Salmonella Contamination of Pigs and Pork in an Integrated Pig Production System

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ABSTRACT

This paper describes the monitoring of Salmonella in a closed pig production system in Belgium over a 2-year period. A sampling scheme including animal feeds and carcasses was designed to cover the entire chain of production from farrow to finishing pigs. Salmonella was detected by a method based on the use of semisolid Rappaport-Vassiliadis as a selective medium. The serotypes of the isolated strains were determined, and the antibiotic resistance of these strains to six antibiotics was also investigated. Feeds were found to be more contaminated than expected (10.2%, 34 of 332 samples). The percentage of positive fecal samples for pregnant sows (8.1%, 11 of 135 samples) was significantly higher than that for young and lactating sows (2.9%, 11 of 378 samples) (P < 0.05). The percentage of positive samples for colon contents collected at the slaughterhouse (47.3%, 88 of 186 samples) was significantly higher than that for feces collected during the fattening stage (5.6%, 18 of 320 samples) (P < 0.001). For carcass swab samples, the observed prevalence was 11.2% (17 of 152 samples). On farms, Salmonella recovery levels were higher for overshoe samples than for fecal samples, except for pregnant sows. Salmonella Typhimurium was the most frequently isolated serotype (32.2%, 55 of 171 samples), while Salmonella Brandenburg was predominant in the colon contents collected at the abattoir (21.4%, 18 of 84 samples). Feeds harbored a wide diversity of serotypes of minor epidemiological significance. Of 55 isolated strains of Salmonella Typhimurium, 11 (20%) were resistant to tetracycline, ampicillin, chloramphenicol, streptomycin, trimethoprim, and nalidixic acid (R Type TeAmCSN), while 12 (21.8%) were resistant to all of these antibiotics except nalidixic acid (R Type TeAmCS). The majority of Salmonella Typhimurium strains that exhibited resistance to more than four antimicrobial agents were characterized as Salmonella Typhimurium DT104 or as being closely related to Salmonella Typhimurium DT104 (7 of 12 isolates). In conclusion, our system of surveillance is effective in identifying most points of contamination in the production chain and will be useful in ongoing efforts to develop a Salmonella-free production system.

Little is known about the relationship between human salmonellosis cases and animal sources in Belgium. In 1996, 601 cases (in 63 outbreaks) of foodborne disease were reported (10). Of these cases, 503 were due to Salmonella. This finding indicates that Salmonella is the leading cause of foodborne disease in Belgium. Apart from these data, little is known about the role of animal products in human salmonellosis and the relative importance of animal species as sources of the disease.

In a study of the importance of bacteria, viruses, and parasites in foodborne illnesses, Mead et al. (34) estimated that nearly 1,500,000 cases of human nontyphoid salmonellosis occur annually in the United States. Salmonella spp. accounted for 31% of all deaths due to acute gastroenteritis. These data confirm the importance of Salmonella as a source of foodborne disease, prompting efforts to implement surveillance and control systems during harvesting. Such efforts are called “preharvest pathogen reduction” efforts (6) by the American authorities.

According to Steinbach and Hartung (42), 20% of human cases of salmonellosis in Germany are caused by the consumption of pork, and in Denmark, pork products were estimated to be responsible for 15% of human salmonellosis cases (9). In a large salmonellosis outbreak in Denmark in 1993, pork meat from live animals infected at the farm level was identified as the principal source of contamination. At this time, a nationwide plan was set up to eradicate Salmonella at the herd level and to ascertain the serological statuses of pigs before slaughter (35, 38).

In recent decades, enzyme-linked immunosorbent assays (ELISAs) and polymerase chain reaction methods have been marketed (4, 7, 8, 35, 38, 47). Furthermore, tests based on the ability of Salmonella to migrate on a semisolid medium have been used. One of the first media developed for such tests was modified semisolild Rappaport-Vassiliadis (MSRV) medium. De Smedt et al. (16) demonstrated that the recovery rate obtained with this medium was better than that obtained with classical enrichment in tetrathionate brilliant green broth. MSRV medium produces results after 48 h. A major advantage of MSRV medium is its ability to detect low levels of Salmonella in samples and its efficiency in isolating Salmonella among important competitive microflora (15). A second medium, whose formula is derived from that of MSRV medium, is Diasalm. Landman et al. (31) and De Zutter and Daube (17) compared this...
medium with the classical Rappaport-Vassiliadis medium broth and observed a better recovery rate for Diasalm, es-
pecially for poultry meat.

