INFECTION OF PIGS FOLLOWING EXPOSURE TO CONTAMINATED PEN FLOORS

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Abstract The objective of these studies was to determine if pigs could acquire infection when exposed to different levels of environmental contamination with Salmonella Typhimurium. In experiment 1, pigs were euthanised after 2, 3 and 24 hours of exposure to a highly contaminated environment (105 organisms/100cm²). Tonsils, caecal contents, ileocaecal junction, lung, liver, spleen and ileocaecal lymph nodes were evaluated. Salmonellae were detected in at least one sample from each pig after exposure for 2 or more hours. Salmonellae were recovered most frequently from tonsils (100% positive), segments of the ileocaecal junction (94.4% positive) and caecal contents (89% positive). In experiment 2, pigs were exposed to a less contaminated environment (102 organisms/100cm²) for time periods of 1, 3, 6 and 24 hours. Tonsils, caecal contents and mesenteric lymph nodes were collected for Salmonella spp. isolation and examined. Salmonellae were not detected in any samples from pigs euthanised after exposure for 1 hour. Salmonella spp. were recovered from the caecal contents of all pigs after exposure for 3, 6 and 24 hours.

Introduction It is recommended that pigs be rested for a minimum of 2 hours prior to slaughter, to recover from the stress of transport and to improve meat quality. However, recent research has shown that pigs reared in an environment free of Salmonella spp. or on farms with low levels of infection may acquire infection during transport to the abattoir or while held in lairage (Hurd et al., 2001a), (Hurd et al., 2002), (Morgan et al., 1987). In the lairage up to 90% of environmental samples collected may test positive for Salmonella spp., indicating that the lairage may be an important potential source of infection for incoming animals (Swanenburg et al., 2001). Experimental infection with Salmonella spp. may be carried out by inoculation per os, intranasal instillation or intraperitoneal injection. However, in order to simulate a natural mode of transmission in the studies described here, animals experimentally infected by intranasal instillation were used to seed the environment with salmonellae. The use of infected animals, which were shedding salmonellae, also ensured that challenged pigs were exposed to Salmonella spp., which had been passage in vivo. Recent data obtained using a simulated lairage model have shown that pigs can become rapidly infected with Salmonella spp. when exposed to an environment contaminated with these organisms (Hurd et al., 2001b). In contrast with these findings, in Denmark Boes et al. (2001) found relatively low levels of cross contamination among pigs from Salmonella spp. negative herds held in lairage for two to four hours in pens contaminated with infected faeces. It appears that the level of environmental contamination present in the lairage and the length of time for which pigs may be exposed to this contamination are important factors in determining the level of cross contamination which can occur in pigs prior to slaughter. The primary objective of this study was to determine if short-term exposure to an environment contaminated with Salmonella spp. such as occurs in the lairage is sufficient to produce infection in pigs. Therefore in order to ascertain the minimum exposure time necessary to produce infection, pigs were exposed for one, two, three, six or twenty-four hour periods. The effect of two different levels of contamination was also investigated.

Materials and Methods Animals These studies were conducted in isolated sheds with solid concrete floors, measuring approximately 4m X 4m, at the Department of Agriculture and Food Laboratory Complex in Abbotstown, Dublin using 31 weaned piglets sourced from a commercial supplier (Hermitage AI, Kilkenny) of Category 1 Salmonella spp. status. Approximately 1 week prior to each trial faecal and blood samples were collected from each pig to ensure that all pigs were negative for infection with Salmonella spp.

Challenge Strain A nalidixic acid resistant S. Typhimurium (PT12) was used as the challenge strain throughout the study. A swab from a pure stock culture held at -70°C was used to inoculate 10ml of buffered peptone water (BPW), which was then incubated for 16-18 hours at 37°C. A 1%
inoculum was then transferred into 30ml of fresh BPW and grown in an orbital shaker for 3.5 hours at 37°C until it reached an optical density of 0.8 at 600nm (OD600) i.e. mid-exponential growth phase. The culture was centrifuged (4000rpm for 15mins), supernatant was removed and the pellet was resuspended in 15ml of phosphate-buffered saline (PBS). The concentration of organisms in the inoculum was estimated by the spread plate method on nutrient agar (NA). In Trial 1, the final concentration of the inoculum was approximately 108 CFU/ml, while in Trial 2 the final concentration was approximately 109 CFU/ml.

