Eradication of multi-resistant *Salmonella* Typhimurium DT104 infections in 15 Danish swine herds


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**Introduction**

Multi-resistant *Salmonella* Typhimurium DT104 (=DT104) was first isolated in United Kingdom in the 1980s. DT104 was isolated in Denmark for the first time in 1996 (1). Retrospective analysis of isolates detected DT104 in a Danish swine herd in 1991. The majority of the Danish isolates are characterized by being resistant to 5 frequently used antibiotics; ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracycline (ACStSuT), but isolates being resistant to e.g. fluorquinolones as well have been detected. DT104 is now known as an important and emerging pathogen in many countries (2, 3, 4, 5). DT104 has spread rapidly between animals within the herd, between herds and to other species (6). *Salmonella* Typhimurium DT104 remains the second most common *Salmonella* in humans in England and Wales in 1997. 95 pctl. of the isolates were resistant to four or more antibiotics with the most common resistance pattern is that of ACStSuT (7). The described combination of the ability to spread rapidly and the multi-resistance towards antibiotics used frequently in animals and humans implies that DT104 can be a serious problem for both animals and humans. By June 1999 DT104 has been detected in 16 swine herds, 12 combined swine and cattle herds and 2 cattle herds in Denmark. Humane DT104 cases have slightly increased in Denmark from 1997 to 1998. DT104 now accounts for 13 pctl. of *Salmonella* Typhimurium phage types compared to 7 pctl. in 1997. This increase is explained by the first community outbreak of DT104 in Denmark (8).

In order to protect the consumers, the Danish Bacon and Meat Council decided to depopulate infected herds, when the first herd was detected in 1996. A research project was established in co-operation with the Danish Veterinary Laboratory in order to gain experience with eradication of DT104 from Danish swine herds. The 15 first infected swine herds are included in the research project. 7 of these herds have cattle as well. Preliminary results have been published in 1998 (9).

**Materials and Methods**

In the 15 swine herds included in the research project, DT104 was detected in the period December 1996 to February 1998. The herds were mainly detected by means of the mandatory Danish Salmonella Surveillance and Control Programme in Denmark and subsequently traced back to contact herds (10). The DT104 project involves 15 swine herds; 5 farrow to finisher, 1 farrow to grower, and 9 grower to finisher. Two of the sow herds belonged to the Specific Pathogen Free category, whereas the rest may be categorized as conventional herds without any external infection barrier.

When a DT104 infection is detected in a swine herd, the farm is closed for contact to other farms by a restriction from the District Veterinary Officer. The eradication programme which will be initiated immediately is divided into 5 phases. The initial phase involves transport and slaughtering under special hygiene precautions of all pigs over 65 kg weight as the last batch of the day. The carcasses are heat treated in order to protect the consumers. Intensive rodent control is established at the farm. The District Veterinary Officer will require that contact herds are examined by a large number pooled pen faecal samples 2 or 3 times. A pooled pen faecal sample consists of 5 x 5 grammes of faecal material collected in a pen.

The second phase involves killing of the rest of the swine, poultry, pigeons and cats. Additional animals e.g. cattle, dogs and horses are examined by culture of individual faecal samples at least 3 times during a period of 3 to 4 weeks. Cattle are placed on pasture if possible.

The third phase involves intensive cleaning of the entire farm by the farmer. All manure and slurry are removed. Manure must be ploughed in, and slurry must be laid out with tubs. This is not allowed on pasture, on fields, where the crops are used to immediate feeding, on sloping fields close to streams or closer than 10 meters to a property-line.

When this is sufficiently achieved and approved by a project veterinarian, phase 4 is initiated, which involves final cleaning and disinfection of internal and external facilities at the entire farm by a professional cleaning and disinfection company. Desinfection is done, when faecal material is no longer visible in the barn, and desinfection is carried out with a mixture of 3 pct. glutaraldehyde and a quaternary ammonium compound. All surfaces are soaked with the disinfectant solution followed by a hot steam desinfection with the same compounds. The desinfection compounds are

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allowed to react in the barn for 24 hours. All manure and slurry contaminated soil at the farm is removed by taking away the upper 5 to 10 cm of the soil and replaced with new clean soil or gravel.

When the disinfection has been approved by intensive microbiological testing at the farm, phase 5 is initiated and new animals arrive. The animals are tested by bacteriological examination of pooled pen faecal samples. The animals are tested intensively during the first 6 months in the grower to finisher herds, and sometimes even longer in farrow to grower herds and farrow to finisher herds. Typically, every pen is sampled on a monthly basis. If all tests are negative for DT104, the herd is finally declared free of DT104.

Results

The 15 swine herds were distributed in three epi-centres, which geographically were well separated. The first epi-centre consisted of five herds. Three farrow to finisher herds and two grower to finisher herd. Two of the herds had a direct connection, as one herd received growers from the other herd. One grower to finisher herd is indirectly connected to the others due to the same machine pool and transport vehicles. The last two herds have no known relationship mutually or to the other herds in the epi-centre.

The second epi-centre consisted of five herds. One farrow to grower herd and four grower to finisher herds. Four of the herds had a direct connection, as three of the grower to finisher herds all received growers from the same sow herd. The last grower to finisher herd infected with DT104 in this area had no known relation to the other herds.

