

# Prevalence of *Salmonella* serotypes on pig carcasses from high- and low-risk herds slaughtered in three abattoirs

## Prävalenz von *Salmonella*-Serotypen in drei Schlachthöfen bei Schlachtkörpern von Schweinen aus Hochrisiko- und Niedrigrisiko-Herden

Quirke, A-M.<sup>1</sup>, Leonard, N.<sup>1</sup>, Kelly, G.<sup>2</sup>, Egan, J.<sup>3</sup>, Lynch, P.B.<sup>4</sup>, Rowe, T.<sup>1</sup>, Quinn, P.J.<sup>1</sup>

<sup>1</sup>Faculty of Veterinary Medicine, UCD, Dublin 4, Ireland, email: nola.leonard@ucd.ie <sup>2</sup>Department of Statistics, UCD, Dublin 4, Ireland, <sup>3</sup>Central Veterinary Research Laboratory, Abbotstown, Dublin 15, Ireland, <sup>4</sup>Teagasc, Moorepark, Fermoy, Co Cork, Ireland

### Summary:

The aim of this study was to compare the prevalence of *Salmonella* serotypes at two different sites on pig carcasses from herds classified as high-risk or low-risk and to elucidate the relationship between carcass contamination levels and serological status. Caecal samples and carcass surface swabs were cultured for *Salmonella* from a total of 210 pigs from low risk herds (< 19 % of pigs in herd *Salmonella* seropositive) and 209 pigs from high risk herds (> 32 % of pigs in herd *Salmonella* seropositive) in three abattoirs. Meat juice samples were collected for analysis by ELISA. The prevalence of *Salmonella* in the caecal contents of 'low-risk' pigs was 10 %, which was significantly lower than the 19% prevalence in 'high-risk' pigs ( $p < 0.01$ ). The corresponding figures for skin samples collected immediately post-evisceration were 2 % and 12 %. The predominant *Salmonella* serotype in the caecal contents of both the low-risk and high-risk pigs was *Salmonella* Typhimurium. *Salmonella* Kentucky and *Salmonella* Derby were the most frequent isolates from the carcass surface swabs of low- and high-risk pigs respectively. There was a positive association between seropositivity of pigs from high-risk herds and caecal carriage ( $p < 0.05$ ). Results showed that herd categorisation based on serological results was useful in predicting *Salmonella* isolation rates from caecal samples and surface swabs of slaughtered pigs.

**Keywords:** Control programme, serology, bacteriology, caecum, skin

## Introduction

A voluntary control programme for *Salmonella* in pork has been in operation in Ireland for approximately 3 years and is due to be put on a statutory basis shortly. Classification according to level of *Salmonella* seropositivity, of herds supplying the major export abattoirs has been carried out since the end of 1997. The Danish mix-ELISA (Nielsen *et al.*, 1995) is the test on which classification is based but no published data is available on the usefulness of this test for monitoring the *Salmonella* status of Irish pigs. In order to implement an effective control programme, data is required as to whether such serological monitoring accurately reflects the *Salmonella* isolation rates of pigs from both highly infected and low prevalence herds. Thus, the objectives of the present study were 1) to compare the bacteriological prevalence of *Salmonella* serotypes at different sites on carcasses from herds classified as high-risk or low-risk and 2) to elucidate the relationship between carcass contamination levels and serological status.

## Materials and methods

### Sampling procedure

Irish pig herds are categorised at slaughter into three levels of *Salmonella* infection, based on ELISA testing of randomly selected meat juice samples: Category 1, < 10% samples positive; Category 2, 10-50 % positive; Category 3, > 50 % positive. A comparison of Category 1 versus Category 3 pigs would have been ideal in order to fulfil the objectives of the study and at the same time, relate the results directly to the national control programme. Unfortunately, this comparison was not possible as the number of Category 3 herds supplying the three abattoirs which agreed to participate in this study was small. Statistical calculations carried out prior to the study suggested that more than 200 Category 3 pigs would be required for a valid comparison. Therefore, the two groups compared were: low-risk herds (Category 1 and low Category 2, i.e. rolling average < 19 % of pigs in herd *Salmonella* seropositive) and high-risk herds (Category 3 and high Category 2, i.e. rolling average > 32 % of pigs in herd *Salmonella* seropositive). There were 27 low-risk herds and 21 high-risk herds. Table 1 outlines the group, category and numbers of pigs sampled in each of the three abattoirs. Samples were collected over a 10 month period.

