**Abstract** The objectives of this study were to determine the prevalence of *Salmonella* spp. in the lairage of a pork abattoir on different days of the week and to investigate the effect of daily washing routines and disinfection procedures on contamination levels with *Salmonella* spp. In total, 359 swabs were collected from lairage pen floors at three time points during the course of two slaughter days. All samples were analysed quantitatively. On day 1 (Monday), following cleaning and disinfection, 6% of samples were positive for *Salmonella* spp. On day 2 (Thursday), lairage pens were subjected to cold water washing in between batches of pigs; 44% of samples were positive for *Salmonella* spp. The number of positive samples isolated increased significantly over the course of the slaughter week (P <0.001). Quantitative analysis revealed that the numbers of salmonellae rose from <0.46 organisms/100cm² to levels of approximately 4 organisms/100cm² over the course of the week.

**Introduction** Studies have shown that the lairage environment in pork plants can be highly contaminated with *Salmonella enterica* (Rostagno *et al*., 2003), (Swanenburg *et al*., 2001a). In addition it has been demonstrated that pigs can become rapidly infected with *S. Typhimurium*, when exposed to a contaminated environment (Hurd *et al*., 2001). Therefore the lairage may represent a significant source of infection for incoming pigs. Little work has been reported on *Salmonella* spp. contamination in Irish pork plants. There are currently no specific statutory guidelines or regulations in place in the Republic of Ireland regarding reduction of *Salmonella* spp. contamination in lairage facilities. A national serological monitoring programme of pigs at slaughter using an ELISA test has been ongoing in Ireland since 1997 and was enacted into law in 2002. Results from this monitoring system are used to assign salmonella status to herds. Herds are categorised according to the number of positive pigs: category 1: <10% positive, category 2: 10 - 49% and category 3: >50% positive. Current legislation requires that pigs from Category 3 (high-risk) herds be slaughtered separately from other pigs and in a manner that minimises the risk of cross contamination. Abattoirs must therefore nominate a specific day or portion thereof for slaughter of Category 3 herds. This measure was introduced as evidence has shown that when pigs testing seronegative for salmonellae were slaughtered after seropositive pigs, the pork produced was contaminated (Swanenburg *et al*., 2001b). The aim of this study was to investigate the prevalence of *Salmonella* spp. in the lairage of a pork abattoir on different days of the week (including one day where Category 3 herds were slaughtered) and to investigate the effect of daily washing routines and intensive disinfection procedures on environmental contamination levels with *Salmonella* spp.

**Materials and Methods**

**Sample collection** Samples from the floor of the lairage were collected using a sterile premoistened swab with a sterile 10 x 10cm² template as a guide. Samples (n=10) were taken from each of the six most intensively used pens at 3 time points throughout the course of the slaughter day. Samples were taken after the pigs had been moved out of the pen to the point of stunning.

**Microbiological Analysis** All samples were quantitatively analysed by conventional culture methods based on BS EN 12824: 1998 (Anonymous, 1998) using a modified 3-tube MPN technique (Dufrenne *et al*., 2001). Each swab was massaged in 100ml of buffered peptone water (BPW) and subsequently divided into 3 x 20ml, 3 x 2ml and 3 x 0.2ml aliquots. Following incubation at 37°C, 0.1ml from each aliquot was added to 10ml of Rappaport-Vassiliadis (RV) broth and incubated for 24h at 41.5°C. Isolates were obtained by streaking onto brilliant green (BG) and mannitol lysine crystal violet brilliant green (MLCB) agar plates. The agar plates were incubated for 24 hours at 37°C and typical colonies were subcultured onto Mac Conkey agar and subsequently screened biochemically on triple sugar iron agar slopes. Three representative isolates per sample were confirmed serologically with polyvalent and single grouping somatic (O) and flagellar (H) anti-
After confirmation, the number of salmonellae present in each sample was calculated using the MPN table of de Man (de Man, 1983).

**Statistical Analysis** Salmonella spp. prevalence was reported as the percentage of samples that tested positive. Differences in prevalence between each time point and sampling days 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** A summary of the Salmonella spp. isolation results for Days 1 and 2 are shown in Figure 1. Salmonella spp. were isolated from 11/179 (6%) samples taken on Day 1, following intensive cleaning and disinfection. On Day 2, 78/180 (44%) samples taken were positive for Salmonella spp. This increase in prevalence was statistically significant (P<0.001).