The third epi-centre consisted of five herds. Two farrow to finisher herd and three grower to finisher herds, which all received growers from one of the sow herds. Additionally, the last DT104 infected farrow to finisher herd in this area had no known relation to the other herds.

Eight of the herds included in this project were found to be highly seropositive in the Danish National Salmonella Surveillance and Control Programme (10). According to the rules, pooled pen faecal samples were taken in highly seropositive herds, and DT104 was detected in these cases. In five of the herds included in this project pooled pen faecal samples were taken to determine whether the herd was infected or not, because of a directly relation to a DT104 positive herd. In the last two herds pooled pen faecal samples were taken, because of clinical disease with severe bloody diarrhea and death of piglets and gilts. The primary source of infection in the three epi-centres is not determined.

In the last two epi-centres 7 herds with direct relation to one of the infected sow herds were found not to be DT104 culture positive.

The degree of infection varied considerably from farm to farm as measured by the number of seropositive animals and DT104 culture positive pooled pen faecal samples (Table 1). Despite a high number of faecal samples and trapped mice and rats, DT104 could only be isolated from other animal species from four farms; dogs, horses, cattle, pheasants and mice. Several animal species were present at the remaining eleven farms without detectable DT104 infection.

Table 1: Degree of infection level in the 15 examined herds

<table>
<thead>
<tr>
<th>No. of herds</th>
<th>Degree of infection level</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>++</td>
</tr>
<tr>
<td>5</td>
<td>+++</td>
</tr>
</tbody>
</table>

Percentage culture positive pooled pen faecal samples:
+: 0-10%, ++: 11-25%, +++: > 25%

Two variants of resistant DT104 were isolated from the herds during the study. The typical multi-resistant DT104 with resistance to ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracycline was found in all herds, whereas a DT104 variant resistant only to spectinomycin, streptomycin and sulphonamides was isolated from three farms as well.

By March 1998, all 15 herds have been de-populated. Two herds have stopped swine-production and two herds have not decided whether to continue with swine-production or not, and the remaining eleven herds have all been re-populated. One herd became re-infected during the first month in August 1998. DT104 was subsequently isolated from the farmers dogs, pheasants and mice. To reduce the possible sources of infection at the farm, the dogs and pheasants were killed, the standard of the buildings was improved to ensure that rodents and other animals are kept outside, the rodent control was intensified, and the cleaning and disinfection was repeated. By June 1999, 9 herds are declared free of DT104 based on bacteriological examination of pooled pen faecal samples taken every month (Table 2), and the two remaining herds have been tested negative after re-population for 7 months now.

Discussion

Multiresistant Salmonella Typhimurium DT104 in Denmark is mainly characterised by being resistant to 5 antibiotics; ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracycline. In a few farms, DT104 variants expressing fewer antibiotic resistance determinants were found as well as DT104 with resistance to ACStSuT. Resistance to fluoroquinolones was not detected in these 15 herds, but has later been found in DT104 infected swine herds, which are not included in this research project. By
Table 2: Nine herds declared free of DT104 based on culture negative pooled pen faecal samples. Type of herd and number of animals, number of pooled pen faecal samples and months of bacteriological monitoring after re-population.

<table>
<thead>
<tr>
<th>Type of herd (number of sows / number of finishers pr. year)</th>
<th>Total number of pooled pen faecal samples</th>
<th>Months of bacteriological monitoring after re-population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grower to finisher (-/3540)</td>
<td>109</td>
<td>6</td>
</tr>
<tr>
<td>Farrow to grower (250/-) Grower to finisher (-/5000)</td>
<td>282</td>
<td>6</td>
</tr>
<tr>
<td>Farrow to finisher (100/1100)</td>
<td>383</td>
<td>8</td>
</tr>
<tr>
<td>Grower to finisher (-/1400)</td>
<td>94</td>
<td>6</td>
</tr>
<tr>
<td>Grower to finisher (-/2400)</td>
<td>167</td>
<td>7</td>
</tr>
<tr>
<td>Farrow to finisher (450/8000)</td>
<td>512</td>
<td>10</td>
</tr>
<tr>
<td>Grower to finisher (-/3800)</td>
<td>154</td>
<td>6</td>
</tr>
<tr>
<td>Grower to finisher (-/3600)</td>
<td>266</td>
<td>6</td>
</tr>
<tr>
<td>Grower to finisher (-/3500)</td>
<td>223</td>
<td>6</td>
</tr>
</tbody>
</table>

June 1999, DT104 has been detected in 16 swine herds, 12 combined swine and cattle herds and 2 cattle herds in Denmark since 1991. Under Danish conditions, DT104 has not spread rapidly between animals within the farm or between different farms. The results from this research project indicate that eradication of DT104 infections in Danish swine herds is possible.

A national screening of swine herds for DT104 was carried out in Denmark in 1998-1999 revealing a very low prevalence of DT104 in Danish swine herds (11). In consequence of the apparent low prevalence of DT104 in Danish swine herds, the succes with eradication of DT104 in the Danish swine herds and the food safety aspects the Danish Bacon and Meat Council decided to continue the eradication programme in Danish swine herds.

References