On-farm sampling is necessary to pinpoint sources of contamination in a pig production system and has been used by Letellier et al. (32, 33) in some studies. After identifying several risk factors, such as insufficient hygienic procedures and the presence of flies or rodents, these authors found a higher contamination rate for breeding pigs such as replace-
ment gilts. This finding was confirmed by Davies et al. (11).

Another study has proved that the constant exposure of pigs to fecal sources may prolong the shedding state of the an-
imals (12). Insufficient farming hygiene and the use of broad-spectrum antibiotics were also considered important risk factors in another study (5). Scandinavian countries have set up control programs to estimate the prevalence of Salmonella in living pigs (21, 26).

The aim of the present work was to design an efficient protocol to determine as accurately as possible the bacte-
riological statuses of herds at each stage of a closed system for the production of pigs and to report the results of a 2-
year surveillance project.

MATERIALS AND METHODS

Structure of the production system followed. Fourteen herds, comprising 11 pure fattening herds, 1 breeding-and-mul-
tiplying herd, 1 farrow-to-finishing herd, and 1 collecting unit of weaned pigs, were followed from 1 November 1999 to 31 October 2001. The number of pigs per fattening herd ranged from 50 to 1,000. The all-in–all-out systems practiced involved periods of <2 days for two herds, 2 to 5 days for seven herds, and >5 days for four herds. This included sanitizing of free animals’ barns. The majority of working units (for 10 of the 13 herds) were >20 years old.

Except for the farrow-to-finishing herd, which represented a complete production system and produced its own piglets, the production systems were those of producer groups adhering to common quality programs, with the same feed being delivered consistently and a large number of handling specifications being described (e.g., with a fasting period being defined as at least 12 h). The pigs belonged to the company that slaughtered the finished pigs and then processed the meat. This situation can be defined as a vertical integration system.

The herds were located in the eastern part of Belgium within a perimeter of ≤200 km from the slaughterhouse, so the average transport time to the slaughterhouse did not exceed 2 h. In total, 6,800 slaughter pigs are produced each year in this production system. The breeding-and-multiplying herd provided all of the weaned piglets, with weaning taking place at 4 weeks of age. Piglets from the weaning stage, after being gathered in the collecting unit for weaned pigs, were distributed at a weight of 20 kg among the 12 fattening herds involved in the production sys-
tem. This breeding-and-multiplying herd was composed of only 600 sows, a sufficient number for the provision of pigs to the production group.

To produce the breed used in the present study, Pietrain and Large White pigs were crossbred with Belgian Landrace pigs. Housing structures for the growing and fattening of pigs consisted of integral duckboards for six herds and consisted of duckboards limited to half the pen for eight herds. The number of barns ranged from 1 to 30, and open communications between barns took place for 9 herds. In such cases, the producers had to walk outside the

buildings in order to visit other barns. Pest control programs were applied for 12 of the 14 herds, and complete disinfection between batches was carried out for 11 of the 14 herds. The disinfectants used were composed of aldehydes and quaternary ammonium compounds authorized for agricultural use. An average of 8 to 10 pigs occupied a pen. The feed was dry except for pregnant and lactating sows, for which a wet feed was produced by premixing the meal with water. Two approved growth factors were used in the feed: avilamycine (40 ppm) in the feed of piglets weighing 7 to 20 kg and salinomycine (20 ppm) at the beginning of the fattening period for pigs weighing 20 to 35 kg. No other antibiotics were used systematically in the feed. Otherwise, the formulation of feed was classical and included 80% soya and cereals.

