AN EPIDEMIOLOGICAL SURVEY ON THE PREVALENCE OF SALMONELLA IN SWINE IN CENTRAL ITALY

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Abstract The aim of the study was to evaluate the prevalence of Salmonella spp. in swine herds in Italy. Samples of faeces from finishing swine farms, cecal contents and ileo-cecal lymphnodes from pigs at slaughter were analysed for Salmonella. Samples of blood serum and meat juice were collected from the same groups, and tested for antibodies against Salmonella O-antigens with an ELISA test kit. 6.5% of faeces from finishing swine resulted positive, with 40% of the groups showing at least one positive sample, while Salmonella was isolated from 28.5% of cecal samples (85% of the groups) and 26.4% of lymph nodes (75% of the groups). The results of serology showed positivity in 100% of the herds and 95% of groups at slaughter. S. Typhimurium, S. Infantis, S. Derby were the most frequent isolated serotypes. The lowest frequencies of susceptibility were observed for tetracycline, sulfonamides, streptomycin and ampicillin.

Introduction The recent European Regulation, regarding the control of zoonosis in animal populations (Regulation CE 2160/2003 and Directive 2003/99), will require, in the next years, the implementation of monitoring programs for Salmonella in swine. From this the present study has been carried out to preliminarily evaluate the presence of Salmonella spp. in swine in central Italy. Finishing herds and groups at slaughter were considered in the investigation; within these, bacteriological and serological tests (ELISA) have been carried out, to investigate the prevalence of infection, the Salmonella serotypes, and the antimicrobial resistance of the isolates. In addition, the possible use of an ELISA test to detect antibodies against Salmonella has been also considered.

Materials and Methods

Sampling Finishing herds and groups of pigs at slaughter have been included in the study. In each farm, 5 samples of faces have been collected from each single box (maximum 20) and pooled before bacteriological testing. In addition, 15 samples of serum have been collected at random from the animals; 21 farm in central Italy have been tested, for a total number of 318 sera and 328 pools of faeces.

In three different slaughterhouses, 20 groups of pigs, originating from different farms, have been sampled. For each group, 15 pigs have been randomly selected to collect faeces and ileocecal lymph nodes (295 samples); 10 grams of diaphragm have been also collected from the same animals to obtain “meat juice” to be tested for antibodies in ELISA as described by Nielsen (1998).

Salmonella isolation and identification Routine procedures have been adopted for the isolation of Salmonella from the samples. Briefly, the material has been pre-enriched in buffered peptone water and incubated at 37°C for 20 hours. After incubation, the culture has been enriched in Rappaport-Vassiliadis soy broth at 41.5°C for 24 hours and then plated onto Brilliant Green Agar and Salmonella Chromogenic medium (Biolife). After incubation at 37°C for 24-48 hours, 3 suspect colonies have been selected, to be further confirmed through biochemical testing (Quinn et al., 1999) and serum agglutination using polyclonal antisera (Dade-Behring).

Salmonella strains have been serotyped using monoclonal antisera, according to the Kauffmann-White scheme (Popoff and Le Minor, 1997; Popoff, 2001). The resistance of Salmonella strains to several antimicrobials has been evaluated using the Kirby-Bauer agar diffusion test, according to the M31-A2 NCCLS procedures (NCCLS 2002)

ELISA test The IDEXX HerdChek® Swine Salmonella test kit has been used for the detection of antibodies from serum and “meat juice”. As indicated by the supplier, a cut-off ≥10 % has been adopted.

Results The results obtained in the present study, both in the farms and at the slaughterhouse, are reported in table 1. The serotypes isolated in the farms were S. Typhimurium (7 farms), Salmonella Group B O:4 H1:i H2:- (1), S. Derby (1), S. Infantis (1).

At the abattoir, from the faeces S. Derby was the most frequently isolated serotype (11
groups), followed by S. Typhimurium (7), S. London (3), S. Brandenburg (2), S. Rissen (2), S. Bredeney (2), S. Give (1), S. Meleagris (1) S. Manhattan (1), S. Arizonae (1). From the lymph nodes the following serotypes have been isolated: S. Derby (11 groups), S. Typhimurium (10), S. Bredeney (2), S. Muenchen (1), S. London (1), S. Give (1), S. Meleagris (1), Salmonella Group B (1), Rissen (1), Salmonella 4,5,12; i,-.

The results of antimicrobial susceptibility testing are summarised in Figure 1.

**Discussion**

The present study demonstrated high prevalence of infection by Salmonella, shown through bacteriological testing, in swine herds in Central Italy both at the farm level (42.5%) and at slaughter (85%). ELISA testing, using a ≥10% cut-off level, confirmed these results, moreover showing higher levels of positivity both in herds (93.7%) and at the abattoir (95%). This could be justified by previous contact of the animal with the microorganism, no more detectable at the time of sampling. According to the results obtained at the abattoir, the cecal content seems to be a more suitable sample for Salmonella detection compared to lymph nodes, giving a better view of the infection status of the group. However, testing limphonodes remains a key element, in order to detect serotypes involved in previous infections, different from the serotypes isolated from the faeces, which could be linked to recent infection of pigs during transport or lairage before slaughtering (Sorensen, 2004).

In the farms, S. Typhimurium appeared to be the most frequent serotype, while at the abattoir S. Derby was mostly isolated from faeces and limphonodes, confirming the observations of other Italian Authors (Decastelli, 2004; Bonardi, 2003). Both these serotypes have been also frequently observed in outbreaks of human salmonellosis in Italy (Luzzi, 2004).

With regard to the antimicrobial susceptibility of the isolates, it resulted low to streptomycin (42%), sulfonamides (40.6%), tetraciclina (21.7%), trimethoprim (66.7%) ed ampicillina (71%). The occurrence of Salmonella strains resistant to these molecules has been widely reported in Italy both in animals and humans, suggesting a possible role of the pigs as a reservoir of Salmonella resistant strains for humans (Graziani, 2003; Busani, 2004; Dal Vecchio, 2004).

**Conclusions**

The present study demonstrates the high prevalence of infection by Salmonella in swine in Central Italy. Therefore there is a future need to better investigate the health status of these animal populations and clarify the possible links between Salmonella infection in pigs and outbreaks in humans. As required by EU regulation, in the next years every country will have to implement monitoring programs for Salmonella in this species, both in farrow to finish and finishing herds. The attention will have to be particularly focused on serotypes and antibiotic resistant strains, relevant for human infection, and this investigation has been carried out to have a preliminary view in this topic. In addition, there is a need for individuating rapid and reliable laboratory tests to be used in the field on a large scale; our experience shows that the ELISA test screening on serum or “meat juice” could be helpful to accomplish such a task.

**References**


<table>
<thead>
<tr>
<th>Sample</th>
<th># of samples (single animals)</th>
<th>Herds/groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Herd Faeces</td>
<td>32 (9,8%)</td>
<td>296 (90,2%)</td>
</tr>
<tr>
<td>Sera</td>
<td>109 (34,3%)</td>
<td>209 (75,7%)</td>
</tr>
<tr>
<td>Abattoir Cecal contents</td>
<td>84 (28,5%)</td>
<td>211 (71,5%)</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>78 (26,4%)</td>
<td>217 (73,6%)</td>
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<tr>
<td>Meat juices</td>
<td>183 (64,2%)</td>
<td>102 (35,8%)</td>
</tr>
</tbody>
</table>

Table 1: Collected samples, tests carried out and results