Development of on-farm control measures for the reduction of Salmonellosis in slaughter pigs

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End of Project Report RMIS No. 5153

The support of Research Stimulus Fund of the Department of Agriculture (NDP) in the financing of this project is gratefully acknowledged

January 2007

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1 Summary

The purpose of this study was to assess on-farm control measure for the reduction in the incidence of Salmonella on commercial pig units which were in Category 3 (high incidence) based on the slaughter-plant meat juice Elisa test under the national Salmonella control scheme.

In Task 1, a survey was carried out on 86 pig units of known Salmonella status, 45 were in category 3 or high Category 2 (high incidence) and 41 were in Category 1 (low incidence). Information was collected on the physical facilities, location, ownership and management practices on these farms with a view to identifying risk factors associate with a high prevalence of Salmonellosis.

Task 2 was the development (in conjunction with the farm owner/operator and his veterinary adviser) of control programmes for selected farms (n = 14). Farms were selected on the basis of being in Salmonella level Category 3 and the willingness of the operator to participate.

Task 3 involved monitoring of the Salmonella incidence on the farms in Task 2 for a 24 month period. This involved collection of blood and faeces samples from pigs from each production stage on the unit at approximately 6-month intervals.

Task 4 was an assessment of the costs to the pig industry (and individual producer) of measures associated with the Salmonella control programme.

Task 5 was a study of the effect of hygiene, transport and lairage practices on Salmonella prevalence in slaughtered pigs.
2 Introduction

Over 50% of herds supplying pigs to the major processing plants in Ireland were infected with Salmonella (i.e. at least one positive sample when the herd was sampled) at the commencement of the project. Typhimurium was the predominant serotype isolated (>50%), and a significant percentage of isolates were the multi-drug resistant phage types 104 and 104b. The high carriage levels of Salmonella present in pigs was considered to pose a significant risk to the HACCP controls in plants. A survey of 4 plants showed that 20% (range 9% to 30%) of carcasses were contaminated with Salmonella on entering the chill (Quirke et al., 2001). This is a serious food safety issue with pork being a significant contributor to human salmonellosis in Ireland.

Legislation (SI 165 of 2002) was implemented in 2002 requiring processing plants to introduce special control measures when slaughtering pigs from infected (Category 3) herds, in particular these pigs must be slaughtered on a separate day or later in the day than pigs from Category 1 and Category 2 herds. High-risk meat cuts from pigs from these herds have to be heat treated or discarded. This was expected to result in a severe financial penalty on suppliers of Category 3 pigs.

Contact with pig suppliers indicates that conflicting advice on Salmonella control was being given to farmers and some on-farm recommendations were unlikely to be effective. There had been no studies in Ireland to determine the farm factors contributing to the high Salmonella level and few husbandry or other strategies have been identified to assist farmers in controlling Salmonella. Vaccines are not available and antibiotic use is not an option.

The object of this project was to undertake a study of infection in a number of herds to identify factors contributing to the carriage of salmonellae and then, to devise and optimize suitable control strategies.
The project team worked with meat processors, farm advisers and herd owners to:

- Identify factors associated with high levels of *Salmonella* infection on farms through detailed investigations on selected farms and including reference to work completed in other countries on factors associated with high *Salmonella* prevalence.
- Examine the effect of factory lairage practices on *Salmonella* incidence
- Devise and evaluate control measures for selected high infection farms

The implementation of new controls at processing plants was expected to impose severe financial penalties on 15-20% of suppliers. Application of additional control measures at the herd level will impose additional costs. As experiences elsewhere have shown that *Salmonella* control at the herd level is difficult, it is imperative that any additional measures recommended for application are effective.

References


3 Survey of Risk Factors for *Salmonella* Infection

A comprehensive questionnaire for pig units was compiled and revised following initial use. This covered routine management practices, physical description of the unit, diet, biosecurity and disease status. Survey forms from 86 farms were completed by Teagasc pig advisers. Initially high Salmonella farms (HIGH; n = 50; category 3 and high category 2) were targeted. Later (with 6 months) category 1 and low category 2 farms (LOW; n = 36) were surveyed. Data from these farms was coded and input on computer and statistical analysis of the data was carried out using the chi square and PROC GLM procedures of SAS Inc., Cary, N. Carolina. Not all respondents answered all questions so some totals may not add to 86.

Among the fifty farms in the HIGH group were 42 integrated units (i.e. rearing pigs from birth to slaughter) and eight specialised finisher units while 32 of 36 in the LOW group were integrated. The average size of HIGH units was 271 sows while LOW units averaged 545 sows.

HIGH Units quarantined incoming stock for 2.1 weeks on average compared with 3.7 weeks for LOW units (P<0.05). There was little difference between groups in distance from the public road (HIGH 201m; LOW 235m) or from the nearest neighbouring pig unit (HIGH 659m; LOW 540m).

LOW units tended to wash the pens more frequently than HIGH units. This was true for each production stage - Weaner stage 2 (7.4 v 6.4 times/year), finisher (4.5 v 3.8 times/year), service area (22 v 10 times/year) and boar pens (9.3 v. 5.1 times/year).

Among the HIGH units 24 had closed finisher buildings and 26 had open-front buildings while among the LOW group 23 were closed and 11 were open-front. Cattle were present on 33 HIGH units but not present on 17 units. In the LOW
group there were cattle on 19 farms and no cattle on 17. This may reflect their smaller herd size.

Twenty eight LOW farms had cats and/or dogs which were allowed access to the unit and an equal number did not while 28 HIGH farms allowed access by pets but 22 did not.

Thirty five HIGH farms held back runt pigs and allowed them to mix with incoming pigs and 13 did not i.e. operated an all-in all-out policy. Only 12 LOW units held back runts and 17 operated an all-in all-out system.