**Experimental Design**

**Trial 1:** Pigs in Trial 1 were randomly allocated to 1 of 5 categories: shedders (n=2), exposure for 2 hours (n=6), exposure for 3 hours (n=6), exposure for 24 hours (n=6) and negative control (n=1). Approximately 1 week prior to the start of the trial, 2 pigs were challenged intranasally with 5ml of *S. Typhimurium* cells (~108 CFU/ml). These pigs were housed separately from the rest of the trial pigs and faeces were allowed to accumulate. On the day of the exposure trial, the infected shedder pigs were removed from the trial pen and euthanised. The remainder of the trial group (n=18) were moved into the trial pen. These pigs were euthanised 2, 3 and 24 hours later. The negative control pig was housed in a separate shed throughout and euthanised 24 hours after the exposure trial started. Tissue samples from each pig were collected aseptically using sterile instruments, gloves and sample containers.

**Trial 2:** As in Trial 1, pigs were randomly allocated to specific categories: shedders (n=2), exposure for 1 hour (n=3), exposure for 3 hours (n=2), exposure for 6 hours (n=2), exposure for 24 hours (n=2) and negative control (n=1). Two pigs were inoculated and allowed to shed for approximately 3 weeks. Faecal material was monitored quantitatively until an environmental load similar to commercial lairage conditions was achieved.

On the day of the exposure trial, the infected pigs were removed from the trial pen and euthanised. The remainder of the trial was performed as described for Trial 1 with the exception that animals were euthanised after 1, 3, 6 and 24 hours of exposure.

In both trials, floor swabs were collected using a sterile premoistened swab with a sterile 100cm2 template as a guide. These samples were analysed quantitatively in order to estimate the bacterial load present on the pen floor.

**Microbiological Analysis**

Samples were analysed by conventional culture methods based on BS EN 12824: 1998 (Anonymous, 1998). All tissue specimens were inoculated into BPW in a ratio of approximately 1:9 w/v. Samples subjected to qualitative analysis were incubated for 18-24 hours at 37°C. Following incubation, 0.1ml from each pre-enrichment homogenate was added to 10ml of Rappaport-Vassiliadis (RV) broth and incubated for 24h at 41.5°C. A portion of the RV broth was then streaked onto brilliant green plates containing nalidixic acid (25µg/ml). Plates were incubated for 18h – 24h at 37°C. Following incubation, suspect colonies were subcultured onto Mac Conkey (MAC) agar and subsequently screened biochemically on triple sugar iron agar slopes (TSI). Caecal contents (Trials 1 & 2), ICJ (Trial 1), ICLN (Trial 1), tonsils (Trial 2) and MLN (Trial 2) were examined quantitatively using a modified 3-tube MPN method (REF). Following homogenisation in BPW each sample was divided into 3 x 10ml, 3 x 1ml and 3 x 0.1ml aliquots (tonsils, MLN and ICLN) or 3 x 20ml, 3 x 2ml and 3 x 0.2ml aliquots (caecal contents, ICJ). Enrichment and selective plating were performed as described. Following biochemical and serological confirmation, the number of salmonellae present in each sample was calculated using the MPN table of de Man (de Man, 1983).

**Statistical Analysis**

The prevalence of *Salmonella* spp. in infected pigs was reported as the number of tissue samples which tested positive. Differences in prevalence between each time point were compared using the chi square option of the frequency procedure of Minitab Release 14 Statistical Software for Windows. All statistically significant differences were reported at the P<0.05 level of error.

**Results**

**Trial 1:** The challenge strain was not detected from any pig before exposure or from any sample taken from the negative-control pig. The bacterial load present in the trial pen was estimated at 5.4 log 10 CFU/100cm2.

The frequencies of recovery of *S. Typhimurium* from tissue samples are presented in Table 1. Salmonellae were detected in at least one sample from each pig after exposure for 2 or more hours. *Salmonella* spp. were not detected in any spleen samples and only in the lung and liver of one animal following 24 hours of exposure.

Overall the tissue samples from which *S. Typhimurium* was recovered most frequently were...
the tonsils (100%), ICJ (94%), ileocaecal lymph nodes (89%) and the caecal contents (89%). A large proportion of lung (5/6), liver (5/6) and spleen (6/6) samples were infected after 2 hours of exposure. However, the number of infected lung, liver and spleen samples decreased significantly (p = <0.001) after 24 hours of exposure, with only one lung sample and one liver sample testing positive for *Salmonella* spp. In contrast the number of infected gut-associated tissues (lymph nodes, caecal contents, ICJ and the tonsils) did not alter significantly within the same time period.

All samples of caecal contents, ICJ and ICLN were analysed quantitatively. The numbers of organisms in lymph node and caecal contents tended to increase over the course of time. However, the ICJ was consistently infected at high levels throughout the course of the experiment.