The following samples were collected: caecal contents, a sample of diaphragmatic muscle and carcass skin swabs. The carcass skin was swabbed immediately after evisceration, after the final wash and again after approximately 18 hours in the chill. Carcass swabs were taken from a 0.1 m<sup>2</sup> area running 10 cm up and across from the midline, starting at the level of the elbow. This was followed by sampling of the jowl area. Sterile moppet sponges (Parkside Hygiene, Dublin) moistened with buffered peptone water (BPW) (LM; Lab 46) were used as swabs. All samples

were kept at ambient temperature until return to the laboratory on the same day when culture was begun. Occasionally, culture was delayed until the following day.

### **Laboratory methods**

A 1 g sub-sample of caecal contents and the entire carcass swab were pre-enriched in BPW and incubated for 16 h - 24 h at 37 °C. This was followed by selective enrichment in both Rappaport Vassiliadis broth (LM; Lab 86), and semi-solid Rappaport Vassiliadis medium (LM; Lab 150) at 41.5 °C for 18 h - 24 h and then subculture onto brilliant green agar (LM; VT44111) and mannitol lysine crystal violet brilliant green (LM; Lab 116) at 37 °C for 20 h - 24 h. Five suspect *Salmonella* colonies from each plate were inoculated onto MacConkey agar (LM; Lab 2) and incubated at 37 °C for 18 h - 24 h. The identity of suspect colonies was confirmed by inoculation of triple sugar iron agar slopes (LM; Lab 53) and lysine decarboxylase broths, followed by serotyping.

Muscle samples were placed in specific containers (Christensen ApS, Denmark) and frozen at -20 °C until analysis. The fluid collected after thawing was sent to a government-approved commercial laboratory, for ELISA testing (Nielsen *et al.*, 1995). The positive-negative cut-off used was OD % = 40.

### **Statistical analysis**

Statistical analysis was carried out using the GLIMMIX macro from SAS (SAS Institute Inc., 1996). A generalised linear mixed model i.e. logistic regression with herd and factory as the random effects was used to calculate the proportions of sites positive for *Salmonella* and the proportions of different serotypes isolated from the positive sites, together with 95 % confidence intervals. The outcome variable was the result of the individual sites of each pig. The independent variable was the risk group of the pig (high or low). This model was also used to determine the relationship between the bacteriological and serological results and the relationship between the frequency of *Salmonella* in the caecal contents and on the skin of the carcasses.

## **Results**

### **Descriptive analysis**

*Salmonella* serotypes were isolated from the caecum of pigs in 24 of 48 herds tested. The proportion of high-risk herds containing pigs from which salmonellae was isolated was higher (14 of 21 herds) than in the low-risk herds in which 10 of 27 contained *Salmonella* positive pigs.

The prevalence of *Salmonella* serotypes at the various sites sampled on pigs from low- and high-risk herds is shown in Table 2. The predominant serotype isolated from the caecal contents of 'low'- and 'high-risk' pigs was *Salmonella* Typhimurium, 5 % and 9 % of samples respectively, followed by *Salmonella*

Derby, 3 % and 9 % respectively. The prevalence of salmonellae on the skin of pigs from both high- and low-risk groups by the time of final sampling after 18 hours in the chill, was low (< 0.5 %), as can be seen in Table 2. Serotypes isolated from the skin of pigs included Typhimurium, Derby, Kentucky, Bredeney, Havana, Livingstone and London. *Salmonella* Kentucky was the serotype most frequently isolated from the skin swabs of pigs from low-risk herds (5 % of post-evisceration samples) while *Salmonella* Derby predominated in skin swabs of pigs from high-risk herds (7 % of post-evisceration samples). *Salmonella* Typhimurium and *Salmonella* Livingstone were isolated from 4 % and 5 % respectively of post-evisceration carcass samples of pigs from high-risk herds.

