The suggested alternative pooling methods should be carried out in the laboratory. Veterinarians should still collect 20 pen samples in the herd using the standard procedure, to ensure that a representative number of pigs are sampled from each herd.

**O 02 Surveillance of zoonotic bacteria in finishing pigs in The Netherlands**


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**Summary:** In The Netherlands, from 1998 till 2002, a surveillance programme for zoonotic bacteria in finishing pigs was conducted at herd level. In 2000-2002, the prevalence of Salmonella spp. approximated 30%, while a significantly decreasing trend was observed when standardizing data for herd size, age and quarter of sampling. Serotype discrimination showed the predominance of S. Typhimurium with an increasing role for phage type DT104. Prevalence estimates for Campylobacter spp. were 97% in 1998 (4th quarter only) and 45% in 1999. For STEC O157, prevalence estimates were 2% and 0% in 1998 and 1999, respectively. By using the samples from this study, a comparison study was conducted in which three different selective enrichment media, i.e. RV, MSRV and DIASALM, were compared for the isolation of Salmonella spp. from pig feces. Both MSRV and DIASALM scored significantly better compared to RV. By using logistic regression analysis of farm and herd specific data, potential risk factors for Salmonella spp. in finishing pig herds were identified and quantified.

**Introduction:** Salmonella spp. and Campylobacter spp. are recognized world-wide as important zoonotic bacteria causing gastro-enteritis in humans. In 1999-2000, a study was conducted in the Dutch general population indicating a total number of cases of campylobacteriosis and salmonellosis of approximately 100,000 and 50,000, respectively (de Wit et al., 2001). Based on typing results, it has been estimated that about one quarter of the human infections with Salmonella spp. in The Netherlands results from the consumption of pork or pork products (Van Pelt et al., 1999). In order to provide critical information for the control of zoonotic bacteria in primary production, a national programme for surveillance of Salmonella spp., Campylobacter spp. and Shiga-toxin-producing Escherichia coli (STEC) O157 in farm animals was implemented in 1997 (Bouwknegt et al., 2003). The main objectives of this programme are to monitor trends in the occurrence of these zoonotic micro-organisms and to identify risk factors for infection of farm animals. Finishing pigs were included in the programme from October 1998.

**Materials and Methods:** A two-stage sampling scheme was used for estimation of prevalence at herd level. Each year, the primary sample size (number of herds to be sampled) was calculated for estimation of the Salmonella prevalence based on the expected prevalence, a desired accuracy of 5% and a confidence level of 90%. Pig farms were randomly selected from a national database stratified according to farm size and geographical region. On each farm, one herd (i.e. finishing pigs housed in the same barn, usually divided in several compartments) was randomly selected for sampling. Yearly, approximately 150 to 200 herds of finishing pigs were sampled. However, due to an
epidemic of Foot and Mouth disease, no samples were obtained from March 2001 till July 2001. Within a herd 12 to 60 samples were taken depending on the herd size, allowing by approximation the detection of ≥ 5% shedding animals at a 95% confidence level. Fresh fecal samples were randomly collected from the floor and equally divided over 2 to 5 pooled samples. From 1998 till 2002, samples were examined for the presence of Salmonella spp. and additionally, in 1998 and 1999, the samples were examined for the presence of Campylobacter spp. and STEC O157. For the detection and typing of these micro-organisms, RIVM standard operating procedures were used. For the isolation of Salmonella spp., in 1998 and 1999, only Rappaport Vassiliadis (RV) was used as selective enrichment medium. In 2000 and 2001, in addition to RV, two semi-solid selective enrichment media, i.e. Modified semisolid RV (MSRV) and Diagnostic semisolid Salmonella medium (DIASALM), were used for comparison. In 2002, both RV and MSRV were used. In addition to the collection of microbiological data, at each farm visit, farm and herd specific information was collected in co-operation with the farm manager by using a questionnaire. Totally, data from 347 farms, collected in 2000 and 2001, were used in logistic regression analyses following the procedure of Hosmer and Lemeshow (1989).

Results: Figure 1 shows the crude annual prevalence estimates for Salmonella spp. in finishing pigs. No significant differences in crude estimates were observed between 2000 and 2002, approximating 30% in the last two years (based on the use of RV and MSRV). However, adjusting these crude estimates for the non-sampling period in 2001 resulted in a continuous decrease within this period. Trend analysis of the results standardized for herd size, age and quarter of sampling, showed a significantly decreasing trend in these three years (P=0.0343). Serotype discrimination showed a two-fold increase in the prevalence of S. Typhimurium DT104 between 2000 and 2001. In 2002, a level comparable to 2001 was observed. Prevalence estimates for Campylobacter spp. were 97% in 1998 (4th quarter only) and 45% in 1999 with a predominance of C. coli. For STEC O157, prevalence estimates were 2% and 0%, respectively.

Figure 1. Crude annual prevalence estimates for Salmonella spp. in fattening pigs.

Table 1 shows the results of the comparison study for the isolation of Salmonella spp. from pig feces. Totally, 1591 samples were examined. Both MSRV and DIASALM scored significantly better than RV (p < 0.01). There was no significant difference between the results of MSRV and DIASALM. Combination
of two selective enrichment media yielded a higher number of positives in all cases. The combination of MSRV and DIASALM scored significantly better than the combination of either MSRV or DIASALM with RV (p < 0.01). The latter two combinations did not differ significantly from each other.

