STUDY ON *SALMONELLA* CONTAMINATION IN PORK SAUSAGES CHAIN BY PFGE ANALYSIS *

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SUMMARY: *The presence of* Salmonella *spp. in food may result from accidental contamination at any step in the food-production chain. This study was carried out in order to evaluate the relationship between* Salmonella *strains isolated in pig farms, slaughterhouses and sausage making plants. From April 2005 until November 2005, 22 strains of* Salmonella *spp. were isolated and subtyped by PFGE to evaluate their genetic relationship and to determine the phases in the production chain where contamination occurred.*

Our study with this approach made it possible to detect where in the food production chain Salmonella *contamination of two sausage samples occurred.*

Keywords: *Salmonella*, PFGE, sausages.

Résumé *: La présence de* Salmonella *dans les aliments peut être due à la contamination accidentelle qui peut se produire pendant plusieurs phases de la production des aliments. Cette étude se propose d'évaluer l'association entre les* Salmonella *détectées dans les élevages de porcs, les abattoirs et les entreprises fabriquant des saucisses. Pendant la période avril 2005 - novembre 2006, 22* Salmonella *isolées d'échantillons de saucisses ont été examinées par PFGE pour étudier une éventuelle corrélation génétique et pour identifier le point de la filière où la contamination s'est produite.*

Mots-clés : *Salmonella*, PFGE, saucisses.

I - INTRODUCTION

Foodborne diseases represent a problem for public health because of their sanitary and economic consequences. The possible solution is more attention in every point of the food chain (from farm to fork).

Salmonella is one of the pathogens most frequently involved in foodborne diseases. Recently, in the EU, the number of salmonellosis cases has increased to 200 000 per year and evaluations of their social cost vary from 2 to 10 billions euros a year. In order to prevent and control the sources of infection and disease, the EU has issued Directive 2003/99/CE (November 17^{t}, 2003) on the monitoring of zoonoses.

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* Texte de la communication affichée présentée lors des Journées de l'AEEMA, 31 mai-1er juin 2007

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Furthermore, Regulation 2160/2003 specifies which animals have to be included in the sampling programs for control purposes. Finishing pigs are among those animals.

Concerning Italy, according to data from the National Surveillance System for Entero-Pathogens [EnterVet, 2005], the most frequently isolated strains were *S.* Typhimurium (17.5%), *S.* Livingstone (9%) e *S*. Derby (8.5%). In the Piedmont Region, where this study was carried out, in 2004-2005, the serotypes most frequently isolated in pork products were *S*. Derby and *S*. Typhimurium followed by other serogroups (See table 1).

In the years 2001-2002, the serogroups most frequently isolated in human samples were *S.* Typhimurium (46.8%), *S.* Enteritidis (26.6%), *S.* Infantis (5%) and S. Derby (2%) [Galetta *et al*., 2003].

However, since 2001 in Europe the serotype most commonly found was *S*. Enteritidis. Eggs and egg products are often regarded as responsible for human infections (ISTISAN 05/27). This somewhat unique situation in Italy is probably due to the presence of various sources of human Salmonellosis.

In Piedmont, in the period July 2003 to June 2004 the *Salmonella serogroups* most frequently isolated in human samples were S. Typhimurium (44.4%), *S*. Enteritidis (29.5%) followed by *S*. Derby (4.2%) [Crespi *et al*., 2004].

Table 1

Isolation of *Salmonella* serogroups in the Piedmont Region (2004-2005)

Serogroups	Year 2004	Year 2005	Year 2006
S. DERBY	3	8	4
S. TYPHIMURIUM	3	7	1
S. RISSEN	-	2	-
S. n. d. 4,5,12: i -	-	1	2
S. ANATUM	1	1	-
S. BREDENEY	-	1	-
S. COLINDALE	-	1	-
S. ENTERICA subsp. HOUTENAE	-	1	-
S. ENTERICA subsp. SALAMAE ;	-	1	-
S. INDIANA	-	1	-
S. INFANTIS	1	1	-
S. LONDON	-	1	-
S. BERTA	1	-	-
S. BREZANY	1	-	-
S. GOLDCOAST	1	-	-
S. MASSENYA	1	-	-
S. TOMPSON	-	-	1
S. LIVINGSTONE	-	-	1
TOTAL	12	26	9

Swine are considered a significant source of contamination for *Salmonella* spp. [Pearce et Revitt, 1998]. Pork products are implicated in human salmonellosis [Wall *et al*. 1994]. In the USA many studies demonstrated that the contamination of pork products is about 4,4% in fresh, refrigerated and processed meat [Helms *et al*., 2003]. *Salmonella* spp. in swine, is present in tonsils (15%), on carcass surface (8%), in the liver and diaphragm (17%) [Christnsen and Luthje, 1994].

