Salmonella monitoring in pigs in the Veneto Region of Italy: results of three monitoring campaigns from 2002 to 2006

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Abstract
From 2002 to 2006 three monitoring campaigns have been performed in the Veneto Region of Italy to define the prevalence of Salmonella in pigs slaughtered in this area. The monitoring scheme applied allowed to assess the prevalence for Salmonella, and was adjusted after each year of application in order to detect defined variations in prevalence, with a reduced number of samples. In the first (2002-2003) monitoring campaign the sample size (384 slaughtered batches) was assessed on the basis of the following criteria: expected prevalence = 50%; accuracy = 5% and confidence interval = 95%. Samples were stratified according to the capacity of each slaughterhouse, and equally distributed in a 12 months period. One animal was sampled for each batch, collecting 25 grams of caecal content. During the following year (2004), starting from the results of the previous campaign the sample size was reduced in order to be able to evaluate a prevalence variation of at least 12%. In 2005 a new monitoring campaign was set up and the sample size was established on the basis of the results of the previous campaign, as previously described; this new study started in September 2005 and finished in December 2006. In this last campaign it was decided to sample 15 animals for each slaughtered batch, when possible, in order to be able to detect at least a 20% within batch prevalence; moreover, with the aim of avoiding any risk of cross-contamination of samples during slaughtering, it was decided to collect a sample of ileocaecal lymph nodes for each selected animals. The bacteriological investigation on lymph nodes should better reflect the sanitary status of the herd of origin than the information obtained analysing the caecal content. In this paper the results of the three monitoring campaigns in the swine populations are described and compared.

Introduction
Salmonella is one of the major causes of foodborne illnesses in humans and in 2004 nearly 200,000 human cases of salmonellosis were notified in the 25 EU Member States (EFSA 2005). The zoonoses legislation (Directive 99/2003/EC and Regulation EC No 2160/2003) obliges all Member States to carry out monitoring programmes for zoonoses and zoonotic agents all along the food chain. Data from the surveys will be used by the Commission to set Community targets for the reduction of the prevalence of Salmonella in animal populations. For this perspective in the Veneto Region of Italy a monitoring programme, involving all the major animal species farmed in the area, has been carried out since 2002. This monitoring programme aims to establish Salmonella and Campylobacter levels across the region and the diffusion of the antimicrobial resistance in pathogen and indicator bacteria. The data collected can be used as a baseline against which future changes in prevalence at slaughter can be monitored and specific control strategies can be defined. The programme was adjusted progressively in order to define the better methodology to estimate as accurately as possible the Salmonella status of animals slaughtered in our region. In this paper the sampling methods adopted to monitor Salmonella in swine population and the results obtained are presented.
Material and methods

Sampling scheme

A three-step monitoring programme was used to accurately estimate the prevalence of Salmonella. In the first campaign (2002-2003) the number of samples to be collected was calculated considering an expected prevalence of 50%, an accuracy of 5% and a confidence level of 95%. Since accurate information regarding the Salmonella status of swine population in our region was not available, we supposed a predicted prevalence of 50%, which is the situation with the highest variance.

In the second (2004) and third campaigns (2005-2006) it was possible to reduce the number of samples considering the prevalence obtained in the previous phases of the study. The sample sizes adopted allowed to detect either the increase or the decrease of prevalence of 12% (bilateral interval).

The criteria used for the definition of the three sampling schemes are summarized in table 1.

<table>
<thead>
<tr>
<th>Sampling campaign</th>
<th>Prevalence</th>
<th>IC confidence limit</th>
<th>Accuracy</th>
<th>Difference of prevalence to be detected</th>
<th>Interval</th>
<th>Sample size (number of batches to be sampled)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2002-2003</td>
<td>(expected prevalence)</td>
<td>50%</td>
<td>95%</td>
<td>5%</td>
<td></td>
<td>384</td>
</tr>
<tr>
<td></td>
<td></td>
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<tr>
<td>2004</td>
<td>prevalence obtained in the previous campaign</td>
<td>95%</td>
<td>5%</td>
<td>12%</td>
<td>Bilateral</td>
<td>187</td>
</tr>
<tr>
<td>2005-2006</td>
<td>prevalence obtained in the previous campaign</td>
<td>95%</td>
<td>5%</td>
<td>12%</td>
<td>Bilateral</td>
<td>171</td>
</tr>
</tbody>
</table>

Table 1. Criteria adopted to define the sample size in the three campaigns of the monitoring programme

In the first and second campaigns of the monitoring programme one sample of faeces was taken from one animal per batch, while in the third phase, in order to increase the sensitivity of the sampling plan, samples of mesenteric lymph nodes were taken from 15 pigs per batch.

Laboratory methods for detection and typing of Salmonella spp.