On Day 1, the number of positive samples isolated increased as the day progressed. Conversely on Day 2, the number of positive samples isolated at each time point decreased throughout the course of the day However, these changes were not statistically significant on either day.

All samples were analysed quantitatively using a modified 3-tube MPN method. The average MPN values/100cm² of samples collected on Day 2 were approximately 4 times greater than those detected on Day 1 at time-point 1.

A total of 89 isolates, representing 5 serotypes were isolated. Results are shown in Table 1. The most frequently isolated serotypes were S. Typhimurium and S. Manhattan. Other serotypes detected included S. Derby, S. Bredeney and S. Livingstone. Phage type analysis of S. Typhimurium isolates revealed that DT12 was the most common phage type recovered.

**Discussion** The objectives of this study were to determine the prevalence of Salmonella spp. in lairage pens in a commercial abattoir as the week progressed. The effect of daily washing routines and intensive cleaning and disinfection on the level of environmental contamination was also investigated, in particular the effectiveness of procedures implemented to reduce contamination in pens which held Category 3 pigs. These studies were conducted, as no published data are available regarding the prevalence of this pathogen or the effect of current hygiene measures in Irish lairage facilities.

The level of contamination recorded at the start of the slaughter week (Day 1, Monday) was relatively low with only 6% of samples testing positive for the organism. This low rate of contamination was attributed to the intensive cleaning and disinfection measures, which were carried out at the weekend prior to sampling. However, as the week progressed, the level of contamination significantly increased. By sampling Day 2 (Thursday), 44% of samples analysed tested positive for salmonellae. Therefore, daily washing routines consisting of high pressure hosing of pens with potable water were not effective in controlling the prevalence of the pathogen. However, it is important to note that the contamination levels may have been much greater in the absence of high-pressure hosing.

These findings have important implications for the Irish Salmonella Control Programme regarding the slaughter of low-risk herds. Currently under Irish legislation, all Category 3 herds must be slaughtered separately, however, animals of Category 1 and Category 2 status are not separated. In practice, pigs from different suppliers are usually held in separate pens; therefore animals from different farms are not mixed in lairage. However, as pens are continuously used throughout the day we noted that pigs from Category 1 herds were held in pens that had been previously occupied by pigs of Category 2 status. The results of this study indicate that on sampling Day 2, the prevalence of Salmonella spp. in the lairage had increased significantly compared to the contamination level reported for Day 1. Thus contamination levels rose over the course of the week despite the fact that all of the herds slaughtered in the days prior to sampling on Day 2 were of Category 1 and 2 status. These data presented here suggest that Category 1 herds could be exposed to high levels of contamination when held in pens contaminated by Category 2 animals or if slaughtered towards the end of the week, as cold water washing of the pens was not successful in reducing or eliminating the pathogen. In order to reduce the risk of exposure to environmental contamination for all herds, in particular those of Category 1 status, it is suggested that different categories of pigs should be assigned to specific holding pens within the lairage facility. This segregation would help to ensure that low risk herds are not exposed to new sources of Salmonella spp. contamination at the abattoir.
In the study abattoir the slaughter of Category 3 herds was confined to the last working day (or portion thereof) of the week. No other animals were slaughtered after Category 3 herds until the lairage and slaughterline were thoroughly cleaned and disinfected. Analysis of samples taken on Day 1 after cleaning and disinfection showed that the level of contamination with *Salmonella* spp. in the lairage was significantly reduced. This finding has important implications concerning the hygiene practices in the lairages of Irish abattoirs and the logistics of separate slaughter of Category 3 herds under the Irish *Salmonella* Control Programme. Based on the results presented here it appears that if cleaning and disinfection is carried out at the weekend, pigs with the lowest level of infection i.e. Category 1, should be slaughtered first, followed by Category 2 pigs and finally herds with the highest rate of infection i.e. Category 3 pigs at the end of the week, immediately prior to intensive cleaning and disinfection. This ensures that at the start of a new week, the level of contamination is significantly reduced and incoming low-risk herds are held and slaughtered in effectively cleaned and disinfected facilities.