Although numbers were too small to allow statistical analysis, there was no apparent correlation between the serotypes isolated from the caecal contents and the skin of individual pigs.

### Results following statistical analysis

Although approximately 200 pigs from each risk group were sampled, differences in *Salmonella* isolation rates between factories and between farms were large. Thus, there were insufficient data to allow inclusion of farm and factory as random effects in calculating prevalence using a random effects model; both random factory and farm effects were omitted in the calculation of caecal prevalence, and factory effects were omitted in the calculation of skin prevalence. A comparison of the percentage of samples positive for *Salmonella* from sites sampled on pigs from low- and high-risk herds is given in Table 3 using the glmm model. Pigs with *Salmonella* positive caecal contents were more likely to have positive carcass surface samples (16 % positive skin samples, 8.9 % - 27.4 % CI) than pigs with negative caecal contents (11.2 % positive skin samples, 8.2 % - 15.1 % CI) ( $p \leq 0.08$ ).

There was a significant difference between the mean ELISA OD % readings from low-risk pigs (19.5, 9.8 - 29.2 CI) and high-risk pigs (43.2, 32.5-54.0 CI) ( $p < 0.01$ ).

In this study, 69 % of the seropositive pigs from high-risk herds and 55 % of the seropositive pigs from low-risk herds had *Salmonella* isolated from caecal contents. Results of logistic regression using herd categorisation or seropositivity of pig, or both, as predictors/determinants of positive caecal contents showed that seropositivity of pigs (taking OD % = 40 as cut-off) from high-risk herds was a significant predictor (Table 4).

### Discussion

Half of all herds sampled contained pigs which were positive for *Salmonella* in caecal contents. These results are comparable to those of Davies *et al.* (1999) who found that at least 45 % of farms sampled in one abattoir had evidence of infection in their pigs. The percentage of farms from which salmonellae were isolated from

caecal contents was high for farms categorised as high-risk as would be expected but 37 % of low-risk herds also contained pigs with salmonellae in the caecum. However, the prevalence of *Salmonella* serotypes on the carcasses was low, particularly after they had been 18 hours in the chill. Nevertheless, the fact that a considerable number of farms contain pigs which carry salmonellae in the caecum, means that the potential for carcass contamination exists and that special slaughter procedures for farms consistently found to have high numbers of *Salmonella*-positive pigs are justified.

There was a marked difference in the prevalence of *Salmonella* in both the caecal contents and on the carcass surface in the two risk groups ( $p < 0.01$ ) (Table 3). Sorensen *et al.* (1999) found an association between the meat-juice sero-prevalence of Category 3 herds and the risk of a carcass being *Salmonella* positive. Our results from this study agree with this finding, as there was a significantly higher ( $p < 0.01$ ) seroprevalence of *Salmonella* in the high-risk herds compared to the low-risk herds, which was in turn associated with caecal isolation rate.

*Salmonella* Typhimurium and *Salmonella* Derby were the two most common isolates from samples of caecal contents, similar to findings in other European countries (Baggesen *et al.*, 1996; Davies *et al.*, 1999). However, *Salmonella* Derby was numerically the most common isolate from the skin of pigs from high-risk herds, in contrast to the findings of Davies *et al.* (1999), who found *Salmonella* Typhimurium to be the predominant isolate from the skin of carcasses. Clarification as to which serotypes most commonly contaminate carcasses is of importance as there are major differences between serotypes in their ability to cause human food poisoning, with *Salmonella* Typhimurium being a major cause of human disease whereas *Salmonella* Derby is rarely isolated from humans. However, in the study reported here, 17 of 21 of the *Salmonella* Derby positive caecal contents and 13 of 15 of the positive skin swabs were from a single abattoir. Sampling of a larger number of factories and including greater sample numbers would be required to determine the true prevalence of different serotypes on pig carcasses.