The samples were collected in major regional pig slaughterhouses irrespective of the origin of the animals (either regional or extra-regional) and were transported to the Italian National Reference Laboratory for Salmonellosis where the samples were analysed.

For each sample (faeces or lymph-nodes) the presence of Salmonella spp. was qualitatively determined according to the Amendment of the ISO 6579:2002. In summary 5 g of sample were added to 45 ml of Buffered Peptone Water and incubated for 18 ± 2 h at 37 ± 1°C. 0.1 ml (3 drops) of pre-enriched broth-culture was inoculated on MSRV and incubated for 24/48 ± 2 h at 41.5 ± 1°C. Suspect white culture around the inoculation spots in MSRV were plated on XLD and BGA and after 18 ± 2 h of incubation at 37 ± 1°C colonies with morphology typical of Salmonella were screened biochemically and serotyped following the Kauffman-White scheme. Salmonella Enteritidis and Salmonella Typhimurium strains were then phagetypeped following the method provided by HPA, Colindale, London.
Results

During the first campaign of the programme 208 batches were sampled and the prevalence of Salmonella resulted to be 29.33%. The most frequent serotypes were S. Typhimurium (24.21%), S. Derby (18.95%), the monophasic strain 4,5:1:- (10.53%).

In 2004, a total of 173 pig faecal samples, representing the same number of batches, were analysed for the detection of Salmonella spp., and the prevalence of the bacterium resulted to be 25.43%. The strains isolated were serotyped mainly as S. Typhimurium (22.22%), S. Anatum (20%), S. Derby (13.33%)

In the last campaign of the monitoring programme 1557 samples of lymph nodes were collected from 107 batches. A total of 65 batches resulted to be positive for Salmonella (prevalence of 60.75%), and 442 strains of Salmonella were isolated and serotyped. The most prevalent serotypes were S. Derby (21.48%), S. Typhimurium (14.09%), S. Anatum (10.74%), S. London (6.71%), S. Rissen (5.37%). In 28 of the 65 positive batches one or more strains belonging to the same serovar were isolated, while in the other batches two or more serovars were identified. In the 9% of the positive batches 5 (2 batches), 6 (2 batches) and also 7 (2 batches) different serovars were identified.

Figure 1 Number of serovars isolated in the same batch simultaneously during the third campaign of the monitoring programme

Discussion

In the last campaign of the monitoring programme a Salmonella prevalence almost three times higher than the one obtained in the previous phases (2002-2003 and 2004) was detected. The reason for this increase of prevalence is clearly due to the rise in the number of samples collected from each batch. Such increase in the number of samples per batch was decided in order to improve the sensitivity of the sampling plan, to assess the extent of the infection within the single groups and to estimate whether different serovars are spread within the single groups.

Another possible explanation for the increase in Salmonella prevalence can be found in the type of matrix investigated, since in the first two phases of the programme we analysed faecal samples while in the last campaign we collected samples of lymph nodes. Usually Salmonella infect swine herds subclinically so pigs at slaughterhouses present chronic infection characterized by a low intermittent excretion of the bacterium in faeces (EFSA 2006). Therefore, the examination of individual faecal samples from pigs has a poor sensitivity. However a better bacteriological estimation of the Salmonella status of a swine herd seems to be obtained analysing intestinal lymph nodes (Nollet et al., 2005), since these tissues reflect a localised intestinal infection, a
previous exposure to Salmonella or, possibly the spread from intestinal organs as consequence of generalised infection (EFSA 2006). Considering the most common serovars detected in the three campaigns of the programme we can note that they are the serotypes identified most frequently in pigs also in the other EU Member States (EFSA 2005).

Conclusion
Both the high prevalence of Salmonella detected in the herds sampled and the presence in several batches of different Salmonella serovars point out the need to carry out specific preventive strategies to control Salmonella in swine production. The possible control strategies, that should be focused mainly at the primary production level, may be for instance the improvement of the hygienic and management procedures in the herds, the improvement of the health status of animals, the adoption of strict biosecurity measures, the control of Salmonella contamination of feed, the use of vaccines and eventually the implementation of specific monitoring programmes to check the effectiveness of the strategies adopted. Finally, the reduction of Salmonella-prevalence at the primary production is one of the means which can facilitate the respect of certain microbiological criteria fixed by Regulation (EC) No 2073/2005, in force in the EU since 1st January 2006. In fact, this Regulation applies a zero tolerance policy for Salmonella contamination in meat and in particular in minced meat and meat preparations. Therefore, in order to respect these criteria, the implementation of specific control measures of Salmonella reduction in the swine population farmed and slaughtered in our region seems to be definite and urgent. In addition, at the end of 2007 the European target for the reductions of Salmonella in pigs will be fixed, and then all the Member States will have to define their control programmes.

References

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