Among units using dry feeding for weaner stage 2 pigs 22 were in the HIGH group and 12 LOW, 18 using wet-dry feeders were in the HIGH category and six in LOW. All 14 units using wet feeding for weaner stage 2 pigs were in the LOW category.

The trend was similar with regard to finisher feeders. Six of nine using dry feed were in HIGH, as were 40 of 54 using wet-dry feeders but only 3 of 17 used wet feed.

Feed source is confounded with unit size, form of feed and feeding system since home compounders are bigger and tend to feed meal in a wet mix. Forty seven HIGH herds used purchased feed while two used home-mixed feed. Twenty four LOW herds used purchased feed while ten used home-mixed feed.

Pelleted weaner feed was used by 14 LOW herds while 18 used meal. Among the HIGH herds 39 fed pellets and only three fed meal. In the case of finisher feed 42 HIGH herds fed pellets and eight fed meal while 14 LOW herds fed pellets and 18 fed meal.
On 28 HIGH herds staff worked with other farm animals in addition to pigs while they worked with pigs only on 22 units. Staff on 10 LOW units worked with other farm animals in addition to pigs and on 26 units they worked with pigs only.

Visitors to the unit took some biosecurity precautions on 32 of 36 LOW units and on 35 of 50 HIGH units. Rest times after washing on weaner stage 1, weaner stage 2, finisher and service/mating areas were all short (on average 1 to 3 days) but in each case were longer on LOW farms.

Conclusions:
Results of this analysis suggest that farms in the high-incidence group units were smaller (P<0.01), had more Trobridge (open-front) housing (P<0.05), had poorer pig flow ((P<0.05) and mixing practices (P<0.05), had less stringent biosecurity practices (P<0.05), used more wet-dry feeders and pelleted feeds (P<0.05), used more in-feed Salmonella control measures for weaners (P<0.05), washed mating/service areas less often (P<0.05) and rested these areas for shorter periods (P=0.06) than low-incidence farms.
4 Development and Monitoring of Control Programmes for Selected Pig Farms

4.1 ABSTRACT

A longitudinal study of the prevalence of *Salmonella* spp. was carried out on 12 Irish pig farms, which included farrow-to-finish herds and associated fattening units. The main objective of the project was to evaluate the efficacy of control measures implemented at farm level on highly infected farms. Control measures included the use of in-feed additives and/or improved hygiene and biosecurity measures. Prevalence of infection was monitored bacteriologically and serologically. Blood and faecal samples were collected from pigs at all production stages at 6-month intervals and in addition, serological status of finishing pigs was monitored at slaughter. Preliminary results suggest that some improvement occurred on all farms following implementation of controls for 12 months. Results for one of the in-feed additives (Formi™) appear promising although as complete data is not yet available this preliminary conclusion should be interpreted with caution.

4.2 Introduction

The presence of *Salmonella* on farms and in abattoirs is a serious food safety issue with pork being a significant contributor to human salmonellosis in Ireland (Boughton et al., 2004; Duffy et. al., 1999; Berends et al., 1998). *Typhimurium* is the predominant serotype isolated from Irish pigs and pork and a significant percentage of isolates are the multidrug resistant phage types 104 and 104b (Boughton et al., 2004; Department of Agriculture and Food, unpublished data).

A National Salmonella Control Programme was enacted into law in Ireland in 2002 [SI 165/2002: Abattoirs Act Veterinary Examination (Amendment) Regulations 2002]. Results from a serological monitoring system of slaughter
pigs are used to assign salmonella status to herds. The Danish mix-ELISA is the test on which categorisation is based (Nielsen et al., 1995) and herds are categorised according to the number of positive pigs: category 1: <10% positive, category 2: 10 - 49% and category 3: >50% positive. Processing plants must implement special control measures when slaughtering pigs from Category 3 herds and thus, the costs of processing meat from such herds are higher. It is envisaged that these costs will be transferred to the producer in the future but currently the abattoir bears all the cost.

Although substantial information is available on the factors affecting the prevalence of infection with *Salmonella* spp. on pig farms in other countries (Lo Fo Wong et al. 2004; Van Der Wolf et al. 2001; Beloel at al. 2004), few studies have been conducted in Ireland to determine the farm factors contributing to high levels of infection with *Salmonella* spp. In addition, there have been no studies in Ireland examining the success rate of special measures implemented on farms to control infection with *Salmonella* spp. The principal objective of this project was to evaluate the efficacy of control measures implemented at farm level on highly infected farms. The study was limited in that it was conducted on commercial pig farms which did allow split-herd trials to be implemented. Nevertheless, the study involves monitoring of farms over a prolonged period (at least two years) and thus useful information on the ability of farmers to maintain improvements in salmonella status can be generated.

### 4.3 Materials and methods

**Farm description & Control measures**

Pig farms were included in the study only if they were classified as Category 3 at time of selection. As can be seen from Table 1, 4 of the 12 farms had changed to Category 2 status at time of first sampling but these data only became available after initial sampling had been completed. The farms comprised farrow-to-finish herds and their associated fattening units.
Selected farms broadly fell into 3 groups based on the type of control measures implemented for *Salmonella* spp. infection. Group 1 included 2 units: Farm A, a 500-sow unit and Farm B a 200 sow unit. Control measures on these farms consisted of improved biosecurity and cleaning and disinfection procedures, as to how attempted to control *Salmonella* on their sites. Group 2 farms relied principally on the in-feed additives Bact-A-Cid™ (Agil Products, Nutrifarm Trading) and/or Prefect™ (Agil Products, Nutrifarm Trading): Farm C, a 230 sow unit improved hygiene measures and fed Bact-A-Cid™ at levels of 5kg/tonne of feed to finishing pigs, Farm D, a 130 sow unit, increased washing frequency and fed Bact-A-Cid™ at 2kg/tonne to sows and Prefect™ at 2kg/tonne to growing and finishing pigs, Farm E, a 250 sow unit, increased use of disinfection and fed Prefect™ at 5kg/tonne to just-weaned pigs and Bact-A-Cid™ at 4kg/tonne to growing and finishing pigs and at 5kg/tonne to sows. Group 3 farms (Farms F to K) employed the in-feed additive Formi™ (BASF) as a control measure. Farms F, G, H and I all belonged to the same farmer with Farm F being a 530-sow unit which supplied growing and finishing pigs to units G, H and I. On these farms Formi™ was fed to sows at 8kg/tonne, to piglets at 9kg/tonne and to growing and finishing pigs at 6kg/tonne. Farm J, a 360-sow unit, fed Formi™ at 6kg/tonne to finishing pigs. Farm K, a 900-sow unit and Farm L, a 120-sow unit fed Formi™ at 6kg/tonne to finishing pigs. Farms F, G, H, I and K relied solely on Formi™ for control whereas farms J and L employed improved hygiene and biosecurity measures also.

