Persistence of a *Salmonella enterica* Serovar Typhimurium DT12 Clone in a Piggery and in Agricultural Soil Amended with *Salmonella*-Contaminated Slurry

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Prevalence of *Salmonella enterica* on a Danish pig farm presenting recurrent infections was investigated. A comparison of the pulsed-field gel electrophoresis patterns of fecal isolates from piggeries, waste slurry, and agricultural soil amended with *Salmonella*-contaminated animal waste (slurry) and subclinical isolates from the same farm (collected in 1996 and later) showed identical patterns, indicating long-term persistence of the *Salmonella enterica* serovar Typhimurium DT12 clone in the herd environment. Furthermore, when *Salmonella*-contaminated slurry was disposed of on the agricultural soil (a common waste disposal practice), the pathogen was isolated up to 14 days after the spread, indicating potentially high risks of transmission of the pathogen in the environment, animals, and humans.

The distribution and prevalence of *Salmonella enterica* in food production animal herds are challenges for safe food production (8, 19). Human food-borne disease outbreaks that are associated with pig products not only are a public health concern but also have economic importance worldwide and in Denmark, where the annual production is about 23 million pigs (2). A wide variety of phage types and genotypes of *S. enterica* serovar Typhimurium have previously been identified in Danish pig production (7, 15, 17), and the most frequently isolated phage type of serovar Typhimurium in 1993, 1994, and 1998 was definitive phage type 12 (DT12) (4, 5). Previous investigations have shown that this phage type mainly has been spread clonally by trading of animals (3, 8), while Mutalib et al. (13) have reported the isolation of identical phage types of *S. enterica* serovar Enteritidis in clinical and environmental samples from poultry, indicating clonal survival. Although the same genotype of multiresistant serovar Typhimurium DT104 has repeatedly been isolated from several herds for up to 16 months (4), it is not clear whether persistence was due to chronic subclinical infection in pigs or to the persistence of *Salmonella* in the herd environment. Furthermore, the survival of *Salmonella* in agricultural soil has been monitored at least 10 samples in all were collected each time at regular quarterly intervals from 1998 to 2000. After screening of all samples using the conventional culture methods mentioned below (5), the results were recorded as *Salmonella* positive or negative and not as percent positive, due to the apparent complexity of the environmental samples. We were aware that there could be a continuous “replenishing” of *Salmonella* organisms as a result of fecal shedding by asymptomatic (carrier) animals. However, the purpose of these (environmental) investigations was to see how long the pathogen persisted or could be isolated from the herd environment and the animal waste. As a result, serological tests on animals were not performed or planned in these studies.

Furthermore, investigations were carried out to determine the risks associated with the spreading of *Salmonella*-contaminated slurry on agricultural soil by monitoring the length of persistence and survival of *Salmonella* in the treated soil. Ten samples each were collected from untreated soil (agricultural soil on which no slurry had been spread for at least a year) and treated soil (agricultural soil on which slurry had been spread during the past year or annually). The soil samples were taken by removing an upper 10-cm layer from the surface. On day 0, i.e., the day on which slurry was spread, the 10 agricultural soil samples were collected to ascertain the absence of *Salmonella* in soil prior to the spreading of *Salmonella*-contaminated slurry. Similarly, the slurry was also tested for the presence of *Salmonella* cells. In the case of treated soil, the samples were collected the same day shortly after the slurry had been spread, and later samplings were collected from soil that had been...
ploughed to mix and distribute the fertilizer. The treated soil was monitored for the presence of *Salmonella* by sampling on a weekly basis. These soil samples (untreated and treated soil) were collected from the same area; i.e., 10 samples were collected on each occasion from predetermined sites that were 1 m apart.

As a general practice after collection of samples from the animals, their environment and feed were tested for the presence of *Salmonella* by preculture in enriched, buffered peptone water and the use of selective media (5). The isolates characterized in the present investigation are listed in Table 1. The strains were identified as serovar Typhimurium according to the Kauffman-White typing scheme (16). Phage typing was performed according to the scheme described by Callow (6) and modified by Anderson et al. (1).

