Research Note

Salmonella Diagnosis in Pig Production: Methodological Problems in Monitoring the Prevalence in Pigs and Pork

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ABSTRACT

Salmonellosis is an important foodborne infection in industrialized and developing countries. Especially for human Salmonellosis caused by Salmonella enterica serovar Typhimurium, pigs and pork are the major sources of infection. Mitigation and control strategies that result from surveillance programs attempt to reduce or even eradicate Salmonella in pork to lower consumers’ risks. The methodology for Salmonella screening in pigs is generally based on antibody detection at slaughter with meat juice as the sample matrix. The instructions to most commercial enzyme-linked immunosorbent assay (ELISA) kits for the detection of Salmonella antibodies state that their product is suitable for antibody detection in meat juice and sera. In the present study, we show that it is essential to recalculate the percent optical density (OD%) data obtained from meat juice by the ELISA (IDEXX HerdCheck swine Salmonella) by the following regression equation: OD%sera = −70.5587 + 128.1490/[1 + exp((−18.8969 − OD%meat juice)/27.6032)]1.1771, r = 0.87, to compare results with those obtained from sera. By this regression equation, we were able to compare the Salmonella antibody levels (classified as <10, 10 to <20, 20 to <40, and ≥40 OD%) for sows, growers, and slaughter pigs. We identified significantly higher numbers of growers with lower OD% levels than for sows and slaughter pigs. Without a recalculation of the meat juice results, the higher fraction of samples with low OD% values led to an underestimation of the actual seroprevalence.

Salmonellosis is the most important bacterial foodborne infection in industrialized and developing countries (3, 12, 14). In industrialized countries, pork—next to poultry meat and eggs—is the third cause of transmission to humans. With regard to infections caused by Salmonella enterica serovar Typhimurium, pork is reported to be one of the main infection sources for humans (6, 19, 20, 28). Salmonella-shedding pigs are known to constitute a major risk factor for contaminating pork carcasses during slaughter, e.g., following the evisceration process (9, 28). Nevertheless, care must be taken during the entire slaughter process to reduce fecal contamination.

For developing mitigation strategies in reducing Salmonella in pork, different monitoring and surveillance systems have been established. They aim to control Salmonella infections already at the herd level (2, 8, 18, 21, 30). Most of these programs have the objective of identifying Salmonella-infected herds and establishing herd management directives for eradication (5, 10). Nevertheless, it must be conceded that most programs at best reduce the incidence of Salmonella in pigs rather than achieve the elimination of this pathogen (29).

In general, monitoring and surveillance systems for Salmonella in pigs rely on enzyme-linked immunoassays (ELISA) testing of carcasses with meat juice as a sample matrix (1), even though these test systems have been developed for the detection of specific Salmonella antibodies in sera. They are based on the detection of antibodies against Salmonella O-antigens. Various ELISA systems for the detection of Salmonella in pigs have been established, and some are commercially available and have been validated against one another (e.g., Salmotype Pig Screen, VetSign, IDEXX HerdCheck swine Salmonella, Salmonella Covalent Mix-ELISA) (2, 11, 26, 27).

The aim of this study was to assess the antibody titer against Salmonella in growers, slaughter pigs, and sows and to compare the results from sera and meat juice. Results of the study should bring new information on Salmonella epidemiology in pig production in Austria and further ascertain the comparability of the results obtained from different sample matrices (sera and meat juice) with a view toward increasing the reliability of Salmonella ELISA assays.

MATERIALS AND METHODS

Animals and herds. A total of 3,762 pigs were analyzed for Salmonella antibodies in sera, meat juice, or both. From these, 617 were sows that originated from 51 different breeding facilities and 827 were growers (weight, 25 to 30 kg) from 201 different grower facilities. Samples from 2,318 slaughtered pigs (carcass weight, 80 to 105 kg) were taken according to a statistical sampling plan from eight different slaughterhouses across Lower Aus-
TABLE 1. Distribution of sera and meat juice OD% values for 473 slaughtered pigs

<table>
<thead>
<tr>
<th>OD%</th>
<th>Sera (n)</th>
<th>Meat juice (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10</td>
<td>211</td>
<td>373</td>
</tr>
<tr>
<td>10 to &lt;20</td>
<td>143</td>
<td>32</td>
</tr>
<tr>
<td>20 to &lt;40</td>
<td>78</td>
<td>43</td>
</tr>
<tr>
<td>≥40</td>
<td>41</td>
<td>25</td>
</tr>
<tr>
<td>Total n</td>
<td>473</td>
<td>473</td>
</tr>
</tbody>
</table>

tria (30). There was no relation between sows, growers, and slaughter pigs.

**Sample collection.** Blood samples taken from 617 sows and 827 growers at the farm level and sticking blood from 473 slaughtered pigs were sampled. Per animal, 1 to 10 ml of blood was collected in sterile blood vials (Sarstedt, Germany). From the 473 and 2,318 carcasses of slaughtered pigs, meat juice was collected by sampling 10 g of the diaphragm muscle.