**Sampling scheme**

Farms were sampled at approximately 6-monthly intervals. Bacteriological samples consisted of composite pen faecal samples. On average, 16 samples were collected from first stage weaned pigs (W1, 4 to 7 weeks old), 20 samples from second stage weaned pigs (W2, 7-12 weeks old), 40 samples from finishing pigs, 10 samples from dry sow houses, 15 from farrowing houses,
5 from gilt pens and 2 from boar pens. Blood samples for serological testing were collected from 20 W1, 20 W2, 60 finishing pigs, 20 dry sows and 10 gilts.

**Procedures for the recovery of Salmonella from samples**

Briefly, 10g faecal samples were pre-enriched in 90ml BPW and incubated for 18-24 h at 37°C, followed by selective enrichment in Rappaport-Vassiliadis broth 41.5°C for 24 h. Samples were plated onto mannitol lysine crystal violet brilliant green agar (MLCB) and brilliant green agar (BG) after both 24 h and 48 h of selective enrichment. Up to five suspect colonies per plate were identified by subculture onto MacConkey agar and inoculation of triple sugar iron agar slopes followed by serotyping.

**Serological testing**

Samples were tested using an in-house mixed-ELISA test as described by Nielsen et al. (1995).

**4.4 Results**

Preliminary results only are available to date. Data presented in Table 4.1 show that no farm is in Category 3 following implementation of control measures for approximately 12 months. Bacteriological and serological results for finishing pigs tend to support the improvements recorded in official salmonella status on most farms, although increases in serological prevalence are recorded for Farms C and D and increases in bacteriological prevalence on farms E and K. Improvements appear to be more marked for those farms using Formi™ than for farms in groups 1 or 2.
Table 4.1. Bacteriological and serological prevalence of *Salmonella* spp. in finishing pigs on 12 farms highly infected with *Salmonella* spp. before implementation of control measures and following implementation of control measures for approximately 12 months. Percentages are given in brackets.

<table>
<thead>
<tr>
<th>Farm and Group</th>
<th>Salmonella status before controls</th>
<th>Salmonella status after controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Categor y</td>
<td>Bacteriological relevance</td>
</tr>
<tr>
<td><strong>Group 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farm A</td>
<td>3</td>
<td>19/28 (68)</td>
</tr>
<tr>
<td>Farm B</td>
<td>3</td>
<td>3/40 (8)</td>
</tr>
<tr>
<td><strong>Group 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farm C</td>
<td>3</td>
<td>5/23 (22)</td>
</tr>
<tr>
<td>Farm D</td>
<td>2</td>
<td>9/30 (30)</td>
</tr>
<tr>
<td>Farm E</td>
<td>3</td>
<td>3/40 (8)</td>
</tr>
<tr>
<td><strong>Group 3</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farm F</td>
<td>3</td>
<td>12/12 (100)</td>
</tr>
<tr>
<td>Farm G</td>
<td>3</td>
<td>27/35 (77)</td>
</tr>
<tr>
<td>Farm H</td>
<td>2</td>
<td>8/25 (32)</td>
</tr>
<tr>
<td>Farm I</td>
<td>2</td>
<td>25/35 (71)</td>
</tr>
<tr>
<td>Farm J</td>
<td>2</td>
<td>4/40 (10)</td>
</tr>
<tr>
<td>Farm K</td>
<td>3</td>
<td>0/30 (0)</td>
</tr>
<tr>
<td>Farm L</td>
<td>3</td>
<td>2/45 (4)</td>
</tr>
</tbody>
</table>

<sup>1</sup> Group 1 controls = improved hygiene and disinfection procedures, Group 2 controls = improved hygiene measures and feeding of Bact-A-Cid/Prefect, Group 3 controls = Feeding of FORMI

<sup>2</sup> ND = not done

<sup>3</sup>Unavailable as pigs sold to Northern Ireland

Data presented in Table 4.2 show a progressive increase in the number of seropositive animals as age increases. A similar trend is seen in the number of bacteriologically positive samples with the exception that bacteriological prevalence is less in samples collected from sows compared to those collected in finishing pigs and gilts.
Table 4.2. Prevalence of infection with *Salmonella* spp. in 12 herds sampled on 3 occasions at approximately 12 monthly intervals, according to type of pig. Percentages are given in brackets.