Bacteriological sampling on the farm and at the slaugh-
terhouse. The sampling scheme was defined at the beginning of the project (Table 1). One of the goals of the scheme was to follow the entire production system, from feed to finishing pigs and carcasses. Sample traceability was complete up to the last step. In order to decrease the workload while maintaining a general view of contamination on the farm, on-farm fecal samples (ca. 300 g) were composite samples from five portions of fresh piles of feces collected from five different pens in the same barn, with each barn containing animals of the same origin and age. Before entering a pen to sample feces, the collectors put on overshirts with which to swab the ground surface. The oversieve samples corresponding to the feces collected in a barn were placed in another stomacher bag for further processing. The single-use oversieve used (Ref GMEOP 010, VWR International, Leuven, Belgium) were white, polypropylene, not weaved, and without insole. Pooled colon content samples and carcass swab samples were collected at the slaughterhouse. Carcass-swabbing areas (Fig. 1) were based on those described by Korsak et al. (30). This sampling method has been used since 1998 for the official monitoring plan of the zoo-
notic agent surveillance program in Belgium (22). For one car-
cass, the area swabbed, including four different zones, totaled 600 cm². Swabs from five carcasses of the same origin were collected aseptically and pooled in the same sterile stomacher bag. All sam-
pleswere transported in a refrigerated box and analyzed after a delay of ≤12 h.

Bacteriological analyses. The protocol for bacteriological analyses was based on SP-VG-M002, the official method of the Belgian Ministry of Public Health for the detection of Salmonella in foods. The Diasalm medium (Lab 537, Lab M, International Diagnostics Group PLC, Lancashire, UK) is used in this method. Diasalm, whose composition was described by De Zutter et al. (18), is a semisolid medium that is used for the identification of motile Salmonella enterica in food material and is closely related to MSRV medium. The formula is completed with the addition of novobiocin (Novobiocin 5g, N-1628, Sigma-Aldrich GmbH, Steinheim, Germany).

After nonselective preenrichment of a 25-g sample in 225 ml of buffered peptone water (CM509, Oxoid, Basingstoke, UK) for 18 to 20 h at 37°C, 0.1 ml was streaked onto the center of a Diasalm plate, which was incubated at 42°C for 24 h. The isola-
tion was carried out with xylose–lysine–tergitol 4 medium (XLT4 medium, 63654, Biorad, Marnes La Coquette, France) after 22 ± 2 h of incubation at 37°C. Confirmation was achieved with clas-
sical, biochemical (e.g., API20E, bioMérieux, Lyon, France), and serological methods according to the Kaufmann-White scheme (39, 41). For overshoe samples, the protocol was the same except that 300 ml of buffered peptone water was added to the stomacher bags before the samples were analyzed.
TABLE 1. Sampling plan for the production system

<table>
<thead>
<tr>
<th>Sampling point</th>
<th>Type of sample</th>
<th>Frequency and methoda</th>
<th>Sampling quantity</th>
<th>Analytical quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed-production</td>
<td>Feed</td>
<td>About three samples a week; control samples were kept after feed components were mixed</td>
<td>About 500 g</td>
<td>25 g</td>
</tr>
<tr>
<td>factory</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breeding</td>
<td>Feces, overshoes</td>
<td>Once a month (with each farrow being inspected); five fresh feces samples were collected on the ground and pooled in one bag; overshoes worn by sample collectors were also analyzed</td>
<td>About 300 g for feces; overshoes disposed in one analytical bag</td>
<td>25 g for feces; the entire bag for overshoes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fattening</td>
<td>Feces, overshoes</td>
<td>Twice for each batch (after 2 and 4 mo of fattening); five fresh feces samples were collected on the ground in five different pens and pooled in one bag; overshoes worn by sample collectors were also analyzed</td>
<td>About 300 g for feces; overshoes disposed in one analytical bag</td>
<td>25 g for feces; the entire bag for overshoes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slaughterhouse</td>
<td>Contents of large intestine</td>
<td>One for each batch; five pooled samples of colon contents of five different pigs were collected</td>
<td>About 100 g</td>
<td>25 g</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcasses</td>
<td>Swabs</td>
<td>Once for each batch; four surface swabs (total for one carcass: 600 cm$^2$) of five different carcasses were pooledb</td>
<td>20 pieces of gauze (5 carcasses x 4 swabs per carcass) in one analytical bag</td>
<td>The entire bag</td>
</tr>
</tbody>
</table>

a A batch is defined as a group of pigs of the same age, fattened together in the same barn and fed with the same feed throughout the fattening period.

b The same pigs sampled for large intestine contents.

