PRESENCE OF *SALMONELLA* SPP. IN PIG CARCASSES DURING SLAUGHTER: DIRECT OR CROSS CONTAMINATION?

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Introduction

Salmonella spp. infection is one of the main causes of human gastroenteritis worldwide and pork is an important source of contamination. At the slaughterhouse swine can carry *Salmonella* spp. in several tissues, which can be a source of contamination of closely related pigs in the slaughter process, and may be responsible for carcass contamination. The tissues mostly affected by *Salmonella* are those from the digestive tract and the corresponding lymphatic tissue such as tonsils and mesenteric- and mandibular lymph nodes (Berends *et al.*, 1996). Molecular typing methods are useful for defining clonal relationships between strains and provide more epidemiological information about the nature of contamination found at the slaughterhouses. Macrorestriction profiling by pulsed-field gel electrophoresis analysis (RFLP-PFGE) is now recognized to be one of the most reliable and discriminating tools for epidemiological typing of *Salmonella* isolates belonging to various serovars (Giovannacci *et al.*, 2001). This research focuses on the use of numerical analysis of macrorestriction profiles resolved by PFGE to identify clonal relationships between strains of the same *Salmonella* serotype, isolated from the carcass and from pig samples (ileum, tonsils, mandibular and ileocolic lymph nodes) in order to define the source of carcass contamination (direct or cross contamination).

Materials and Methods

From June 2003 to September 2004, several visits were made to an abattoir in the North of Portugal. In each visit a randomly selected group of pigs were sampled during the slaughter procedure. A total of 101 pigs were sampled. For each pig, samples of the: ileum (25g), ileocolic lymph nodes (25g), mandibular lymph nodes (10g) and tonsils (10g) were collected. In the corresponding half carcass an internal surface swab was performed, with a cotton sterilised gauze (hydrated in 25 ml of Buffered Peptone Water with 0.1% Tween).

At the laboratory all samples, except the carcass swab, were submerged in boiling water for 10 seconds to decontaminate the surface. Afterwards culture method was performed according to ISO 6579:2002 typical and suspicious *Salmonella* isolates were serotyped according to the Kauffmann-White scheme in the Laboratório Nacional de Investigação Veterinária (Lisbon, Portugal), the Portuguese Reference Laboratory for *Salmonella*.

Two main groups of samples were considered: the pig samples (ileum, ileocolic lymph nodes, tonsils and mandibular lymph nodes), which reflects the pig status as an asymptomatic carrier of *Salmonella* and the carcass, where the presence of *Salmonella* can be associated to pig infection and/or to carcass contamination. To perform RFLP-PFGE genotyping BHI broth was inoculated with several typical colonies of *Salmonella* and incubated overnight at 37°C. Cells immobilised in agarose plugs were obtained from a bacterial suspension with $5x10^9$ cells/ml, according to the method previously described by Smith *et al.*, (1988). DNA fragments were separated in 1% agarose in 0.5X TBE (50 mM Tris, pH 8; 50 mM boric acid; 0.02 mM EDTA). Pulsed-field gel electrophoresis of the DNA fragments was performed on the Gene Navigator system, with 2,5 L of 0.5X TBE buffer for 22 hours at 200 V and 14°C, with a linear increase in switching time from 10.3 to 64.0 seconds, as described before by Bender *et al.*, (2001). After PFGE, the gel was stained with ethidium bromide (0.2 μg/ml), photographed under UV transilumination and the image digitalisation was processed with KODAK 1D 2.0 software. Macrorestriction profiles (MRPs) were analysed visually and each band position was carefully determined. Profiles of different strains were compared over several gels to ensure the reliability of the band matching process. The percentage between positive matched samples was estimated, as well as the association between two different positive samples using the Fisher-exact test.

Results and Discussion

Internal surface contamination with *Salmonella* spp. was detected on 13 (12.9%) carcasses. Table 1 summarises the information related to the positive carcasses: date of sample collection, pig identification, *Salmonella* serotype and genotype identified in the positive carcasses and in the positive pig samples related to carcass contamination.

Table 1: Positive carcass information: date of sample collection, pig identification, serotype and genotype identification.

Date of collection	Pig	*Salmonella* serotype	*Salmonella* genotype	
			Carcass	Pig samples
11/06/2003	22	Rissen	R_1	
	24	Rissen		R_1
2/07/2003	40	Typhimurium	Ty_1	Ty_1
	41	Typhimurium	Ty_1	Ty_1
	42	Typhimurium		Ty_1
	43	Typhimurium	Ty_1	Ty_1
	44	Typhimurium	Ty_1	Ty_1
	45	Typhimurium	Ty_1	Ty_1
	46	Typhimurium		Ty_1
	47	Typhimurium	Ty_1	Ty_1
12/07/2004	75	Rissen	R_2	
	76	Rissen	R_1	
	77	Rissen	R_3	R_1
19/07/2004	81	Rissen		R_1
	83	Rissen	R_1	R_1
13/09/2004	94	Rissen	R_1	
	95	Rissen	R_1	R_1

Among the positive carcasses, 8 (69%) belonged to *Salmonella*-positive pigs where the same genotype was identified in, at least, one corresponding pig sample, suggesting that direct contamination or internal infection had occurred (highly significant association; Fi = 0,356; $p < 0.001$). *Salmonella* spp. isolated in the remaining 5 (31%) positive carcasses was related to cross-contamination processes. From these, 3 presented the same genotype as the one identified in pig samples from animals slaughtered in the same day. These results emphasise the importance of the slaughter of *Salmonella*-infected pigs as an important risk factor for carcass contamination and, consequently, to the introduction of this foodborne pathogen into the human food chain.

In addition, it was observed that R_1 genotype was identified in several *Salmonella* Rissen isolated at different dates along the study period from pigs that belonged to different farms and, in some cases, that were transported in different vehicles (data not shown). For these reasons, it was hypothesised to consider it a resident strain in the slaughterhouse lairage, the only possible common source of *Salmonella* contamination for the pigs. With respect to the other MRPs (R_2 and R_3) identified in two *Salmonella* Rissen isolates, it was considered that they could represent clonal descendants from the resident strain (R_1) since these isolates were identified one year after the identification of R_1 and a high similarity among them was observed (data not shown). The lairage was identified as an important source of pig infection, that could occur 2-3 hours prior to slaughter, therefore more attention should be paid to the hygienic management of the lairage area and encourage the implementation of corrective measures.

Conclusions

The study indicates the usefulness of RFLP-PFGE to define, with more accuracy the possible dynamic routes of carcass contamination with *Salmonella* spp. and its relationship to the slaughter of positive pigs, that, according to the results, may be related to the pigs infection in lairage prior to slaughter. This study indicates that corrective measures could be more efficiently implemented in order to reduce pig infection and consequently carcass contamination.

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