TRACING OF *SALMONELLA* SPP. IN PORK SLAUGHTERING AND CUTTING PLANTS USING SEROTYPING AND MACRORESTRICTION GENOTYPING.

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Background

Non-typhoid *Salmonella* spp. are documented as the leading cause of foodborne bacterial disease and zoonosis in developed countries (D'Aoust, 1994). Among a variety of foodstuff, raw and manufactured pork meat products can be the vehicles of human salmonellosis (Desenclos *et al.*, 1996; Berends *et al.*, 1998).

Effective epidemiological surveillance and control of *Salmonella* spp. require accurate subtyping of strains in order to determine potential pathways of infection. Powerful subtyping molecular techniques are available to assess the distribution of *Salmonella* strains within food processing environments and to define bacterial clonal relationships (On and Baggesen, 1997).

Objectives

The purpose of this study was to further the knowledge concerning the origin of *Salmonella* spp. present on pork cuts by tracing this foodborne pathogen in pork slaughtering and cutting plants using molecular typing methods (Giovannacci *et al.*, 1999).

Materials and methods

Samples were collected from two slaughtering and cutting plants (S1 and S2) on seven different sampling times. Sampling was performed both on pork (pigs, carcasses at different stages and cuts) and on environmental surfaces in slaughtering, chilling, and cutting rooms. Most samples were collected with the cheesecloth swabbing technique, which allows to investigate very large surfaces, either on pork or in the environment. The *Salmonella* detection method was based on the AFNOR NF V08-052 technique (Anonymous, 1997), slightly modified. Up to nine isolates per positive sample were preserved for further typing. Serotying was applied to every isolate according to the Kauffmann-White scheme. Isolates belonging to the most predominant serotypes, *i.e.* Typhimurium and Derby, were characterised by macrorestriction with both *Spe*I and *Xba*I and pulsed field gel electrophoresis (CHEF DRIII, Bio-Rad, USA). Clonal relationships were further determined by numerical analysis of the combined macrorestriction profiles (Molecular Analyst, Bio-Rad).

Results and discussion

Salmonella spp. were isolated both from pork (pigs, carcasses, cuts) and from the environment along the slaughtering and cutting lines. Serotyping allowed to identify eight serotypes, predominantly Typhimurium and Derby.

Subsequent molecular typing, *i.e. Xba*I and *Spe*I macrorestriction, identified 20 genotypes of *Salmonella* Typhimurium (t1 to t20) (Figure 1) and 16 genotypes of *Salmonella* Derby isolates (d1 to d16) (Figure 2). A major cluster (II) grouped all the *Salmonella* Typhimurium genotypes common to both plants and all pig-related genotypes (*i.e.* isolated from mesenteric nodes) (Figure 1). Moreover, a predominant pig-related *Salmonella* Derby genotype (d1) was common to both plants (Figure 2).

Genotyping allowed to trace *Salmonella* contamination in plants S1 and S2 and to show its evolution from time to time in these plants. *Salmonella* pork cuts contamination was always closely linked to the slaughterhouse contamination, itself associated with the *Salmonella* genotypes brought by live pigs. A turnover of the contamination by *Salmonella* was observed in both plants. Indeed, none of the identified *Salmonella* strains persisted for long periods in the investigated pork processing environments.

Conclusions

This study showed that macrorestriction has practical applications for short term epidemiological studies and has a potential to delineate clonal lines for *Salmonella* Typhimurium and *Salmonella* Derby, as also stated by On and Baggesen (1997).

The contamination of live pigs, amplified through bacterial spread due to the slaughtering process, is the major identified cause of *Salmonella* contamination of pork cuts, which supports the findings of Berends *et al.* (1998).