Characterization of isolated strains. The strains were tested by the disk diffusion method to establish antibiotic resistance profiles according to the recommendations of the National Committee for Clinical Laboratory Standards (36). For this purpose, six antibiotics commonly used in human and veterinary medicine were tested: tetracycline (Te, 30 μg), ampicillin (Am, 10 μg), chloramphenicol (C, 30 μg), streptomycin (S, 10 μg), trimethoprim (TMP, 5 μg), and nalidixic acid (Na, 30 μg). All disks (Sensi-Disc) were provided by Becton Dickinson (Meylan, France), and Mueller-Hinton II agar was used (254032, Becton Dickinson). Breakpoint diameter values were 14 mm for Te, 13 mm for Am, 12 mm for C, 11 mm for S, 10 mm for TMP, and 13 mm for Na (values below these levels indicated that strains were resistant). Phage typing was carried out by the Institut Pasteur of Belgium with the use of the bacteriophage-typing scheme proposed by Anderson et al. (1).

Statistical analyses of surveillance results. Chi-square tests with fourfold tables were used to determine statistical differences between results obtained along the production chain from fecal samples for pregnant sows to large intestine contents collected at the slaughterhouse and from overshoe samples for pregnant sows to overshoe samples for fattening pigs. Comparisons of prevalence levels obtained for 25-g feces samples and those obtained for overshoe samples were also carried out for the breeding and fattening stages.

For the 138 batches of fattening pigs from the 13 herds, different combinations were established (e.g., intestinal contents positive or negative during fattening and positive or negative at slaughter), and chi-square tests were also carried out in order to establish concordance between Salmonella status during fattening and that at the abattoir. For statistical analyses of serotypes recovered at different stages and of serotypes with different anti-
TABLE 2. Surveillance of Salmonella contamination along the production chain (November 1999 to October 2001); prevalence levels for feeds, feces collected from herds, large intestine contents collected at the slaughterhouse, and carcasses.

<table>
<thead>
<tr>
<th>Sampling point</th>
<th>No. of samples positive/no. of samples analyzed (% positive)</th>
<th>Chi-square valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feeds</td>
<td>34/332 (10.2)</td>
<td></td>
</tr>
<tr>
<td>Pregnant sows</td>
<td>11/135 (8.1) vs 9/84 (10.7) vs 1/61 vs 11/378 (2.9)</td>
<td>0.16 NS</td>
</tr>
<tr>
<td>Lactating sows</td>
<td>10/317</td>
<td></td>
</tr>
<tr>
<td>Young sows</td>
<td>2/125 (1.6) vs 16/82 (19.5) vs 1/36</td>
<td></td>
</tr>
<tr>
<td>Lactating and young sows</td>
<td>11/378 (2.9) vs 18/259 (6.9) vs 1/36</td>
<td>5.77*</td>
</tr>
<tr>
<td>Weaned pigs</td>
<td>11/84 (10.7) vs 17/223</td>
<td></td>
</tr>
<tr>
<td>Fattening stage</td>
<td>2/125 (1.6) vs 16/82 (19.5) vs 1/36</td>
<td></td>
</tr>
<tr>
<td>2 mo</td>
<td>7/165 (4.3) vs 15/122</td>
<td></td>
</tr>
<tr>
<td>4 mo</td>
<td>11/155</td>
<td></td>
</tr>
<tr>
<td>2 and 4 mo</td>
<td>18/320 (5.6) vs 25/236 (10.6)</td>
<td>4.70*</td>
</tr>
<tr>
<td>Slaughterhouse</td>
<td>88/186 (47.3)</td>
<td></td>
</tr>
<tr>
<td>Carcasses</td>
<td>17/152 (11.2)</td>
<td></td>
</tr>
</tbody>
</table>

a Comparison of results for feces and those for overshoes.

b Large intestine contents.

RESULTS

Field study results. Field survey results are presented in Table 2. The percentage of positive samples for pregnant sows (8.1%, 11 of 135 samples) was significantly higher than the percentage for lactating sows and young sows combined (2.9%, 11 of 378 samples) (P < 0.05). The percentage for pregnant sows was also significantly higher than that for weaned pigs (1.6%, 2 of 125 samples) (P < 0.05).

The percentage of colon content samples testing positive was always significantly higher for samples collected at the slaughterhouse (47.3%, 88 of 186) than for samples collected during the breeding, weaning, and fattening stages (5.6%, 18 of 320 samples) (P < 0.01). For all sampling sites, moderate levels of positive samples were obtained before pigs were transported, and an increase was observed at the slaughterhouse.