All samples were analysed using a modified 3-tube MPN method in order to obtain quantitative data on the numbers of organisms present in the lairage. This type of quantitative data is time-consuming to acquire and infrequently published. It is useful in assessing levels of contamination present and the possible risk of acquisition of infection by pigs exposed to such environments. In the study described here all samples, which tested positive for *Salmonella* spp. on Day 1 following cleaning and disinfection, contained on average 0.46 CFU/100cm². On Day 2, where a greater number of positive samples were isolated, an increase in the number of salmonellae isolated from positive samples was also observed. The numbers of organisms isolated from positive samples ranged from 0.2 to 55 CFU/100cm² (Day 2). These findings indicate that the risk of contamination increased as the week progressed and that the average contamination level was four times greater than that observed at the start of the slaughter week. Despite the high number of positive samples, the level of environmental contamination present was low. Similar low levels of contamination were reported by Rajkowki *et al.* (1998), where the level of contamination present in pig trailers before and after disinfection was examined using both qualitative and quantitative methodologies. It is unknown if the level of contamination reported in the study described here is sufficient to produce infection in exposed pigs. Recent experimental data has shown that as few as 102 CFU/100cm² was sufficient to infect naïve pigs exposed to a contaminated environment similar to a commercial lairage situation (Boughton *et al.*, 2005, this proceedings).

**Conclusions** From the results presented and discussed here it is evident that the lairage can be highly contaminated with *Salmonella* spp. As experimental studies have shown that pigs can rapidly acquire infection following exposure to a contaminated environment, it is highly probable that abattoir lairage facilities represent a significant source of infection for incoming pigs, particularly those originating from low-risk herds. Therefore abattoir lairage pens may represent a potential control point for the reduction and elimination of *Salmonella* spp. in the pork production chain. Daily washing routines were not effective in reducing or eliminating *Salmonella* spp. However, intensive cleaning and disinfection was found to be highly effective in reducing the prevalence of *Salmonella* spp. contamination within the lairage pens. Therefore it is recommended that the current practice of separate holding and slaughter of Category 3 herds be extended to all categories of pigs under the National *Salmonella* Control Programme. In addition, the slaughter of all pigs should be conducted in a manner that allows the slaughter of low-risk herds at the start of the slaughter week, following weekend cleaning and disinfection. Improvements in day-to-day cleaning and disinfection measures may further reduce the potential for cross-contamination to occur. Further work is necessary to definitively demonstrate that reduced levels of contamination in the lairage pens can significantly reduce the number of infected pigs and consequently lead to fewer contaminated carcasses at slaughter.

**References**


Dufrenee, J., Ritmeester, W., Delfgou-van Asch, E., van Leusden, F. and de Jonge, R. 2001. Quantification of the contamination of chicken and chicken products in the Netherlands with *Salmonella* and *Campylobacter*. *J Food Prot.* 64: 538-541
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Figure 1. The prevalence of Salmonella spp. in a total of 359 floor swab samples (60 samples/timepoint) collected from the lairage of a large pork export abattoir. Day 1: Monday, start of slaughter week, following intensive cleaning and disinfection, Day 2: Thursday, end of slaughter week.

Table 1. Serotypes of Salmonella spp. isolated from 359 floor swab samples from the lairage of a large pork export abattoir on two sampling occasions.

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Number (%) of isolates</th>
</tr>
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<tbody>
<tr>
<td>Typhimurium</td>
<td>40 (42%)</td>
</tr>
<tr>
<td>Manhattan</td>
<td>31 (32%)</td>
</tr>
<tr>
<td>Bredeney</td>
<td>8 (8%)</td>
</tr>
<tr>
<td>Derby</td>
<td>6 (6.3%)</td>
</tr>
<tr>
<td>Livingstone</td>
<td>6 (6.3%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Untypeable</td>
<td>3 (3.2%)</td>
</tr>
</tbody>
</table>

Table 1. Serotypes of Salmonella spp. isolated from 359 floor swab samples from the lairage of a large pork export abattoir on two sampling occasions.