Evaluation of the use of serological and bacteriological investigation for monitoring and controlling Salmonella in Italian pig herds

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Abstract
At the European level the control of foodborne diseases is defined by the new zoonoses legislation (Directive 2003/99/EC and Regulation (EC) No 2160/2003), which points out the necessity to establish surveillance programmes for zoonotic agents in animal populations. Recently Commission Decision 2006/668/EC concerning a baseline study on the prevalence of *Salmonella* in slaughter pigs has been published.
Many different strategies have been developed and applied by EU Member States in order to implement monitoring and/or control programmes for *Salmonella* in pigs; these strategies are mainly based on bacteriological analysis (performed on caecal content, ileo caecal lymph nodes or carcass swabs collected at slaughterhouse) and/or on serological analysis (mainly performed on meat juice obtained from diaphragm muscle).
Very few data are published about the comparison among different strategies so that it is still difficult for a country wanting to implement a monitoring/control programme to choose the most cost-effective methodology.
The objective of the present study was to develop an effective methodology to evaluate *Salmonella* spp. prevalence in slaughter pigs comparing bacteriological and serological strategies with the aim of identifying the most effective methodology to apply.
To detect the presence of the infection, both bacteriological examination of faeces and ileocaecal lymph nodes and serological investigation of meat-juice and blood sera were used.
Samples of diaphragm muscle, blood, caecal content and mesenteric lymph nodes were collected from 150 pigs of 10 batches in two slaughterhouses of the Veneto Region of Italy and comparisons were made between isolation of *Salmonella* in faeces and lymph nodes and the capability to detect Salmonella antibodies in sera and meat juice using three different commercial ELISA kits.
In this paper the results of bacteriological and serological investigations are presented emphasising the comparison among the three different commercial ELISA kits.

Introduction

Salmonella spp. is one of the major causes of foodborne illnesses in humans. According to the Community Summary Report on Trends and Sources of Zoonoses a total of 192703 cases of human salmonellosis were reported by the 25 EU Member States in 2004. Pork, after eggs and poultry meat, is a major source of human foodborne salmonellosis in the European Union (EU) (EFSA 2005). The zoonoses legislation (Directive 2003/99/EC and Regulation EC No 2160/2003) points out the necessity to establish specific monitoring programmes at primary production for some zoonotic agents. Two very different approaches for *Salmonella* detection in pigs can be applied: bacteriological or serological analysis. The choice of method to use depends mainly on the epidemiological situation of the monitored population. The bacteriological method detects all the spreading serovars and allows to define the actual infection *status* of the animal, but this analytic procedure is laborious.
The serological analysis expresses the previous exposure to the infection agent by detecting specific antibodies against *Salmonella*; it is cheaper and easier to perform.

Therefore the use of bacteriological investigation is a prerequisite to estimate exactly the prevalence of infection and the serovars involved in order to identify the suitable ELISA kit to employ for large scale monitoring programmes.
To date in Italy only few serological monitoring programmes have been performed in swine population (Cibin et al. 2005, Magistrali et al. 2005), so in this study we tried to compare bacteriology and serology in order to assess the possibility of serological application in future monitoring programmes.

Materials and Methods

The study was carried out in two slaugtherhouses located in the Veneto region of Italy. Samples were collected from 10 batches (15 pigs/batch). From each pig, on the slaughter line, blood samples were taken at the exsanguination, ceacal content and mesenteric lymph nodes after evisceration and cubes of approximately 3 cm of edge of diaphragmatic muscle at the post-mortem inspection.
For bacteriological examination 5 g of faeces and 5 g of lymph nodes were cultured according to the Amendment of ISO 6579:2002. Colonies with typical *Salmonella* morphology were screened biochemically and serotyped following the Kauffman-White scheme.
Serology was performed on serum and meat juice by means of three commercial indirect mix-ELISA tests (Kit 1: Salmonella Covalent Mix-ELISA-SVANOVA; Kit 2: Porcine Antibody ELISA Vestigen™ –GUILDAY-; Kit 3: HerdCheck Swine Antibody Test Kit –IDEXX) following the manufacturer's recommendations.
Meat juice for the serological analysis was obtained by freezing and thawing the diaphragmatic muscle as described by Nielsen et al. (1998).

Results

Bacteriological analysis

Considering as positive one batch in which Salmonella was detected in at least one sample of faeces or/and lymph nodes, 100% of the batches resulted positive.
The prevalence of positive cultures was higher for lymph node samples (44%; IC 35,91-52,32) than for faecal samples (20% IC 13,91 – 27,3) and this discrepancy between the bacteriological results obtained from the two matrixes (table 1) was confirmed also by the statistical analysis (k value: –0.0057). Although several serovars were identified in lymph nodes and in faeces, S. Typhimurium, S. Derby, S. Anatum and S. London represented the great majority of the strains isolated from both the matrixes.