**Trial 2: *Salmonella* spp.** were not recovered from any sample from the negative-control pig. The bacterial load present in the trial pen on the morning of the exposure trial was confirmed at ~2.65 log10 CFU/100cm2.

Results of samples for all challenge-exposed pigs are shown in Table 2. The challenge strain was not detected in any samples from 3 pigs euthanised after exposure for 1 hour. *Salmonellae* were detected only in the caecal contents of 2 pigs that had been exposed for 3 hours, and from 2 pigs, which had been exposed for 6 hours. The organism was cultured from the tonsils from one pig that had been exposed for 6 hours.

*S.* Typhimurium was isolated from both pigs euthanised after exposure for 24 hours. Caecal contents showed positive results for both pigs, however salmonellae were not isolated from the mesenteric lymph nodes or from the tonsils.

Quantitative analysis of positive samples showed that the number of organisms isolated from caecal contents from 2 pigs euthanised after 3 hours of exposure was approximately 1.7 log10 CFU/g per pig. After 6 hours of exposure, >1.7 log 10 CFU/g were present in the caecum of one animal and −0.3 log10 CFU/g in the other. The number of organisms isolated from the positive tonsil sample was 0.9 log10 CFU/g. No increase in the number of salmonellae isolated from pigs that had been exposed for 24 hours was observed.

**Discussion**

The tissue distribution of *S.* Typhimurium in pigs following experimental infection has been previously described (Wood *et al*., 1989), (Wood and Rose, 1992). Quantitative data on numbers of organisms in the most commonly infected organs in long-term carrier pigs has been published (Wood and Rose, 1992). Limited data is currently available concerning quantitative analysis on the numbers of *S.* Typhimurium that may be found in internal organs of acutely infected pigs following short-term exposure to *Salmonella* spp. (Fedorka-Cray *et al*., 1994). The results generated from this type of data may be important in implementing control measures following short-term exposure to a contaminated environment, as occurs during transport and lairage. In Trial 1 the finding of high numbers of *Salmonella* spp. in the tissues of all pigs examined after 2 hours was surprising. Previous workers using a similar environmental challenge dose to that described for Trial 1 have successfully isolated *Salmonella* spp. (within 24 hours) from internal organs by qualitative methods but have had difficulty quantifying the actual bacterial load present (Fedorka-Cray *et al*., 1994). In Trial 1, 24-hour samples tended to be more highly contaminated than those taken at 2 or 3-hour intervals as no end points were achieved for these samples. However in Trial 2, this trend was reversed as the numbers of organisms in positive samples decreased with time, with the highest contamination levels occurring within 3 hours. The reasons for this are not clear but it is possible that the lower exposure dose used in Trial 2 was sufficient to initiate but not sustain infection levels.

The results of these studies show that pigs exposed to a contaminated environment can become infected after 2 hours. In Trial 1, all pigs exposed had at least five positive samples within 2 hours of exposure. This result is consistent with

<table>
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<th>24</th>
<th>Total</th>
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**TABLE 1. Number of tissue specimens positive for *Salmonella* Typhimurium from groups of 6 pigs euthanised following 2 to 24 hours of exposure to a contaminated environment.** *Samples are listed in the order in which they were collected from the pig. Sterile technique was maintained throughout in order to avoid cross-contamination of samples.*
previous work, which also demonstrated that a 2-hour exposure period was sufficient to produce infection in pigs (Hurd et al., 2001b). The environmental bacterial dose used in this trial was estimated to be approximately 105 organisms/100cm². Although this may be higher than the actual load that occurs in commercial lairages, other workers have failed to detect a clear relationship between bacterial load in the slurry and acquisition of infection in pigs (Hurd et al., 2001b). In Trial 2, where the environmental bacterial load was reduced to approximately 102 organisms/100cm², *Salmonella* spp. were not detected in any sample from pigs exposed for just one hour. Previous studies by Hurd et al. (2001) (Hurd et al., 2001c) reported the detection of positive samples within 30 minutes of exposure. However, in these studies the environmental challenge dose was estimated at 10⁵ organisms/g, suggesting that a high level of bacterial contamination coupled with a short exposure time is sufficient to produce infection.

**Conclusion** The results reported here demonstrate the rapidity with which *S. Typhimurium* infection can occur in pigs exposed to a contaminated environment. These studies illustrate that exposure to an environment highly contaminated with *S. Typhimurium* can produce infection in pigs within 2 hours, while exposure to a less contaminated environment can produce infection within 3 hours. These findings may be useful in informing changes to improve control measures for *Salmonella* spp. on farm and during transport and lairage of slaughter pigs. Further research is needed to determine the minimum environmental load necessary to produce infection and to investigate other factors affecting possible transmission during transport and lairage.

**References**