<table>
<thead>
<tr>
<th>Pig type</th>
<th>W1</th>
<th>W2</th>
<th>F</th>
<th>G</th>
<th>LS</th>
<th>DS</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
<td>53/434 (12%)</td>
<td>59/457 (13%)</td>
<td>207/2082 (19%)</td>
<td>34/127 (27%)</td>
<td>33/339 (10%)</td>
<td>55/248 (22%)</td>
<td>5/45 (11%)</td>
</tr>
<tr>
<td>Serology</td>
<td>6/547 (1.1%)</td>
<td>36/525 (6.9%)</td>
<td>263/1552 (17%)</td>
<td>117/258 (45%)</td>
<td>-</td>
<td>213/447 (48%)</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)Pig type: W1 - Weaned pigs, 4-7 weeks of age; W2 - Weaned pigs, 7-12 weeks of age; F - Finishers; G - Gilts; LS - Lactating Sows; DS - Dry Sows; B - Boars

4.5 Discussion

The results presented here demonstrate that the novel food additive used on several farms (Formi\textsuperscript{TM}) is showing promising results. Campbell et al. (2003) showed that fumaric acid (an active ingredient of Formi\textsuperscript{TM}) was effective in lowering faecal coliform numbers in pigs but independent data on the commercial product itself do not appear to be available. Formi\textsuperscript{TM} is expensive compared to other feed additives and further work on its cost effectiveness is required. In addition, studies on whether effective control of *Salmonella* spp. can be maintained through the use of this product or whether control could be achieved through using Formi\textsuperscript{TM} periodically rather than continuously would be useful.

Results from farms in Groups 1 and 2, together with other serological data which are not presented in this paper, suggest that control of *Salmonella* levels on these farms was more difficult to maintain. It is well known that improved hygiene and disinfection procedures are important in the control of contamination with salmonellae on pig units (Wray and Wray, 2000). Gray and Fedorka-Cray (2001) stated that the control of all *Salmonella* spp. in the environment must include removal of all organic matter followed by thorough disinfection. Therefore, a lack of efficacy of the measures implemented might
have resulted in failure to significantly reduce levels of *Salmonella*. Labour shortages on pig units and lack of incentive (processing plants currently carry all costs of *Salmonella* control in Ireland) may explain failure to maintain initial improvements.

The efficacy of the in-feed additives, Bact-A-Cid™ and Prefect™ is unclear. Campbell *et al*. (2003) failed to show any effect of Bact-A-Cid™ on the gut flora of pigs. On the other hand, numerous studies (Gardiner *et al*., 2003; Jorgensen *et al*. 2003; Lo Fo Wong *et al*. 2004) found that acidified feeds (produced either by adding lactic acid bacteria, organic acids or whey) significantly reduced *Salmonella* seroprevalence compared to standard pelleted feed.

Results in Table 4.2 are consistent with findings by other authors (REFS). Reduced bacteriological prevalence in lactating sows is probably associated with the high standard hygiene of farrowing houses on most farms. The opposite is true of gilt pens. The pens tend to be overcrowded and cleaned infrequently. The low level of seropositive results in W1 stage (Table 2) might be associated with relatively small amount of positive faecal samples found in farrowing pens.

### 4.6 Conclusions

Some improvement occurred on all farms following implementation of controls for 12 months. Results for one of the in-feed additives (Formi™) appear promising although further longer-term studies are required. Maintenance of improvements using improved hygiene procedures may be difficult. Expected implementation of fines for Category 3 farms (according to legislation SI 165 of 2002) may change the approach of farm owners towards *Salmonella* contamination.

In spite of the limitations of the study, the data collected under this task will be useful in the formation of *Salmonella* control guidelines for Irish pork.
producers. Findings were communicated to specialist pig veterinary surgeons and agricultural advisers during an information workshops. In order to reduce the level of contamination with Salmonella serotypes on highly infected farms it is necessary to combine proper washing and disinfection routines with biosecurity measures and use of feed additives.

Each farm should be assessed individually as to the best possible strategy, with farm factors being an important indication of the likely success rate of methods implemented.
4.7 References


5 The Efficacy of Cleaning and Disinfection on Pig Farms

5.1 Abstract

Little is known about the effectiveness of the cleaning and disinfection methods in use on commercial pig farms either in Ireland or worldwide. A National Salmonella Control Programme was implemented in Ireland in August 2002 to monitor and control infection with Salmonella spp. in pigs. In Ireland, all commercial pig herds must be categorised according to their Salmonella status. Herds in category 1, 2 and 3 have a serological Salmonella prevalence of infection of \( \leq 10\% \), \( > 10-\leq 50\% \) and \( > 50-\leq 100\% \), respectively.

The aim of this study was to assess the efficacy of washing and disinfecting finisher units on category 1 and category 3 farms, in reducing or eliminating levels of Enterobacteriaceae. Enterobacteriaceae counts were used as indicators of the contamination of the environment with enteric bacteria, which could include Salmonella spp. Samples were taken from the pen floors and feeder/drinker units of four category 1, two high category 2 and 1 category 3 farms. Enterobacteriaceae and salmonellae were enumerated in each sample. Limited results available on enumeration suggest that there was a decrease in levels of Enterobacteriaceae on pen floors after cleaning and disinfection, regardless of category. However, significant residual contamination remained on the surfaces of the feeder/drinker units following cleaning and disinfection on all farms.

5.2 Introduction

A national programme to reduce Salmonella contamination in pork and pork products should include monitoring and intervention from the farm to the factory. In Ireland, the national Salmonella control programme is based on the categorisation of all commercial pig herds according to their Salmonella status. Finishing pigs in herds in category 1, 2 and 3 have a serological Salmonella prevalence of \( \leq 10\% \), \( > 10-\leq 50\% \) and \( > 50-\leq 100\% \), respectively.
The prevalence of *Salmonella* within a herd from farm to slaughter is governed by many factors, one of the most important being an effective hygiene programme. Cleaning and disinfection have an important part to play in the control of this disease. It has been found that uninfected pigs, which remained in disinfected pens, usually stayed free of *Salmonella* (Linton et al. 1970). However, achieving a sufficient reduction in *Salmonella* levels by hygienic and management procedures alone can be quite difficult. In a study aimed at reducing *Salmonella* at farm level, Dahl *et al.* (1997) found that problem herds that improved hygiene combined with all in-all out measures did not achieve the same success as those that used organic acids in the water or feed.