All isolates were typed using pulsed-field gel electrophoresis (PFGE) to investigate the relationships among different isolates. Preparation of total DNA and the experimental setup were as described in reference 10 except that plugs had a final agarose concentration of 0.7% and 0.1 mg of proteinase K per ml was used for proteolysis. Restriction enzyme digestion was performed according to the scheme described by Callow (6) and modified by Anderson et al. (1). The molecular size markers (kilobases) (Lambda Ladder PFG marker; New England Biolabs). Lanes 2 to 5 contain DNA from selected *Salmonella* serovar Typhimurium isolates from piggeries, slurry, and agricultural soil amended with *Salmonella*-contaminated slurry (i.e., treated soil), as follows: lane 2, isolate S 83—piggery sample (tested on 10 March 2000); lane 3, isolate S 86—slurry sample (tested before being spread on agriculture soil on 11 March 2000); lane 4, isolate S 88—treated soil sample (sampled on 11 March 2000); lane 5, isolate S 114—treated soil sampled 14 days after spreading of slurry. All serovar Typhimurium DNA samples shown were digested with the restriction enzyme *Bln*I.

![FIG. 1. PFGE typing of selected *Salmonella* serovar Typhimurium strains isolated from piggeries, slurry, and soil amended with *Salmonella*-contaminated slurry (treated soil). Lane 1 contains molecular size markers (kilobases) (Lambda Ladder PFG marker; New England Biolabs). Lanes 2 to 5 contain DNA from selected *Salmonella* serovar Typhimurium isolates from piggeries, slurry, and agricultural soil amended with *Salmonella*-contaminated slurry (i.e., treated soil), as follows: lane 2, isolate S 83—piggery sample (tested on 10 March 2000); lane 3, isolate S 86—slurry sample (tested before being spread on agriculture soil on 11 March 2000); lane 4, isolate S 88—treated soil sample (sampled on 11 March 2000); lane 5, isolate S 114—treated soil sampled 14 days after spreading of slurry. All serovar Typhimurium DNA samples shown were digested with the restriction enzyme *Bln*I.](http://aem.asm.org/)

**TABLE 1. *Salmonella* serovar Typhimurium isolated from piggeries, slurry, and agricultural soil samples of the investigated Danish pig farm with recurring *Salmonella* infections**

<table>
<thead>
<tr>
<th>Isolate(s)</th>
<th>Source</th>
<th>Sampling date</th>
</tr>
</thead>
<tbody>
<tr>
<td>9622953</td>
<td>Clinical sample</td>
<td>1 October 1996</td>
</tr>
<tr>
<td>9720733</td>
<td>Clinical sample</td>
<td>24 February 1997</td>
</tr>
<tr>
<td>97720424</td>
<td>Clinical sample</td>
<td>29 October 1997</td>
</tr>
<tr>
<td>S 55</td>
<td>Slurry</td>
<td>29 June 1998</td>
</tr>
<tr>
<td>S 75-77</td>
<td>Slurry</td>
<td>11 February 2000, 10 March 2000</td>
</tr>
<tr>
<td>S 78-85</td>
<td>Piggery</td>
<td>10 March 2000</td>
</tr>
<tr>
<td>S 86</td>
<td>Slurry</td>
<td>11 April 2000</td>
</tr>
<tr>
<td>S 87-103</td>
<td>Treated soil</td>
<td>11 April 2000, 13 April 2000</td>
</tr>
<tr>
<td>S 105</td>
<td>Slurry</td>
<td>17 April 2000</td>
</tr>
<tr>
<td>S 106-S 116</td>
<td>Treated soil</td>
<td>17 April 2000, 25 April 2000</td>
</tr>
<tr>
<td>S 118-S 123</td>
<td>Piggery</td>
<td>12 May 2000</td>
</tr>
</tbody>
</table>

* Agricultural soil amended with *Salmonella*-contaminated slurry.
and fields should give rise to further control measures related to the handling of manure and slurry and the disinfection of pig production facilities. It is thus important to point out that despite the limitations of the methods of isolation and detection encountered for the environmental samples mentioned above, the isolation of viable *Salmonella* cells from agricultural soil under natural environmental conditions even after 14 days of the spread of contaminated slurry is a great risk factor. This is important, since our laboratory investigations using terrestrial microcosms under controlled conditions indicate that serovar Typhimurium clones DT104 and DT12 can survive up to 299 days (S. Baloda, unpublished data). The survival of the pathogen in contaminated soil can facilitate the spread of pathogens (infection) via grazing farm animals, birds, cats, dogs, rodents, and even humans. As a result, effective waste management practices should be devised in view of the long-term survival potential of this zoonotic pathogen in soil amended with *Salmonella*-contaminated slurry.

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REFERENCES


