

A FIELD STUDY OF *SALMONELLA ENTERICA* CONTAMINATION OF PIG SLURRY IN FRANCE

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Introduction

Salmonellosis is one of the most important and most-frequently reported human foodborne diseases worldwide (1). Salmonellosis outbreaks have been associated with the consumption of pork and pork products (7, 5). Contamination of pork products is related to asymptomatic intestinal carriage of *Salmonella* by living pigs arriving at the slaughterhouse (2). In order to reduce the occurrence of *Salmonella* in pork, a decrease of *Salmonella* carriage at the farm level is needed. On the other hand, the spread of *Salmonella* contaminated slurry on fields and crops may constitute a threat for environmental preservation. Therefore, efforts undertaken at the farm level to reduce *Salmonella* shedding contribute to increase both human food safety and environmental safety. Few data are available regarding the contamination level of finishing pigs and their slurry in France. The aim of our study was to identify *Salmonella* contaminated finishing pig batches and to assess the level of contamination of their slurry.

Material and methods

A study was carried out from April 2003 to November 2004. 61 batches of finishing pigs from 50 farrow-to-finish French farms were involved in the survey. The farms were selected on voluntary basis. *Salmonella* shedding was assessed on the one hand with an environmental sampling procedure: sterile pairs of gauze socks (Sodibox, La Forêt Fouesnant, France) were used to wipe faecal material on the slatted floor of each pen housing the batch of interest. On the other hand, in each pen a pool of faeces collected on the floor was prepared and placed into sterile bags. In addition 4 litres of slurry stored in the pit below the followed pigs were collected in sterile bottles. Environmental swabs, 25 g of the homogenized pools of faeces and 25 mL of mixed slurry were analysed for the presence of *Salmonella enterica* in a four steps protocol. Following a pre-enrichment step (20 hours at 37°C in buffered peptone water), two selective media were used: Müller-Kauffman Tetrathionate Broth (MKTB) and Modified Semi-Solid Rappaport Vassiliadis agar (MSRV), incubated respectively 24 hours at

42°C and 48 hours at 41.5°C. The migrated plates of MSRV were isolated on Rambach agar plates and each MKTB on Xylose-Lysine-Tergitol4 (XLT4) agar plates. Both media were incubated 24 hours at 37°C. The presumptive colonies (one per selective media) were biochemically confirmed on Kligler-Hajna medium (AES Laboratoires, Combourg, France). All isolates were serotyped by agglutination following the Kauffman-White scheme (6). A quantification of *Salmonella* was additionally used according to the most probable number method to better describe positive pools of faecal material and positive samples of slurry (3).

Results

At least one environmental sample tested positive in 17 batches (27.9 % of tested batches) *i.e.* 12 out of 50 farms. In 10 batches (16.4 %), *Salmonella* shedding was detected in pooled faeces. *Salmonella* quantification was possible in 6 of these batches with levels ranging from 2.4 to 350 *Salmonella*/gram. In 8 batches (13.1 %), *Salmonella* was identified in slurry samples. Quantification was achieved in 2 samples of slurry and we found respectively 1.6 and 110 *Salmonella*/mL. Quantification in pooled faeces or in slurry could be observed when at least 40 % of environmental swabs tested *Salmonella* positive. *Salmonella* Typhimurium and *Salmonella* Derby were the most common serotypes isolated. Results of positive samples are presented Table 1.

Table 1: Description of *Salmonella* serotypes isolated in swabs, pooled faeces and slurry samples and *Salmonella* quantification in pooled faeces and slurry (19 positive batches, April 2003 - November 2004)

Farm	Batch	Swabs	Pooled faeces	Slurry samples		
		% Positive - <i>Salmonella</i> serotype	mpn* (<i>S.</i> /gram and CI at 95 %)	<i>Salmonella</i> serotype	mpn (<i>S.</i> /mL and CI at 95 %)	<i>Salmonella</i> serotype
A	1	100 - S.T**	2.4 (0.66 - 8.5)	S.T	-	S.T
A	2	12.5 - S.T	-	-	-	S.T
A	3	75.0 - S.T	8.3 (2.7 - 25)	S.T	-	S.T
B	4	50.0 - S.T	350 (94 - 1300)	S.T	-	S.T
B	5	40.0 - S.T	-	S.T	-	-
B	6	12.5 - S.T	-	-	-	-
C	7	25 - S.Bredeney	-	S.Bredeney	-	-
D	8	41.7 - S. Derby	350 (94 - 1300)	S. Derby	1.6 (0.38 - 6.9)	S. Derby
D	9	8.3 - S. Derby	-	S. Derby	-	-
D	10	0.0 - .	-	S. Derby	-	-
D	11	16.7 - S. Derby	-	-	-	-
E	12	12.5 - S. Derby	-	-	-	-
F	13	8.3 - S.T	-	-	-	-
G	14	0.0 - .	-	-	-	S. Derby
H	15	8.3 - S. Derby	-	-	-	-
I	16	16.7 - S. T	-	-	-	-
J	17	37.5 - S.T	-	-	-	-
K	18	75 - S.T	7.6 (2.5 - 23)	S.T	-	S.T
L	19	83.3 - S. Derby	350 (94 - 1300)	S. Derby	110 (35 - 360)	S. Derby

* mpn : most probable number ; ** S.T : *S.* Typhimurium

Discussion - Conclusion

Our study indicates that pig slurry may be contaminated by *Salmonella enterica*. However, the percentage of positive samples was rather low. These results suggest that *Salmonella* could only be detected in slurry stored in the pit under the slatted floor of moderately or highly shedding batches of pigs. Since storage without introduction of new fresh slurry is known to reduce *Salmonella* survival (4), the probability of spreading the bacteria in the environment is expected to be low as far as adequate storage is applied.

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References

1. Baird Parker, A. C., 1990. Foodborne salmonellosis. *The Lancet*. 336, 1231-1235.
2. Borch E. et al., 1996. *Int. J. of Food Microbiol.* 30:9-25.
3. Fravalo P. et al., 2003. *J. of Rap. Meth. and Autom. in Micro.* 11, 81-88.
4. Guan, T.Y. and Holley, R.A., 2003. *J. Environ. Qual.* 32:383-392.
5. Hald T. et al., 1999. *3rd International Symposium of the Epidemiology and Control of Salmonella in Pork, Washington DC, USA.* 197-199.
6. Popoff M. Y. and Le Minor L., 1992. *Antigenic formulas of the Salmonella serovars.* Paris, Institut Pasteur. 8p.
7. Van Pelt, W. and Valkenburgh, S.M. (Eds), 2001. *Zoonoses and zoonotic agents in humans, food, animals and feed in the Netherlands.* Available: www.keuringsdienstvanwaren.nl.