For overshoes samples, in contrast to fecal samples, the percentage of samples testing positive for weaned pigs (19.5%, 16 of 82 samples) was significantly higher than that for lactating and young sows combined (6.9%, 18 of 259 samples) (P < 0.01). A significant difference between the percentage for weaned pigs and that for fattening pigs (10.6%, 25 of 236 samples) was also observed (P < 0.05).

Differences observed in comparisons of percentages of positive samples obtained with overshoes and percentages of positive fecal samples were significant at all stages except for the pregnant-sow stage. Levels of Salmonella recovered from overshoe samples were always higher than those recovered from fecal samples.

Analysis of herd statuses during fattening and at the abattoir. To correlate the Salmonella status of pigs during fattening with that of colon contents at the slaughterhouse, different combinations of results were established for all herds. A batch was considered positive during fattening when at least one sample tested positive. Data obtained are presented in Table 3. Concordance of the status results for the fattening stage and those for the slaughterhouse stage was obtained for only 69 of the 138 batches of finishers from the finishing herds followed (i.e., 50% of all batches; cases 1 and 4), and differences were not significant by the chi-square test (χ² = 0.30).

Serotypes isolated. Table 4 presents the distribution of isolated serotypes in feeds, in samples obtained during the breeding and fattening stages (including pooled results for pregnant, lactating, and young sows and for pigs after 2 and 4 months of fattening), in colon contents collected at the abattoir, and in carcass samples. Altogether, 37 serotypes were identified, with 22 different serotypes being isolated from feed samples, 13 being isolated from samples collected during breeding and fattening, 11 being isolated from large intestine content samples, and 5 being isolated from carcass samples. Cumulative results indicate that six serotypes, Salmonella Typhimurium, Salmonella Brandenbourg, Salmonella Derby, Salmonella Infantis, Salmonella Goldcoast, and Salmonella Anatum, accounted for 72% of the isolates. Similar proportions were observed among stages except for feed samples, for which these serotypes represented only 9.9% of the isolated serotypes.

Prevalence levels among stages were significantly different for Salmonella Ibadan, Salmonella Senftenberg, and a pool of minor serotypes that were most often isolated from feed samples. Salmonella Anatum was the serotype that was recovered at the highest levels at the breeding-and-fattening stage, while Salmonella Typhimurium seemed to appear from this same early stage. Finally, Salmonella Brandenburg was recovered only from the colon contents collected at the abattoir.
TABLE 4. Salmonella serotypes isolated from feeds, from samples collected at the breeding and fattening stages, from large intestine contents collected at the slaughterhouse, and from carcasses

<table>
<thead>
<tr>
<th>Salmonella serotype</th>
<th>Feeds (n = 30)</th>
<th>Breeding and fattening samples (n = 40)</th>
<th>Contents of large intestine (n = 84)</th>
<th>Carcasses (n = 17)</th>
<th>Total (n = 171)</th>
<th>Chi-square value a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typhimurium</td>
<td>1 (3.3)</td>
<td>12 (30.0)</td>
<td>36 (42.9)</td>
<td>6 (35.3)</td>
<td>55 (32.2)</td>
<td>15.99***</td>
</tr>
<tr>
<td>Brandenburg</td>
<td>—</td>
<td>—</td>
<td>18 (21.4)</td>
<td>6 (35.3)</td>
<td>24 (14)</td>
<td>21.60***</td>
</tr>
<tr>
<td>Derby</td>
<td>1 (3.3)</td>
<td>8 (20.0)</td>
<td>11 (13.1)</td>
<td>3 (17.6)</td>
<td>23 (13.5)</td>
<td>4.38 NS</td>
</tr>
<tr>
<td>Infantis</td>
<td>—</td>
<td>3 (7.5)</td>
<td>7 (8.4)</td>
<td>—</td>
<td>9 (5.3)</td>
<td>3.61 NS</td>
</tr>
<tr>
<td>Goldcoast</td>
<td>—</td>
<td>3 (7.5)</td>
<td>7 (8.4)</td>
<td>—</td>
<td>6 (3.5)</td>
<td>6.44 NS</td>
</tr>
<tr>
<td>Anatum</td>
<td>1 (3.3)</td>
<td>5 (12.5)</td>
<td>—</td>
<td>—</td>
<td>6 (3.5)</td>
<td>13.23**</td>
</tr>
<tr>
<td>Enteritidis</td>
<td>1 (3.3)</td>
<td>3 (7.5)</td>
<td>—</td>
<td>—</td>
<td>4 (2.3)</td>
<td>7.21 NS</td>
</tr>
<tr>
<td>London</td>
<td>—</td>
<td>2 (5)</td>
<td>1 (1.2)</td>
<td>1 (5.9)</td>
<td>4 (2.3)</td>
<td>3.38 NS</td>
</tr>
<tr>
<td>Ibadan</td>
<td>3 (10)</td>
<td>1 (2.5)</td>
<td>—</td>
<td>—</td>
<td>4 (2.3)</td>
<td>10.13*</td>
</tr>
<tr>
<td>Senftenberg</td>
<td>4 (13.3)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>4 (2.3)</td>
<td>19.25***</td>
</tr>
<tr>
<td>Other serotypes</td>
<td>19 (63.3) b</td>
<td>6 (15.0) c</td>
<td>6 (7.1)</td>
<td>1 (5.9)</td>
<td>32 (18.7)</td>
<td>48.86***</td>
</tr>
</tbody>
</table>