The objectives of this study were 1) to determine and compare the efficacy of cleaning and disinfection procedures in finisher units from ten category 1 and ten high category 2/category 3 farms, using *Enterobacteriaceae* counts as a marker for residual enteric bacteria, and 2) to determine the prevalence of *Salmonella* spp. both before and after cleaning.

5.3 Materials and methods

Farms and sample collection

To date five category 1, two high category 2 and one category 3 farm have been identified and sampled. Visits commenced on obtaining agreement for intensive sampling from farmers. Between 40 and 60 samples were taken both before and after the finisher units were cleaned and disinfected according to the farmer’s usual programme. On average a total of twelve pens were sampled per farm with six floor, one feeder and one water sample being collected per pen. Sterile templates of 100cm² were placed randomly on the pen floor and samples were collected by swabbing the area within with a sterile pre-moistened carcass sponge. An approximately similar area was swabbed within the feeders, although it was not possible to use sterile templates due to their rigid design. Drinkers were sampled by collecting approximately 100ml of water in a sterile sample jar. All samples were stored in a chilled container during
transport and kept at 4°C prior to examination within twenty-four hours of collection.

**Microbiological Analysis**

On arrival at the laboratory each sponge was suspended in 100ml of maximum recovery diluent (0.1% peptone, 0.85% NaCl: MRD: Oxoid, Basingstoke, Hampshire, England). All swab samples were shaken vigorously before analysis.

*Enterobacteriaceae* counts were obtained by preparing violet red bile glucose agar (VRBGA; Oxoid) pour plates using 1ml of swab suspensions, or derived 1:10 dilutions in MRD. Plates were over-poured with VRBGA to create a semi-anaerobic environment, incubated at 37°C for 24 h and examined. The *Enterobacteriaceae* enumeration method had a minimum detection limit of 1 CFU cm⁻² (McEvoy et al. 2004).

*Salmonella* isolation procedures were performed on 25ml of each water sample and on 20ml of each swab suspension according to BS EN 12824; 1998. Briefly, water samples were pre-enriched in 225ml BPW and incubated for 18-24 h at 37°C. Samples of the swab suspension were also incubated for 18-24 h at 37°C. All samples were then selectively enriched in Rappaport-Vassiliadis broth 41.5°C for 24 h. Samples were plated onto mannitol lysine crystal violet brilliant green agar (MLCB; Lab M, Bury, Lancashire, England) and brilliant green agar (BG; Lab M, Bury, Lancashire, England) after both 24 h and 48 h of selective enrichment. Up to five suspect colonies per plate were identified by subculture onto MacConkey agar and inoculation of triple sugar iron agar slopes followed by serotyping.
MPN (Most Probable Number) Analysis

An estimation of the number of *Salmonella* spp. in all *Salmonella*-positive samples was determined using a modified 3-tube MPN method (Dufrenne et al. 2001). Isolation of *Salmonella* serotypes from 3x10ml, 3x1ml and 3x0.1ml aliquots of homogenized sample in BPW or MRD was performed as described above. After confirmation, the number of *Salmonella* present in each sample was calculated using the MPN table of de Man (De Man 1983).

Data Analysis

*Salmonella* prevalence was reported as the number of samples that tested positive. *Enterobacteriaceae* counts were transformed to log$_{10}$ cfu/cm$^2$. Because of the wide range and skewed nature of the data, median values were calculated. Statistical analysis of the results is awaiting collection of further data.

5.4 Results

Table 5.1 shows the results of the samples taken before and after the cleaning procedure from the pen floors in all eight farms. In most cases there was a moderate reduction in *Enterobacteriaceae* levels after cleaning and disinfection procedures. Farm F, which washed only, achieved little / or no reduction in *Enterobacteriaceae* levels following washing only. High category 2 / category 3 farms possibly achieved a greater reduction in levels of *Enterobacteriaceae* following cleaning.

In most cases there was little *Salmonella* detected before and after cleaning and levels ranged from 0.36-1.1 MPN/cm$^2$. However, farm D had a *Salmonella* prevalence of 62% prior to cleaning which dropped to 0.02% following the washing procedure. These results were surprising as although a recent change had occurred in feeding practices it was a category 1 herd at the time of sampling.
Table 5.1. Effect of cleaning procedure on levels of Salmonella and Enterobacteriaceae on the pen floors

<table>
<thead>
<tr>
<th>Category</th>
<th>Farm</th>
<th>Samples tested</th>
<th>Enterobacteriaceae</th>
<th>Salmonella</th>
<th>Before washing</th>
<th>After washing</th>
<th>Before washing</th>
<th>After washing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(n)</td>
<td>Range Median</td>
<td>(n) Range</td>
<td>Median</td>
<td>(n) Range</td>
<td>Median</td>
<td>(n) Range</td>
</tr>
<tr>
<td>3</td>
<td>A</td>
<td>64</td>
<td>1.7-6.6 4.5</td>
<td>0-5.8 0.8</td>
<td></td>
<td>_ c _</td>
<td>_ c _</td>
<td>_ c _</td>
</tr>
<tr>
<td>High 2</td>
<td>B</td>
<td>64</td>
<td>3.5-6.1 4.6</td>
<td>0-1.6 0</td>
<td></td>
<td>_ c _</td>
<td>_ c _</td>
<td>_ c _</td>
</tr>
<tr>
<td>High 2</td>
<td>C</td>
<td>72</td>
<td>0-5.1 1.2</td>
<td>0-1.6 0</td>
<td></td>
<td>1 1.1</td>
<td>_ c _</td>
<td>_ c _</td>
</tr>
<tr>
<td>1</td>
<td>D</td>
<td>84</td>
<td>2.6-6.1 4.6</td>
<td>0-3.6 0.8</td>
<td>26 36-&gt;10^6</td>
<td>1 7.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>E</td>
<td>84</td>
<td>0-3.6 1.6</td>
<td>0-3.2 0</td>
<td></td>
<td>_ c _</td>
<td>_ c _</td>
<td>_ c _</td>
</tr>
<tr>
<td>1</td>
<td>F</td>
<td>60</td>
<td>1.2-5.1 3.3</td>
<td>0.7-4.2 2.9</td>
<td></td>
<td>_ c _</td>
<td>_ c _</td>
<td>_ c _</td>
</tr>
<tr>
<td>1</td>
<td>G</td>
<td>72</td>
<td>0-6.0 2.0</td>
<td>0-3.6 0</td>
<td></td>
<td>_ c _</td>
<td>1 0.36</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>H</td>
<td>48</td>
<td>0.8-4.2 3.7</td>
<td>0.7-4.1 2</td>
<td></td>
<td>_ c _</td>
<td>_ c _</td>
<td>_ c _</td>
</tr>
</tbody>
</table>

a Log_{10} cfu/cm^2.