This study was designed to investigate the *Salmonella* contamination in pork products, and to compare strains isolated from meat with strains isolated from animal samples. Among pork products, sausages were chosen for two reasons: i) since they are made with minced meat, they are subject to contamination during preparation; ii) since in Italy they are usually eaten raw or under-cooked, they are a frequent source of human *Salmonella* infections and are exposed to cross contamination during meal preparation. According to the Regional Food Surveillance Office (See table 2) sausages are found to have a high rate of positive results for *Salmonella* spp.

Table 2

Results of Regional Official Food Surveillance (2004-2006)

Year	N° samples	N° positive	% positive	C.I.
2002	170	12	7.06	5.6-8.8
2003	118	4	3.39	2.1-5.3
2004	118	3	2.54	1.5-4.3
2005	91	5	5.49	3.7-8.1
2006	61	3	4.92	2.70-7.85

II - MATERIALS AND METHODS

1. SAMPLING SCHEME

Samples were collected from processing plants in Northwestern Italy (Piedmont). Plants were subdivided in four classes and given a score based on the following criteria :

- sausage production carried out according to Directive 92/5/CEE or Directive 94/65/CE;
- total production capacity of the plant (q/month);
- yearly production of sausages in the plant.

A total of 35 plants were included in the sample collection.

The number of samples (204) was calculated according to 10% of prevalence of positive sausages and a C.I. of 95%.

During the sample collection period, the number of samples was reduced because the production of sausages slowed down in some processing plants (See table 3).

The sausage samples were collected by the official Veterinary Services in production plants. The samples (150 g) were kept at 4°C, sent to the laboratory of Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle D'Aosta and analyzed within 24 – 48 hours.

Table 3

Sampling plan

Q/ /month	Plants	Selected plants	Samples to be collected	Collected samples
0-5	11	10	33	20
6-10	8	6	40	28
11-100	13	8	104	63
>100	3	1	27	9
Total	35	25	204	120

2. METHODS

The samples were analyzed with mini-VIDAS® *Salmonella*. This method uses monoclonal antibodies directed against somatic antigens "O" and flagellar antigens "H". This *Salmonella* detection method is validated by the Association Française de Normalisation (AFNOR BIO 12/10-09/02). Each sample (25 g) was weighed in a sterile bag, 225 ml of BPW were added and the mixture was homogenized with stomacher for 2 minutes. This pre-enrichment culture was incubated for 16-20h at 37°C. After incubation, 100 µL were transferred into 10 mL of Rappaport Vassiliadis Broth and incubated for 6-8 hours at 41°C. Then, 1 mL of this broth was transferred into 10 mL of M Broth and incubated for 16 h at 41°C. At the end of this incubation period, a VIDAS® strip was inoculated with 100 µl of this boiled (100°C for 15 minutes) M broth.

The VIDAS® method gives results within 45 minutes.

In case of positive result with VIDAS®, confirmation tests were carried out with microbiological method according to AFNOR V08-052 (1997).

All strains of *Salmonella* were serotyped according to the Kauffmann-White scheme 2001 using both monoclonal and polyclonal serum.

Isolated strains were genotyped using pulsed-field gel electrophoresis (PFGE). Genomic DNA of the *Salmonella* isolates was prepared in agarose plugs. DNA was digested with Xbal (Boehringer-Mannheim, Germany) using CHEF-DR III (BioRad) according to the protocol of Hunter *et al*. [2005].

The images of strains were analyzed with software BioNumerics (Applied Maths).

Similarities between profiles were calculated using Dice's coefficient and Dendrograms were constructed using Colplete Linkage algorithm (1.6% position tolerance).

III - RESULTS

1. *SALMONELLA* PREVALENCE IN SAUSAGES

A total of 120 sausage samples were analyzed for *Salmonella*. 22 samples were found to be positive for *Salmonella* spp. (prevalence 18.3%). Their isolation and the determination of their serogroup were carried out.

One sample was found to be positive with the VIDAS® test but viable *Salmonella* cells could not be isolated This particular sample was regarded as negative in our study since no iisolation was achieved. Tables 4 and 5 present the most frequently found serogroups in the strains isolated from pork and pork products

Table 4

Microbiological results

Class of plants	N° of samples	Positive samples	Confirmed positive samples
a	20	5	5
b	28	6	6
c	63	10	9
d	9	2	2
total	120	23	22

Table 5

Distribution of serogroups identified in sausage samples

Serogroup	N° isolation	%	Cumulative isolation	Cumulative %
***S.* Derby**	9	40.91	9	40.91
***S.* Typhimurium**	6	27.27	15	68.18
***S.* London**	2	9.09	17	77.27
***S.* Anatum**	1	4.55	18	81.82
***S.* Livingstone**	1	4.55	19	86.36
***S.* Tinda**	1	4.55	20	90.91
***S.* Enteritidis**	1	4.55	21	95.45
***S.* n. d. 4,5,12:i -**	1	4.55	22	100.00

2. PFGE STRAINS COMPARISON

Strains isolated from pork sausages were analyzed by pulsed field gel electrophoresis and compared with restriction profiles of strains, previously isolated from pig herds in the Piedmont areas active in sausage production.

