should be removed from the carcass at an early stage of the slaughter process and transported separately to a specific cutting room. Authors also suggested that the head meat and tongue should be heat treated before being approved for meat products.

With respect to the results of the Salmonella sp. prevalence in the liver swabs (12.5%) observed in this study, which gives information about hygiene during the slaughter process, were high than the results observed by Swanenburg et al. (2001), which reported an prevalence of 9.3%. Nowadays, according to the Regulation (EC) N.°854/2004, the post-mortem inspection procedure of the pigs liver and its lymph nodes includes visual inspection and palpation. This means that the routine liver palpation during sanitary meat inspection, may lead to cross-contamination through the inspector's hands.

Results from the liver parenchyma (10%) which gives information about infection of the pig before slaughter slaughtering, on the farm, during transport or in the lairage, were very similar to the observed for slaughter pigs infected with Salmonella sp. in ileo-caecal lymph nodes (10.3%) reported on the baseline survey on the prevalence of Salmonella in slaughter pigs, in the EU (EFSA, 2008a).

It is also important to point out that the presence of Salmonella infection in the intestinal lymph nodes, which are removed from the carcass and are not consumed, may only represent a limited public health threat, whilst a contaminated liver is likely to be a greater risk due to dissemination via the food chain.

The results observed in the liver samples highlight the importance of this offal as a vehicle of Salmonella sp. to the food chain, suggesting that hygienic and technical measures should be adopted in order to reduce the risk of cross-contamination and human infection. For instance, during meat inspection the liver should be incised only when strictly necessary and the knife used by the Official Veterinarian during its activity should be carefully and properly disinfected in order to avoid/reduce cross-contamination.

Among the positive samples Salmonella Rissen (15/19; 79%), was the most prevalent serotype, which is in accordance to the EFSA Report (2008a) that indicates S. Rissen as a serotype frequently isolated from slaughter pigs' lymph nodes in Spain and Portugal. Although S. Rissen doesn't seems to be a risk and a cause for Salmonella infections in humans within the European Union, the authors believe that the relevant frequency of occurrence along the pork production chain in Portugal, should incite to further studies on the epidemiology and virulence traits of this serotype.
The presence (3/19; 16%) of S. Typhimurium observed in this study should be emphasised, as it is the second most frequently serotype isolated from reported human salmonellosis in the EU, with increasing importance comparatively to the previous year (EFSA, 2010a). In the reported food-borne Salmonella outbreaks in 2008, pig meat and products thereof, were important food sources and may have contributed to the significant increase in S. Typhimurium outbreaks in humans. Salmonella 4,[5],12:i:- (1/19; 5%) was also detected in the present study. This monophasic S. Typhimurium has been increasing identified in the EU since 2006 as being involved in major food-borne outbreaks in humans in MSs and many non-European countries. The emergence of these new S. Typhimurium strains in pig populations and their subsequent spread to other animal species and humans is of public health significance (EFSA, 2010b). Therefore, it is recommended a special attention and concern to the presence of this serotype along the pork production chain.

Conclusions

Theis study underlines the significance of pig liver and the tongue as a potential vehicle of Salmonella sp. along processing and selling chain and also to the final consumer. The findings of this survey are expected to highlight the importance of the improvement of hygienic and practical measures in order to minimize Salmonella sp. cross contamination and multiplication along liver and tongue processing and selling chain in order to safeguard human health.

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