*Salmonella* Kentucky was the most common serotype isolated from skin swabs collected from low-risk pigs. However, all *Salmonella* Kentucky positive samples were collected on one day in Abattoir B and thus, these findings should not be taken as representative of all abattoirs.

There was a numerical trend ( $p \leq 0.08$ ) for an increased prevalence of *Salmonella* in carcass swab samples when caecal prevalence was high. This suggests that cross-contamination of carcasses is increased when pigs carrying salmonellae in the caecum are slaughtered as found by Baggesen *et al.* (1997). However, in the study reported here, correlation between the serotypes isolated from the caecum and skin of individual pigs was poor, indicating that more comprehensive studies are required to clarify the relationship between caecal carriage and carcass contamination rates.

Random effects models were used in this study to explore the usability of serology to predict microbiological status measured as microbiologically positive caecal samples. The results indicated that there was a positive correlation between serological reaction and positive caecal sample (Table 4). Using seropositivity of pigs (taking OD % = 40 as cut-off) from high-risk herds as a predictor of *Salmonella* in caecal contents gave more statistically significant results ( $p < 0.05$ ) than using seropositivity, not including risk group ( $p < 0.18$ ). These results are based on analysis of data from approximately 400 pigs. When data on larger numbers of pigs become available, the criteria for the classification of a herd as high or low risk and the determination of the ELISA cut-off value will be re-examined.

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Table 1

The group, category and number of pigs sampled in three abattoirs.

Group	Category*	No. of pigs	No. of pigs sampled in each abattoir		
			Abattoir A	Abattoir B	Abattoir C
Low-risk	< 10 % pos. (Cat. 1)	195	95	60	0
	10 - 19 % pos. (Cat. 2)	15	5	10	0
High-risk	32 - 50 % pos. (Cat. 2)	70	70	0	0
	> 50 % pos. (Cat. 3)	139	101	10	28

\* Category determined by results of samples taken for *Salmonella* control programme.

Table 2

Crude prevalence rate of *Salmonella* in sites sampled on 210 'low-risk' and 209 'high-risk' pigs.

Sample collected	'Low-risk' pigs	'High-risk' pigs
	Percentage positive	Percentage positive
Caecal contents	10.0	19.1
Skin after evisceration	7.1	13.1
Skin at entry to chill	2.9	0
Skin after 18 hours in chill	0.5	0
Meat juice	14.3	34.9



Table 3

Comparison of *Salmonella* prevalence in sites sampled on 210 'low-risk' and 209 'high-risk' pigs.

Sample collected	'Low-risk' pigs		'High-risk' pigs		Level of significance
	Percentage Positive	Confidence Intervals	Percentage Positive	Confidence Intervals	
	Caecal contents	10.0	6.6, 14.9	19.1	
Skin after evisceration	1.6	0.5, 4.8	12.0	9.51, 25.6	p < 0.01
Skin at entry to chill	2.9*	-	0*	-	-
Skin after 18 hours in chill	0.5*	-	0*	-	-
Meat juice	7.6	3.5, 15.8	29.7	15.6, 49.2	p < 0.01

\* No statistical analysis possible.

Table 4

Results of logistic regression testing the significance of seroprevalence criteria to predict/determine microbiologically positive pigs (based on culture of caecal contents). Factory and herd were included as random effects in the model.

Predictor	Level of significance
High risk herd	p ≤ 0.08
Seropositivity of pig (taking OD% = 40 as cut-off)	p ≤ 0.18
Seropositivity of pig from high-risk herd (taking OD% = 40 as cut-off)	p < 0.05