**Sample processing.** On the day of collection, blood samples were centrifuged for 8 min at 1,650 × g. The supernatant (blood serum) was either used immediately for ELISA analysis or stored at −20°C until use. Diaphragm muscles from carcasses were sampled with a special meat juice collector (Sarstedt), by which the specimen is obtained through a freeze-and-thaw cycle and either used immediately or stored at −20°C until further analysis.

**ELISA method.** For analyzing blood sera and meat juice, a commercial ELISA kit for the detection of *Salmonella* antibodies (HerdCheck swine *Salmonella*, Idexx Laboratories, Westbrook, Maine) was used, and samples were analyzed according to the instructions of the test kit (17). The test results are presented as the regression analysis of positive and negative reference sera and expressed as the percent optical density (OD%). Results were classified in four levels: <10, 10 to <20, 20 to <40, and ≥40% (22).

**Statistical methods.** Nonlinear regression analysis was conducted on meat juice and blood sera results (OD%) obtained from 473 slaughter pigs by the SigmaPlot software package (Systat Software, Point Richmond, Calif.). Recalculated meat juice and blood sera results were compared by the signed-rank (Wilcoxon) test. Results from the three animal categories were assigned to four groups. For testing the significance of differences between the animal categories, a $3 \times 4$ χ² (chi-square) test with Yates’ correction (15) was used.

**RESULTS AND DISCUSSION**

**Sample matrix.** To compare *Salmonella* seroprevalence in growers, sows, and slaughtered pigs, we compared the correlation between serological testing and antibody detection in meat juice. Hence, we decided to check the antibody level of the sera and meat juice samples of 473 slaughtered pigs (Table 1). In contrast to the manufacturer’s instructions, which state that the test is suitable for both matrices under study (correlation $r = 0.95$ and slope = 0.99) (17), we observed statistically significant differences between the defined OD% levels of one and the other sample matrix (Table 2). The samples were tested with a second commercially available ELISA test system, and discrepancies were even higher (data not shown). This is in accordance with a recent study from Galland and Alt (13). Consequently, it seems appropriate to recalculate sample data by the following regression equation, which was obtained from our data: $OD\%_{sera} = -70.5587 + 128.1490/\{1 + \exp([-18.8969 - OD\%_{meat juice}]/27.6032)]^{1.1771}$, $r = 0.87$. Recalculated meat juice data and serum results did not differ significantly (signed-rank test, $T = 1.722; P = 0.08$ for alpha = 0.05).

**Serological detection of Salmonella in sows, growers, and slaughter pigs.** The frequencies of *Salmonella* antibodies in sows, slaughter pigs, and growers were analyzed; the results are given in Table 2. The group of growers is significantly shifted toward lower OD% values than the group of sows and slaughter pigs. To show how relevant the recalculation of meat juice data is, we also statistically analyzed raw data. The not-recalculated data that rely on meat juice analysis suggest that growers and slaughter pigs differ significantly from sows (Table 2). As most surveillance programs are based on the antibody testing of slaughter carcasses with meat juice, it could be relevant to recalibrate data according to our study to give more precise data for the identification of infected herds and better management directives for the eradication of *Salmonella* in pig production.

Even though infection of slaughter pigs with *Salmonella* might occur within 2 h after animals have been introduced in a contaminated environment (16, 25), the serological response in pigs to *Salmonella* follows at least 2 weeks, but often even more weeks, later—dependent on the *Salmonella* serovar (22). Hence, it is not surprising that growers have significantly lower *Salmonella* ELISA OD values than do sows and slaughter pigs. Other studies of differently aged pigs state similar results (27). For *S. enterica* serovar Typhimurium, it has been reported that transmission from pig to pig is the most important cause of infection (4). A rather high percentage of sows with OD% values >10 indicates that sows are at least an indirect source of infection for piglets and growers—even more so, because in Austria, *S. enterica* serovar Typhimurium is isolated most often from both pigs and pork (7, 23, 24). Nevertheless, other potential risk factors, such as type and origin of feed, stable and herd management, contact between different herds (markets), cleaning and disinfection plans, ventilation sys-
tems, and last, but not least, slaughter technology, are additional risk factors for Salmonella in pork.

Most monitoring and surveillance programs to control Salmonella in pigs are based on antibody detection in meat juice. The present study has verified a main problem in these endeavors, i.e., that the correlation between serological testing and antibody detection in meat juice for identifying pig production units with high Salmonella prevalence is nonlinear. Thus, we recommend that meat juice data be recalculated to precisely detect herds with a high prevalence of Salmonella. Otherwise, the use of nonrecalculated results obtained from meat juice would cause an underestimation of the actual situation, compromising combined efforts to reduce Salmonella prevalence, such as compliance with hygienic standards, control of feeding stuff, or identification of Salmonella carriers.

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REFERENCES