b MPN/cm^2; detection limit, 0.36 MPN/cm^2.

c Negative for Salmonella (detection limit, <0.36 MPN/cm^2).

Following washing the results for the feeder/drinker units did not improve (Table 5.2) and residual contamination appeared to be a major problem. A common trend throughout all farms, regardless of category, was a significant increase in Enterobacteriaceae levels following washing and disinfection.

Salmonella results were highly variable with category 3 farms having higher ranges in washed feeder/drinkers than category 1 farms.

It was not possible to compare the effectiveness of the different disinfectants used by the farms as none of the disinfection programmes were applied in a standardised way.
Table 5.2. Effect of cleaning procedure on levels of Salmonella and Enterobacteriaceae in feeder/drinker units

<table>
<thead>
<tr>
<th>Category</th>
<th>Farm</th>
<th>Samples tested</th>
<th>Samples Positive tested</th>
<th>Enterobacteriaceaea</th>
<th>Salmonellab</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>(n)</td>
<td></td>
<td>Before washing</td>
<td>After washing</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Range</td>
<td>Median</td>
</tr>
<tr>
<td>3</td>
<td>A</td>
<td>16</td>
<td></td>
<td>3.0-5.6</td>
<td>4.4</td>
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<tr>
<td>High 2</td>
<td>B</td>
<td>16</td>
<td></td>
<td>3.7-5.7</td>
<td>5</td>
</tr>
<tr>
<td>High 2</td>
<td>C</td>
<td>24</td>
<td></td>
<td>0-6</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>D</td>
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<td>3.7-6.1</td>
<td>5.5</td>
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<td>E</td>
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<td>1.5-4.9</td>
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<tr>
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<td>F</td>
<td>24</td>
<td></td>
<td>0.5-4.9</td>
<td>3.5</td>
</tr>
<tr>
<td>1</td>
<td>G</td>
<td>24</td>
<td></td>
<td>0.5-5</td>
<td>2.8</td>
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<tr>
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<td>H</td>
<td>20</td>
<td></td>
<td>3.4-4.9</td>
<td>3.9</td>
</tr>
</tbody>
</table>

a Log_{10} cfu/cm^2.
b MPN/cm^2; detection limit, 0.36 MPN/cm^2.
c Negative for Salmonella (detection limit, <0.36 MPN/cm^2).

5.5 Discussion

Although preliminary, and with little published data to compare with, the results of this study of cleaning and disinfection on commercial pig farms indicate that there are particular problems with the cleaning of the feeder/drinker units. This may be due to operator negligence or difficulties accessing all crevices in the feeder units. Whatever the reason, large volumes of contaminated faecal matter are remaining in these units following cleaning of the pen. In contrast, previous studies of disinfection on commercial poultry laying units (Davies et al, 2003) found that equipment was less contaminated than the main house structures even though there was a smaller reduction after cleaning and disinfection.

Healthy pigs can carry Salmonella serotypes in their intestine and may shed this pathogen when stressed. Pigs are subjected to many stress factors during production, which in turn may induce these carriers to shed the bacterium at a higher rate and increase the likelihood of Salmonella-free pigs
to acquiring infection. Fedorka-Cray et al. (1994) showed that pigs that were 
Salmonella-free prior to exposure were likely to acquire infection once exposed to a contaminated environment. Thus, thorough cleaning and disinfection of pens between batches of pigs is an important tool in Salmonella reduction at herd level and if neglected, residual infection may initiate infection in clean stock (Thomas, 1982).

Van der Wolf et al. (2001) reported that a lower Salmonella seroprevalence was associated with herds that never disinfected a compartment after pressure washing as part of an all-in / all-out procedure than herds that sometimes or always used disinfectants. It was suggested that farmers that use disinfectants clean less adequately in the hope that any remaining microbes will be dealt with by the disinfectant. In contrast, the preliminary results from this current study have shown a lower Salmonella and Enterobacteriaceae prevalence in herds that always washed and disinfected than herds that washed only. It has always been accepted that hygiene is important for optimal production results and reduction of infection in the pig industry.

Fedorka-Cray et al. (1997) showed that pigs weaned at 14-21 days and removed to clean accommodation remained free of Salmonella. In a study by Funk et al. (2001) a sizeable reduction in Salmonella-shedding in sows was detected shortly after relocation to the farrowing unit. It was suggested that due to more frequent and thorough cleaning of the farrowing rooms there was decreased environmental contamination. Similarly, Rajkowski et al. (1998) showed a considerable reduction in Salmonella levels in trucks following washing and disinfecting. 41.5% of pens yielded confirmed Salmonella isolates before washing and this dropped to 2.7% after washing and disinfecting.

In summary, although this work is still in progress, we have shown so far that washing and disinfecting pen floors is apparently effective in greatly reducing levels of Enterobacteriaceae. However, contamination of the feeder/drinker units following cleaning is a far greater problem and one that
needs to be better addressed. Further work and intervention in this area could be expected to reduce the potential for spreading of infection and cross-contamination of other animals. This in turn should help to reduce the number of positive carcasses entering the abattoirs.