a * P < 0.05; ** P < 0.01; *** P < 0.001; NS, not significant.
b Agona, Bochum, Hithergreen, Lexington, Livingstone, Mbandaka, Moers, Odozi, Ohio, Paratyphi B, Plymouth (two isolates), Rubislaw, Schwarzengruend, Solt (two isolates), Utah, and Wien (two isolates).
c Agona, Babelsberg, Crewe, Ohio, Putten, and Rissen.
d Bredeney, Broughton (two isolates), Kuesel, Livingstone, and Virchow.
e Africana.

Resistance profiles of isolated strains. Table 5 shows the resistance profiles of the major isolated serotypes. *Salmonella* Typhimurium had a significantly higher percentage of antibacterial resistance (78.1%, i.e., 43 of 55 isolates were resistant to at least one antimicrobial agent) than did other serovars (18.4%, with 21 of 114 isolates being resistant to at least one antimicrobial agent).

Twelve of 23 isolates (12 isolates of R type TeAmCS and 11 of R type TeAmCSNa) that were resistant to more than four antimicrobial agents were phage typed. Of these 12 isolates, 4 were characterized as DT104, and three were similar to DT104 (DT12/104). In contrast, of 10 phage-typed isolates exhibiting resistance to one or two antimicrobial agents, only one was characterized as a DT104 isolate (data not shown).

DISCUSSION

For the present study, microbiological methods were preferred to serological methods for several reasons. First, *Salmonella* herd status depends on the chosen cutoff value. For example, in Denmark, this value had to be changed from 40 to 20% optical density (37). Moreover, van Winsen et al. (46) demonstrated that the Dutch ELISA system was more suitable for *Salmonella* Typhimurium and *Salmonella* Brandenburg and less reliable for other *Salmonella* serotypes, such as Livingstone, Goldcoast, and Panama. Finally, in order to obtain information about transmission epidemiology, it is essential to characterize the isolated strains. With the Diasalm method, results can be obtained quickly and easily.

TABLE 5. Antibiotic resistance profiles for *Salmonella* Typhimurium, *Salmonella Brandenburg*, *Salmonella Derby*, *Salmonella Infantis*, and other serotypes a

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Typhimurium (n = 55)</th>
<th>Brandenburg (n = 24)</th>
<th>Derby (n = 23)</th>
<th>Infantis (n = 9)</th>
<th>Other serotypes (n = 58)b</th>
<th>Chi-square value b</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (%) of sensitive strains</td>
<td>12 (22.4)</td>
<td>19 (79.2)</td>
<td>14 (60.8)</td>
<td>8 (88.9)</td>
<td>52 (89.6)</td>
<td>62.38</td>
</tr>
<tr>
<td>No. (%) of resistant strains</td>
<td>43 (78.1)</td>
<td>5 (20.8)</td>
<td>9 (39.1)</td>
<td>1 (11.1)</td>
<td>6 (10.3)</td>
<td></td>
</tr>
<tr>
<td>Resistance profile (no. of strains)</td>
<td>Te (15), S (1), TMP (1), Na (1), Te TMP (1), Te Am C S (12), Te Am C S NA (11)</td>
<td>Te (5)</td>
<td>Te (6), TMP (1), C TMP (1), Te S (2)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Te, tetracycline; Am, ampicillin; C, Chloramphenicol; S, streptomycin; TMP, trimethoprim; Na, nalidixic acid.
b P < 0.001.