In two cases, a striking similarity between strains was found so that one could reasonably assume that contamination occurred in the sausage processing chain.

In the first case, two *S*. Derby strains presented 100% similarity (samples IZS 19 and IZS 20 in figure 1). The strains were isolated from two sausage samples collected in two different sausage plants (SP1 and SP2). The sausage plants obtained meat for sausage production from two distinct but unrelated herds (H1 and H2). Finishing pigs from the first herd were slaughtered in the same abattoir (A1) as the other pigs (H2) only few days earlier.

In this case, the slaughter operation appeared as the likely source of contamination. Presumably, two batches of meat were infected with the same strains of pathogens present in the slaughterhouse.

The relationship between the sausage plant, the herds and the slaughterhouse in this first case is summarized in table 6.

Figure 1

Genetic relationship among isolated strains

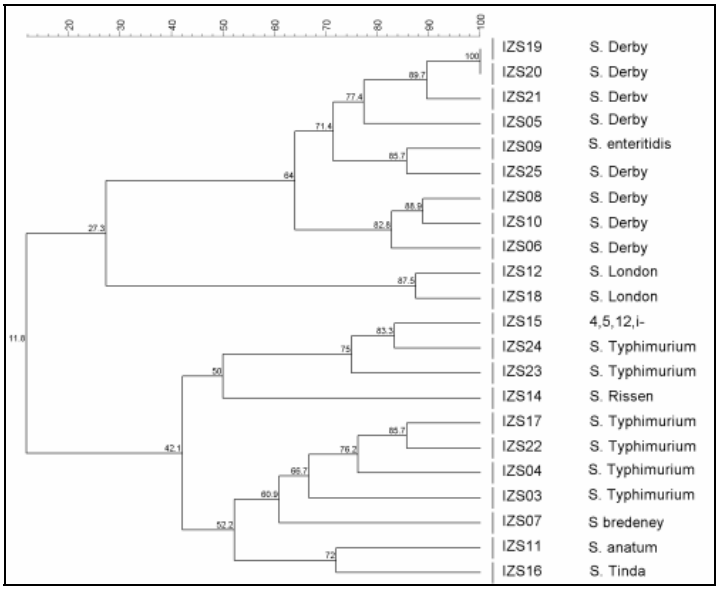

Table 6

Relationship in the first case of contamination

Herds	Abattoirs	Sausage plants
H1	A1	SP1
H2		SP2

The second case features two strains of *S*. Derby that showed 89% similarity (samples IZS 8 and IZS 10 in figure 1). The strains were isolated from two sausage samples collected in distinct sausage plants (SP3 and SP4) that obtained meat from pork slaughtered respectively in abattoirs A1 and A2. Interestingly, both these abattoirs bought and slaughtered animals bred in the same farm (H3), which thus appears as the likely source of contamination.

The relationship between the sausage plant, herds and abattoir in the second case is summarized in table 7.

Table 7

Relationship in the second case of contamination

Herds	Abattoirs	Sausage Plants
H3	A1	SP3
	A2	SP4

IV - CONCLUSION

The results obtained in this study on the prevalence of *Salmonella* spp. confirm that pork sausages can be an important source of *Salmonella* and represent a definite risk for the consumers when eaten raw or under-cooked.

The distribution of *Salmonella* serogroups in our findings reflects the Italian and European distribution of major serogroups in pigs. In our study *S*. Derby was found to be the most frequently isolated strain. S. Derby was often reported to be associated with swine and pork products [Chau PY, 1997; Valdezate *et al*., 2005]. It appears less important, however, as a cause of human salmonellosis [Giovannacci *et al*., 2001; Chen *et al*., 1999]. Its potential pathogenicity must be considered in relation to the presence of strains of *S.* Derby resistant to the most common antimicrobial agents [Ling *et al*., 2001; Michael *et al*., 2006].

The PFGE analysis was useful to highlight relationships among strains of *Salmonella* isolated from different plants, abattoirs and herds. In fact, combining typing methods such as PFGE and information from food chains, it was possible to identify related strains and common source of contamination. This type of approach may be useful in order to improve *Salmonella* spp. surveillance systems. In particular, it was possible to formulate the hypothesis that meat from the same slaughterhouses and/or farm could be the source of food supply chain contamination. This approach might be routinely applied to other food chains in order to identify critical control points and to improve corrective measures.

The tracing system in pork processing plants iwas confirmed to be a very useful and efficient tool to implement *Salmonella* spp. surveillance.

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