5.6 References


Davies, R., Breslin, M., 2003. Observations on Salmonella contamination of commercial laying farms before and after cleaning and disinfection. Veterinary Record 152:283-287


Funk, J.A., Davies, P.R., Nichols, M.A., 2001. Longitudinal study of Salmonella enterica in growing pigs reared in multiple-site swine production ecosystems. Veterinary Microbiology 83:45-60


6 Monitoring of Costs and Returns at Farm Level from Implementing Salmonella Control / Reduction Measures

Costs incurred by individual pig producers as a result of the Salmonella control programme apply only to those in category 3 or those in high category 2 who wish to take precautions lest their categorisation worsen. These costs are mainly due to increased frequency of washing and acidification of feed. In some cases a minor reduction in throughput may occur when all-in all-out stocking is practiced. Restriction on their day of sale is an inconvenience rather than a cost while limited opportunity to change outlets for their pigs due to reluctance of meat plants to accept new suppliers who are in category 3 may be a hidden cost which is difficult to quantify.

Almost all pig producers (regardless of Salmonella category) wash farrowing and first stage weaner housing after every batch. Increasing the frequency of washing will typically mean washing second stage weaner accommodation after each batch (8 batches per year) rather than once or twice per year and washing finisher accommodation after each batch (3 or 4 per year) rather than once per year.

Assuming 100 sows producing 2,000 pigs per year the facility will contain 12 weaner pens (each with 20 pigs) and 24 finisher pens (each with 20 pigs) per 100 sows. The extra washing will involve 12 weaned pens 6.5 times (extra) per year plus 24 finisher pens three times per year or 150 pen-washings. The time required to wash one pen will be about 20 minutes or 50 man hours in total. Water usage will be 18 litres per minute or 54,000 litres water. Spreading of this volume of manure will require 6 vacuum tanker loads @ 30 Euro per load. Based on a French study ("Le Nettoyage et la Disinfection" – ITP, Paris 2003) labour and manure spreading are 53% of the total cost which includes detergents, disinfectants, electricity and water.)
Labour and manure cost of the extra washing is therefore estimated at 50 hours @12 Euro plus 6 tanker loads of manure @ 30 Euro = 780 Euro per 100 sows or 7.8 Euro per sow per year. Total cost (based on the French estimate) is 14.72 Euro per sow per year.

Supplementation of the feed with FORMI (or an equivalent product) is usually at 6kg/tonne costing on average (over the period of the study) €1.40 per kg or €8.40 per tonne of feed. Production of 21 pigs per sow per year will require 1.0 tonnes weaner feed and 3.2 tonnes finisher feed. If both diets are supplemented the cost per sow per year will be €35.28 per sow per year. While this remains to be demonstrated, some pig veterinarians feel that supplementing only the finisher diet and/or supplementing the diet only in the last period of 4 to 6 weeks pre-slaughter may be sufficient. This assessment is consistent with a recent Danish study which concluded that acidification of feed represented poor value from a cost benefit analysis (Goldbach and Alban, 2006).

A financial penalty of 1c per kg on category 3 herds would amount to 72c per pig or €15.12 per sow per year (21 pigs @72kg carcass weight).

Estimates from the pigmeat processing plants are that about 4,000 pigs are slaughtered each week from category 3 herds of which 50% are from herds without a valid certificate. The loss on each category 3 pig (loss of sale value of head and offal; cost of disposal of head and offal) is estimated at €3.50 per pig and this cost is at present absorbed by the plant. Since this cost applies to all pigs from a unit the total is 73.50 Euro per sow per year. For the pig industry the annual loss is about 0.78 million Euro of which the 50% due to invalid certificates is certainly avoidable.
The costs considered may be summarised as (Euro per sow per year):

Increased washing frequency: €14.72
Acidification of weaner and finisher feed: €35.28
Financial penalty of 1c/kg: €15.12
Loss to meat plant from Category 3 pigs: €73.50

Penalties applied to herds with a high incidence of Salmonella in Denmark are especially punitive. The present penalty in Denmark is 2% of the value of the pig in category 2 herds and 4, 6 or 8% of the value for category 3 herds depending on how long the herd has been in category 3 (Hurd et al., 2005).

6.1 References


7 Effect of Hygiene, Transport and Lairage Practices on *Salmonella* Prevalence in Slaughtered Pigs.

A graduate student (veterinarian) to work on this aspect of the project commenced work in November 2003 and her study programme has been agreed with UCD. She is currently receiving training in laboratory techniques and assisting with Tasks 2 and 3.

Months 12-18

Work relevant to this task has been carried out under another project (FIRM project 00/R&D/D/32) since the proposal was submitted to the Stimulus Programme for funding. Results from this work have helped to define the importance of the lairage in acquisition of infection by uninfected pigs on entering the abattoir. Further work is necessary and will be carried out under this task.

However, it has also become apparent while working with the farmers and veterinarians during tasks 2 and 3, that little information is available on the importance of slurry in dissemination of infection in pig units. A survey of the literature yielded little information on *Salmonella* levels in pig slurry although information is available on cattle slurry, particularly in the older literature.

Therefore, it was decided that some work on prevalence and survival of *Salmonella* in pig slurry should be carried out under this task. Results will also inform future studies on *Salmonella* survival on transport vehicles and in lairages.

Thus, after some preparatory work on investigation of *Salmonella* levels in naturally contaminated slurry and investigation of the most suitable methodology to use, an *in-vitro* study has been set up. This study is examining the survival of the two most common serotypes on pig farms, Typhimurium and Derby, which have been inoculated at two different levels ($10^2$ and $10^5$) into slurry obtained from a Category 1 farm. *Salmonellae* are enumerated in all samples and each treatment is replicated 5 times.
Months 18-24

Survival Characteristics of *Salmonella* Typhimurium and *Salmonella* Derby in pig slurry during storage.