Multivariable logistic regression analysis (model based on 271 records) showed significant associations for: the seasons spring (Odds Ratio (OR) = 3.51; P value = 0.0164), summer (OR = 2.74; P = 0.0358) and autumn (OR = 3.76; P = 0.0077) all compared to winter, a moderate farm size (501-1000 animals) (OR = 4.22; P = 0.0032) compared to a small farm size (201-500 animals), one specific feed supplier (OR=12.3; P = 0.0027) compared to the reference supplier, the use of fermented liquid feed (OR=0.37; P = 0.0280) compared to its non-use, the use of natural ventilation (OR=6.18; P = 0.0231) compared to mechanical ventilation,

Table 1. Comparison of media for the isolation of Salmonella spp. from pig feces samples (n = 1591)

<table>
<thead>
<tr>
<th>Selective enrichment medium</th>
<th>no. of pos. samples after 24 h</th>
<th>% salm. Pos. after 24 h of total salm. pos. after 48 h</th>
<th>No. of pos. samples after 48 h</th>
<th>% salm. pos. after 48 h of total salm. pos. after 48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>RV</td>
<td>69</td>
<td>36,9</td>
<td>82</td>
<td>43,9</td>
</tr>
<tr>
<td>DIASALM</td>
<td>108</td>
<td>57,8</td>
<td>151</td>
<td>80,7</td>
</tr>
<tr>
<td>MSRV</td>
<td>110</td>
<td>58,8</td>
<td>150</td>
<td>80,2</td>
</tr>
<tr>
<td>RV + DIASALM</td>
<td>131</td>
<td>70,1</td>
<td>161</td>
<td>86,1</td>
</tr>
<tr>
<td>RV + MSRV</td>
<td>125</td>
<td>66,8</td>
<td>168</td>
<td>89,8</td>
</tr>
<tr>
<td>MSRV + DIASALM</td>
<td>129</td>
<td>69</td>
<td>180</td>
<td>96,3</td>
</tr>
<tr>
<td>RV + MSRV + DIASALM</td>
<td>142</td>
<td>75,9</td>
<td>187</td>
<td>100</td>
</tr>
</tbody>
</table>

presence of a hygiene lock (OR=4.41; P = 0.0038) compared to its absence, presence of cats on the farm (OR=0.25; P = 0.0222) compared to the absence of animals and an age of 2-3 months (OR=0.17; P = 0.0446) compared to an age of 3-4 months.

Discussion: The Dutch national surveillance programme in finishing pigs yielded valuable information on the occurrence of zoonotic bacteria, the suitability of isolation methods for Salmonella spp. and risk factors for Salmonella infection. The use of an additional, more sensitive selective enrichment medium (MSRV) resulted in two-fold higher prevalence estimates. In 2000-2002, the prevalence of Salmonella spp. approximated 30%, while a significantly decreasing trend was observed when standardizing data for herdsize, age and quarter of sampling. The latter finding may be due to an increased awareness of good hygiene practices in the pig primary production sector. Serotype discrimination showed the predominance of S. Typhimurium with an increasing role for phage type DT104, which corresponds with an increase of this specific phagetype in human salmonellosis (van Pelt, 2001). The prevalences measured for Campylobacter spp. indicate that pigs are an important reservoir for this micro-organism. However, since the main species was C. coli and Campylobacter contamination of pork is normally very low (van der Zee et al., 2000), the relative importance of pork in the epidemiology of campylobacteriosis is likely to be small. Furthermore, pigs do not seem to be an important reservoir for STEC O157. The results of the method comparison study clearly indicate that the semi-solid media MSRV and DIASALM are more sensitive for detection of Salmonella spp. in pig feces compared to the liquid RV medium. Similar results have been found for poultry feces (Voogt et al., 2001). However, since the isolation principle of these semi-solid media is based on migration of the bacteria through the agar,
these media are not appropriate for detection of non-motile or less motile Salmonella bacteria. Therefore, a combination of a semi-solid medium with a liquid medium, such as RV, is recommended. Several factors were significantly associated with the presence of Salmonella spp. The increased risk associated with natural ventilation might be related to biosecurity, which is likely to be lower in naturally ventilated barns. The decreased risk associated with an age of 2-3 months compared to an age of 3-4 months might be related to one or more causes, i.e. (i) maternal immunity preventing Salmonella colonisation at a younger age, (ii) a preventive effect of medication administered at an early stage of life, or (iii) enhanced exposure to Salmonella after transportation to a finishing barn (usually around 10 weeks of age). The protective effect found for the use of fermented liquid feed is hypothetically related to the presence of organic acids in this type of feed which may inhibit the presence of Salmonella in the feed and enhance the acid barrier of the stomach (van der Wolf et al., 2001). The preventive effect found for the presence of cats on the premise might be related to the expelling of rodents, which are considered important vectors for Salmonella infection (Lo Fo Wong et al., 2002). For the other associations determined, no hypotheses could be generated.

References:


Campylobacter prevalence in Danish finisher pigs from mixed production herds

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Summary: The prevalence of Campylobacter jejuni was investigated in finisher pigs from 89 herds with pigs and cattle, 68 with pigs and poultry, and 90 with pigs only. C. jejuni was detected in pigs from 8.5% of herds examined. There was no significant difference in C. jejuni prevalence between herd types. Verification of 5 additional colonies from each C. jejuni-positive sample yielded C. jejuni only in 50% of samples, while the remaining 50% were mixed infections. The results suggest that C.