Agona (two isolates), Anatsum (six isolates), Babelsberg, Bochum, Bredeney, Broughton (two isolates), Crewe, Enteritidis (four isolates), Goldcoast (six isolates), Hithergreen, Ibadan (four isolates), Kuesel, Lexington, Livingstone (two isolates), London (three isolates), Mbandaka, Moers, Odozi, Ohio (two isolates), Paratyphi B, Plymouth (two isolates), Putten, Rubislaw, Rissen, Senftenberg (four isolates), Schwarzengrund, Solt (two isolates), Utah, Virchow, and Wien (two isolates).
With the sampling scheme used in the present study, it was possible to obtain a full view of the production chain with respect to *Salmonella* spp. contamination rates. The observed percentage of contamination is not an exact representation of the actual shedding rate. Indeed, Hollinger (25) insisted on the intermittent shedding of *Salmonella* by long-term carrier pigs. Hence, the frequency of sampling has to be increased.

Feeds were found to be more contaminated than previously reported. Indeed, Baggesen et al. (3) reported prevalence levels of 0.1, 2.4, and 2.2% for compounded pig feeds, raw materials, and process control samples, respectively, in Denmark. In a survey conducted in America in 1997, a prevalence rate of 2.9% for pig feeds sampled in farms was found (24). In the present study, the most contaminated feeds were feeds for pregnant sows and those for lactating sows; fish meals were also frequently contaminated (data not shown). Reporting on a study conducted in Sweden, Häggblom (23) mentioned that the prevalence of *Salmonella* in feeds decreased from 2% in 1991 to 0.5% in 1996 owing to the strict application of good hygienic practices and the establishment of hazard analysis critical control point (HACCP) principles in the production of animal feeds.

A difference between sampling sites at the breeding level was observed, with a higher contamination level being observed for pregnant sows. In general, and especially for weaned pigs, the recovery of *Salmonella* from overstocked samples was far more extensive than the recovery of *Salmonella* from feces. This finding might reflect poor hygienic practices and insufficient sanitization modalities. Indeed, Fedorka-Gray et al. (19) reported several studies indicating that the intensive cleaning of accommodations might increase the period during which contamination is absent in piglets, even if sows are shedding. These authors argued for the importance of all-in–all-out systems. In contrast, in a survey involving 23 herds in the United Kingdom, Davies and Wray (14) found *Salmonella Typhimurium* to persist in pig pens after cleaning and disinfection.

An important increase in *Salmonella* contamination at the slaughterhouse was noted. In Canada, Letellier et al. (33) found a *Salmonella* prevalence level of only 5.2% in cecal contents. However, the samples used in their study consisted of only 1 g of cecal content, compared with the 25-g composite samples from five pigs used in the present study. Fortunately, *Salmonella* prevalence decreased at the carcass level, which means that in the slaughterhouse involved in the present study, good hygienic practices lower the probability of the transfer of *Salmonella* from the gut to the carcass. With the same swab sampling scheme, Korsak et al. (30) demonstrated that the overall prevalence for four Belgian pig slaughterhouses was 27%. Moreover, pooled samples will test positive if only one carcass, and not all five, is contaminated.

To explain the increase in the *Salmonella* level for cecal content samples collected at the abattoir relative to that for samples collected during the fattening stage, one may refer to several studies about the role of transport and the lairage prior to the slaughter process. Indeed, Hurd et al. (28) observed an important increase in *Salmonella* prevalence for samples collected at abattoirs relative to that for on-farm fecal samples. However, these investigators noted a protective effect of a lairage period of 18 h in sanitized facilities, with more positive results being obtained with no lairage period. In contrast, if the lairage is contaminated by *Salmonella*, pigs may shed this organism in feces in as little as 2 h after exposure (27). Thus, these authors support the hypothesis of a “visceral infection” of pigs originating from holding pens at the slaughterhouse just before the killing step. A Dutch study revealed that even when good cleaning and disinfection practices are used, holding pens remain contaminated with *Salmonella* (43). In conclusion, this stage should be considered a critical step in the contamination of pigs.