This initial trial appears to show a substantial difference in survival rates between the two isolates especially when inoculated at higher levels. At the higher level of inoculation a 90% drop in levels was shown by day 15 for *S. Typhimurium* and by day 10 for *S. Derby*, with no detectable levels at days 35 and 24, respectively.

A similar pattern in survival characteristics was shown at the lower levels of inoculation. *S. Typhimurium* was undetectable by day 20 and *S. Derby* by day 17.

Efficacy of cleaning and disinfection on pig farms in Ireland

This study aims to determine and compare the efficacy of cleaning and disinfection of finisher units from category 1 and category 3 farms, to reduce or eliminate bacterial levels. To date three category 1 farms, two high category 2 farms and one category 3 farm have been sampled.

Cleaning practices on all farms has significantly reduced levels of *Enterobacteriaceae*, however, the greatest reduction has probably occurred on category 2/3 farms and a greater percentage of samples from clean pens from these farms have negative *Enterobacteriaceae* counts. However, cleaning practices vary substantially from farm to farm. All 3 category 2/3 farms washed and disinfected whereas only one category 1 farm used disinfectant. One category 1 farm, that washed only, showed no reduction in levels of *Enterobacteriaceae*. The category 1 unit that did use disinfectant yielded a total of 35 *Salmonella*-positives (washed & dirty pens) out of 112 samples taken. The 3 category 2/3 units yielded a low number of *Salmonella*-positive samples (1-3 out of 80-90 samples taken and all from clean pens). A common factor
shared by all categories is the high levels of Enterobacteriaceae in the feeder/drinker units of washed and/or disinfected pens. Preliminary results may suggest that category 2/3 farms have overall higher levels of Enterobacteriaceae at the outset, however more farms need to be sampled to confirm this. Visual assessment of the units combined with results from microbiological analyses suggests that the presence of gross faecal matter does not correlate with high levels of bacterial contamination. The ultimate aim is to sample 10 category 1 and 10 category 3/high2 units. There are a number of difficulties obtaining farms, especially those with a cleaning and disinfection programme in place. Many units appear to wash only once/year, which is proving difficult.

Months 24-30

Work continued on the investigation of the efficacy of cleaning and disinfection on pig farms during this reporting period. Sampling has been completed on 13 farms to-date. Because of the consistency of the results obtained to-date and because of the difficulty in finding Category 3 farmers who are both willing to co-operate and who wash and disinfect pens regularly, the initial target of 20 farms has been revised downwards to 16. The trend shown by the data in the last reporting period is being maintained. The most significant finding is the lack of effective cleaning and disinfection of feeding troughs on both Category 1 and Category 3 farms.

Months 30-36

Work on the efficacy of cleaning and disinfection was completed on another farm during this period and the results of the study were written up for presentation at the Safepork Conference in San Francisco in September 2005. In this study, levels of Enterobacteriaceae were used as a marker for the effectiveness of cleaning and disinfection. Results showed that on both
Category 1 and Category 3 farms, pen floors were significantly cleaner after washing and disinfection (p<0.001). However, the feeder-drinker units on both farm categories were not improved after cleaning and disinfection and in fact, feeder units were significantly dirtier after washing on Category 3 farms. Thus, trends reported previously were confirmed when the data were statistically analysed. Feeder-drinker units may be an important source of infection for new pigs entering a 'clean' pen.

In addition, work examining the role of transport and lairage on the Salmonella status of pigs was carried out. A category 3 farm was identified and all pens of pigs to be slaughtered the following week were sampled. One highly positive pen was identified and all pigs in that pen were sampled intensively before and during slaughter. Multiple samples of the following sites were collected and cultured for salmonellae: the truck before and after transport of the pigs and after power washing, the lairage pens before and after holding of the pigs, rectal and caecal contents of the pigs post evisceration. Numerous positive samples were obtained at all sites, which reinforces previous findings from other Irish studies and from other countries on the importance of the carrier pig as a source of contamination at slaughter. Further studies of other farms and complimentary studies of carcass and pork cut contamination rates (the latter to be carried out under another FSPB/FIRM project) will be required to fully assess the significance of these results.

An undergraduate student in his final year of industrial microbiology (no cost to this project) repeated the study on survival rates of S. Typhimurium and S. Derby in slurry in cooler weather than that experienced during the first study. Results of the initial trial were confirmed, with S. Typhimurium persisting for longer than S. Derby, although survival of both strains was greater in the cooler conditions. These findings are of interest as they suggest that elimination of S. Typhimurium from the environment may be more difficult than
other serotypes, which should be borne in mind when dealing with Category 3 farms, most of which appear to be heavily contaminated with *S. Typhimurium* (see results of Task 3).

Months 36-42

Some further work examining the role of transport and lairage on the *Salmonella* status of pigs was carried out. More comprehensive studies than those originally proposed under this task will continue under another project funded by FSPB/FIRM, as mentioned in the last reporting period (project 04-RESR-08, Coordinator Dr G Duffy, Teagasc, AFRC).

Pigs from a further two Category 3 farms were identified and sampled during this reporting period. Results indicate that pigs can acquire infection in as little as two hours in lairage and that washing of trucks as carried out at abattoirs may have little impact on *Salmonella* levels on heavily contaminated vehicles.

8 Conclusions

9 Publications from this project


Relay workshop for the Pig Industry held on April 26th 2006, in CVRL, Backweston, Celbridge.

10 Acknowledgements

The authors acknowledge the assistance of the following in carrying out this project.

Pig producers (who shall remain anonymous) who allowed access to their pig units

Teagasc pig enterprise advisers who collected the farm survey data: P. Tuite, M. Martin, C. Carroll, J. Finn, G. McCutcheon. Mr. J. O'Reilly who coded and entered data from the farm survey
Consultant veterinary surgeons who carried out the on-farm sampling: A. Ahearne, M. Bourke, D. Kelliher, P. Kirwan, P. Spillane,