Quantitative examination of multiresistant *Salmonella* Typhimurium DT104 in slurry from DT104 infected swineherds in Denmark.

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Summary: In order to evaluate the level of multiresistant *Salmonella* Typhimurium DT104 spread by slurry from DT104 infected swineherds, a quantitative examination (MPN) of slurry from 18 DT104-infected Danish swineherds was carried out. The study included the use of different parameters of infection as predictors of the contamination level in the slurry. DT104 was isolated in slurry from 16 of the 18 herds with most probable number estimates from less than 0.02 to 23 cfu per gr. slurry. The prevalence of seropositive meat juice samples in the last month of filling the slurry tank was found to be a significant predictor of the contamination level in the slurry.

Introduction: Slurry from Danish swine herds infected with multiresistant *Salmonella* Typhimurium DT104 (DT104) must be deposited with a hose applicator and ploughed in immediately after spreading on fields according to rules laid down by the Danish Veterinary and Food Administration. To investigate if these special precautions are relevant in order to prevent or reduce the spread of DT104 to wildlife and livestock, a quantitative examination (MPN) of DT104 in slurry was carried out. In addition it was studied if parameters of infection at herd level e.g. serology and bacteriological examination of faecal samples can be used as predictors of the contamination level in the slurry.

Materials and Methods: Slurry-samples were collected from 18 DT104 infected swineherds during February and March 2001. In each herd, nine samples were collected from the slurry tank, three samples from the top, middle and bottom layers respectively.

Information on bacteriological findings in the herd and storage time of the slurry was recorded. For the 14 finisher herds, the prevalence of seropositive meat juice samples (OD%>20) in the last month of filling the slurry tank was obtained from
the Danish Zoonosis Register. Culturing, sero- and phagotyping were done by standard procedures. The three samples from each layer were assessed together by using a three tube MPN-method.

Before the results of the microbiological examination were known, a group, consisting of five of the authors, had agreed on a classification of all 18 herds in to high risk, medium risk and low risk herds based on knowledge of the infection on the individual farm e.g. bacteriological examination of faecal samples, serology, duration of infection and information on last input of contaminated slurry to the slurry tank.

A multivariable regression model with the natural logarithm to cfu/gr. slurry as dependent variable and farm number as random variable was used for the analysis of correlation between MPN-number and other parameters (SAS®, Proc Mixed).

**Results:** DT104 was isolated in slurry from 16 of the 18 herds. The most probable number estimates were ranging from less than 0.02 to 23 cfu per gr. slurry.

All 18 slurry tanks were divided in to three levels based on the most probable number of cfu per gr slurry: high level (>1 cfu/gr.), middle level (0.1-1 cfu/gr), low level (<0.1 cfu/gr.). Table 1 shows the correlation between these three microbiological levels and the risk classification done by the expert group.

<table>
<thead>
<tr>
<th>Bact. exam</th>
<th>High risk</th>
<th>Medium risk</th>
<th>Low risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>High level</td>
<td>4 herds</td>
<td></td>
<td>1 herd</td>
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<tr>
<td>&gt; 1 cfu/gr.</td>
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<td></td>
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<tr>
<td>Middle Level</td>
<td>2 herds</td>
<td>2 herds</td>
<td>1 herd</td>
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<tr>
<td>0.1-1 cfu/gr</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Low Level</td>
<td>2 herds*</td>
<td>2 herds</td>
<td>4 herds</td>
</tr>
<tr>
<td>&lt; 0.1 cfu/gr</td>
<td></td>
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</tbody>
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* In two herds, pig slurry were diluted with non DT104 cattle slurry in the tank

Table 1. Correlation between microbiological examination of slurry and information on infection at herd level

There was a positive correlation between last month serology and ln(cfu/gr) (P=0.0511). There was no significant effect of storage time or sample level (top, middle and bottom) on ln(cfu/gr) in the 14 finisher herds, table 2. When including data from the 4 sow herds in the analysis, there was a tendency that ln(cfu/gr) depended on sample level (P=0.0677), table 3.

Surprisingly we did not demonstrate an association between bacteriological findings in the herds and ln(cfu/gr. slurry).
Discussion: The classification of herds done by the expert group showed a good correlation to the result of the bacteriological examinations. The two most severe misclassifications, where herds with low MPN-value was classified as high risk herds, could be explained by dilution of the slurry with non-DT104 cattle slurry. When analysing the data we found that the last month serology in the 14 finisher herds was the only significant predictor of the DT104-contamination level in the slurry, suggesting that this value can be used to predict contamination level in slurry. The lack of correlation between bacteriological examination on faecal samples and slurry cfu is probably due to the lack of power in the study (small sample-size) and also that the relation between the time of faecal examination and filling the slurry tank varied a lot between herds.

Salmonella dies of in slurry as an effect of storage time. In this study the storage time was ranging from 0 to 12 weeks during the winter, where the average temperature in Denmark is low, causing long bacterial decimation times. The study period thus probable explains the lack of correlation to storage time observed in the present study.

The range of DT104 cfu is comparable to a level previously reported for Salmonella in general in sub-clinically infected herds. There was a tendency that the DT104-contamination of the slurry was higher in the top and bottom layer compared to the middle layer, reflecting that the most recently added slurry is concentrated in the top layer.

All herds in the study having less than 1 cfu/gr. slurry were granted exemptions from the special slurry handling on different conditions. A normal use of slurry in Danish agriculture is approximately 40 ton slurry per 1 hectare (10,000m²). Giving a contamination level of 1 cfu per gram, the spread of DT104 will thus be 4,000 cfu per m² landfill. It has not been possible to evaluate if this constitutes a risk of spreading the infection to livestock and wild life.