There was no correlation between *Salmonella* status during fattening and that at the slaughterhouse. Different hypotheses or explanations for this lack of correlation may be outlined. A first problem may be associated with the amount of *Salmonella* shed in feces rather than with the method itself: the minimum *Salmonella* level recovered from artificially contaminated feces was previously found to be 10 CFU/25 g (data not shown). Second, intermittent shedding during the breeding and fattening stages could have occurred. Third, a preliminary investigation showed a high level of contamination of water arising from the de-hairing system at the slaughterhouse (data not shown). This system uses cold recirculated water, and this water became contaminated with *Salmonella* only 1 h after the start of slaughter. Hence, contamination of the rectum and the end of the colon at the abattoir cannot be excluded, as demonstrated by serotype analysis (data not shown). This finding implies that a better sampling site for the evaluation of the real shedding state of swine at the slaughterhouse might be chosen. Letellier et al. (33) and Quirke et al. (40) sampled pigs on the slaughter chain by incising the cecum in order to obtain 1 or 25 g of cecal contents. Finally, an increase in shedding is possible and could be caused by stress experienced by live animals (e.g., during transport or at the lairage). Isaacson et al. (29) proved that the recovery of *Salmonella* from ileocecal contents was far more extensive when live swine were transported to the abattoir without a fasting period.

The six most isolated serotypes were *Salmonella Typhimurium* (which includes *Salmonella Typhimurium* var. Copenhagen), *Salmonella Brandenburg*, *Salmonella Derby*, *Salmonella Infantis*, *Salmonella Goldcoast*, and *Salmonella Anatum*. This was true for samples obtained at all stages except for feed samples. This discrepancy is in agreement with the study of Häggblom (23), who judged that in Sweden, although the same serotypes may be isolated from samples of feedstuffs and samples obtained from finishing pigs (i.e., *Salmonella* Derby and *Salmonella Typhimurium*), the level of contamination of pigs from feedstuffs is very low.

In a study on slaughtered pigs involving a wide variety of samples, including carcass swabs, rectal contents, and samples of different organs and offals, Swanenburg et al. (45) noted that *Salmonella Typhimurium* and *Salmonella*...
Brandenburg represented nearly 50% of the isolated serovars, while *Salmonella* Virchow was predominant in slaughterhouse environmental swabs. In two surveys conducted in The Netherlands, Swanenburg et al. (44, 45) also noted that at one slaughterhouse the carcass splitter was contaminated with *Salmonella* Infantis corresponding to the unique serotype recovered in carcass swabs.

Davies et al. (11) observed an important difference between the distributions of serotypes during the different stages involved in a multiple-site production system. The most isolated serotype during breeding was *Salmonella* Derby (52.1% of isolates), while during fattening, *Salmonella* Typhimurium represented 75% of the isolates. Our study revealed no statistical serotype distribution difference between the breeding and the fattening stages (data not shown). In a survey conducted in North Carolina, Davies et al. (13), whose intention was to compare multiple-site production systems and farrow-to-finishing farms, also found on rare occasions, like Häggblom (23) in Sweden, that the same serotypes were recovered from feed and from fecal samples collected from swine fed this contaminated feed.

With regard to the susceptibility of isolated strains to the tested antimicrobial agents, one of the major observations was that of all the types of samples tested, feeds harbored the most sensitive strains. It seems that resistance to antibiotics appears during the transition from breeding to fattening, probably owing to the incorrect use of antibiotics at weaning or the use of growth factors in feed. Samples collected at the fattening stage and contents of large intestines collected at the abattoir contained large percentages of isolates exhibiting resistance to more than four antimicrobial agents, and a large proportion of these isolates were characterized as *Salmonella* Typhimurium DT104. This finding is of great concern given that these strains, owing to chromosomally integrated genes, are generally resistant to four or five antimicrobial agents with the phenotype ACESSuT (resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline). Additional resistance, especially to newly marketed fluoroquinolones, may appear (2).

In conclusion, further research is necessary, both to improve the sensitivity of methods for the detection of *Salmonella* in feces, probably by increasing the frequency of the samples, and to better understand mechanisms of increased shedding during transport, in the lairage, and during the slaughter process. The additional information will allow adequate preventive measures to be taken along the production chain. Hygienic measures and HACCP plans in slaughterhouses remain essential in obtaining safe meat, as shown in this study.

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