References

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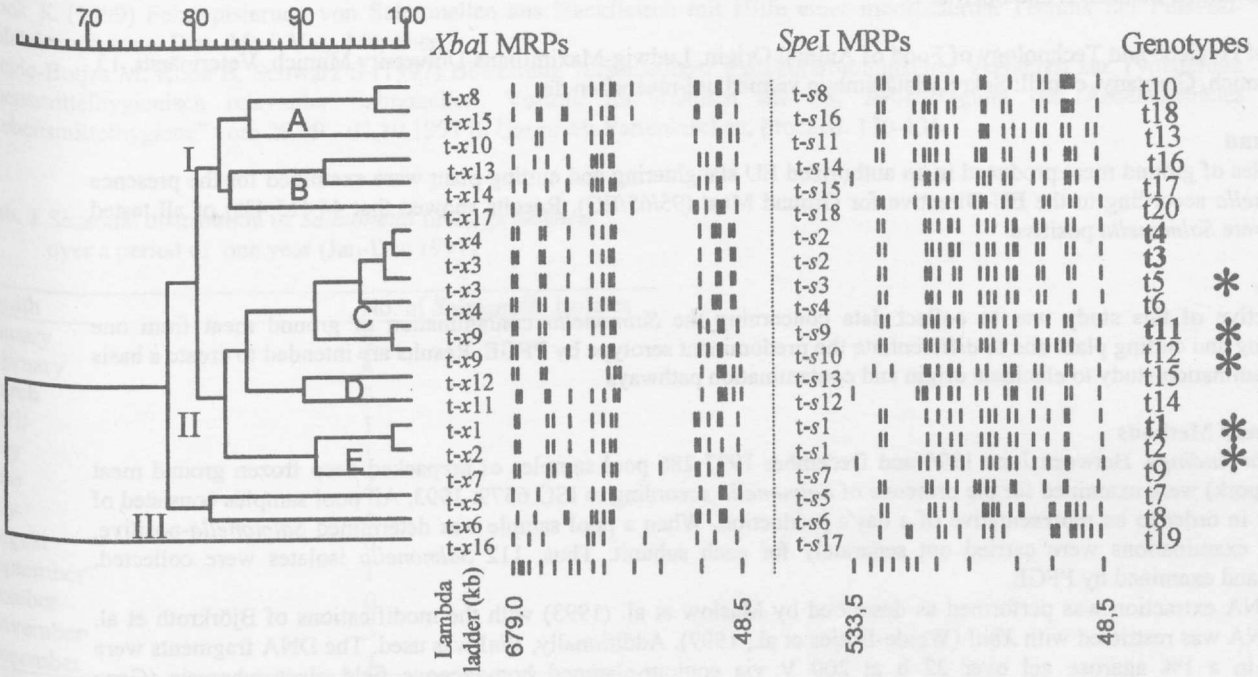
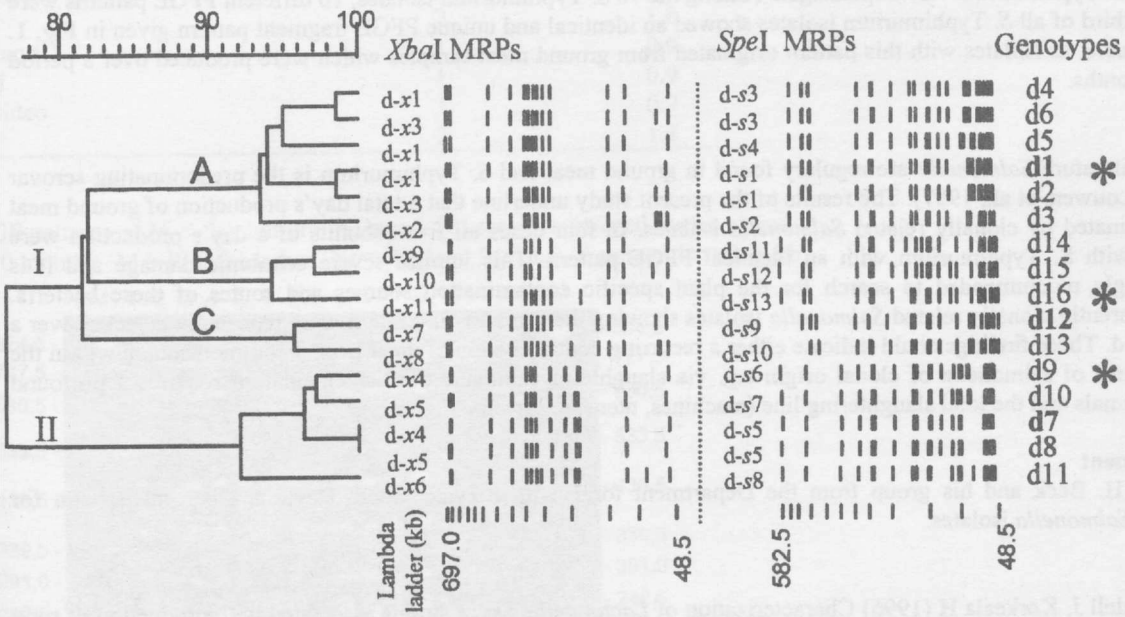
Figure 1. Schematic representation of 18 the *Spe*I and 17 *Xba*I macrorestriction profiles (MRPs) obtained from the *Salmonella* Typhimurium isolates leading to the identification of 20 genotypes (t1 to t20) and dendrogram of the cluster analysis.



The numbers on the horizontal axis indicate the percentage of similarity as determined by the Dice's coefficient and UPGMA clustering. Major clusters were formed at the 80 % similarity level.

Bold-faced genotypes (t4, t3, t11 and t1) were identified both in S1 and S2. The * symbol indicates pig related genotypes, *i.e.* isolated either from pig skin or from mesenteric nodes (t5, t11, t12, t1 and t2).

Figure 2. Schematic representation of 13 the *Spe*I and 11 *Xba*I macrorestriction profiles obtained from the *Salmonella* Derby isolates leading to the identification of 16 genotypes (d1 to d16) and dendrogram of the cluster analysis.



The numbers on the horizontal axis indicate the percentage of similaries as determined by the Dice coefficient and UPGMA clustering. Major clusters were formed at the 80 % similarity level.

Bold-faced genotypes (d1, d16, d9) were identified both in S1 and S2.The * symbol indicates pig related genotypes, *i.e.* isolated either from pig skin or from mesenteric nodes (d1, d16 and d9).