Bacteriological analysis		LYMPH-NODES		
		pos	neg	Tot
FAECES	Pos	14	17	31
	Neg	52	67	119
	Tot	66	84	150

Table 1: Comparison between bacteriological detection of Salmonella on faeces and on lymph nodes

Comparison between serological and bacteriological tests

In order to evaluate the correlation between the bacteriological and serological analysis, the sensitivity and the specificity of the three ELISA kits on serum and meat juice were calculated considering as "gold standard" the bacteriological test performed both in faeces and in lymph nodes. Since the three ELISA kits are specific for serovars belonging to serogroups B, C1 and D1, the sensitivity of serological tests was calculated considering as positive only the samples in which strains belonging to serogroups B, C1 and D1 were isolated (from lymph nodes or faeces). The specificity was determined considering as negative the samples in which *Salmonella* was not isolated from faeces or lymph nodes and those in which *Salmonella* strains belonging to serogroups different from B, C1 and D1 were identified.
For all the three serological tests the specificity values are generally higher than the sensitivity ones. Considering the bacteriological results obtained from lymph nodes instead of the results

obtained from faeces, both the sensitivity and the specificity of the serological tests (both in serum and meat juice) were generally better.

Comparison between serum and meat juice for serological tests
The three ELISA kits were performed both on serum and meat juice and the results were compared. Serological analysis on serum was considered as "gold standard" (Nielsen et al. 1998). The Cohen Kappa value obtained comparing serum and meat juice for the three ELISA kits were respectively k: 0,46 (Kit 1), k: 0,31 (Kit 2) and k: 0,28 (Kit 3).

	Ser. MJ/ Bact. F	Ser. MJ/Bact. L	Ser. S/Bact. F	Ser. MJ/Bact. L
SENSITIVITY				
Kit 1	0,09	0,41	0,65	0,79
Kit 2	0,17	0,16	0,78	0,68
Kit 3	0,09	0,20	0,57	0,88
SPECIFICITY				
Kit 1	0,65	0,79	0,69	0,71
Kit 2	0,78	0,68	0,70	0,79
Kit 3	0,57	0,88	0,83	0,59

***Table 2**. Values of sensitivity and specificity of the three ELISA kits on serum and meat juice determined compare serology with bacteriology*
(Ser. MJ/Bact. F: serological test on meat juice compared to bacteriological test on faeces; Ser. MJ/Bact. L: serological test on meat juice compared to bacteriological test on lymph nodes; Ser. S/Bact. F: serological test on serum compared to bacteriological test on faeces; Ser. S/Bact. L: serological test on serum compared to bacteriological test on lymph nodes)

Discussion

The bacteriological results indicated that analysis of lymph nodes could be more sensitive than analysis of faeces for *Salmonella* detection, since lymph nodes represent the tissues most consistently colonised by *Salmonella* in infected animals and these organs often harbour *Salmonella* in carrier animals (Nollet et al., 2005) .
Comparing bacteriology and serology for *Salmonella* diagnosis, a weak agreement was found but the sensitivity and the specificity of serological tests (in serum and meat juice) were generally higher when the results of serology were compared with those of bacteriological analysis of lymph nodes. This data confirm what was previously observed by other authors, who demonstrated that in a herd a better estimation of the *Salmonella* prevalence can be carried out by isolation of the bacterium in the lymph nodes (Nollet et al., 2005).
Several explanations for these discrepancies can be hypothesized.
A positive serological result in a bacteriological negative animal may be due to:

- the cross reactivity between *Salmonella* and other bacteria of the *Enterobacteriaceae* family (Van Der Hejden 2001);
- the use of a too low cut off value of the serological test adopted;
- the presence in the herd of intermittent shedders that may harbour infection, produce antibodies against Salmonella without excreting the bacterium (Lo Fo Wong et al., 2003);
- the persistence of a detectable level of antibodies in pigs that may be no longer infected.

On the other side, a negative serological result in a bacteriological positive animal may be due to:

- the stage of infection: the interval between the peak of the bacteriological and serological response ranges from one to approximately two months (Kranker et al., 2003);
- the antibody clearance in infected animals;
- the presence of animals with a low serological response to *Salmonella* spp. (Lo Fo Wong et al., 2003);
- the presence of Salmonella strains that doesn't belong to the serogroups detectable by the ELISA kit used.
- the adoption of a too high cut-off value of the serological test used.

This weak agreement between the two methods demonstrates that no prediction concerning the *Salmonella* carrier *status* can be made with confidence using serological tests at the individual level.-Other previous studies agree that serology is suitable for the screening on a herd basis (Nollet et al., 2003, Lo Fo Wong et al., 2003).
The sensitivity and the specificity values obtained by the three serological tests were very different and these results confirm the great variability between the serological tests previously observed by other authors (Mejia et al. 2005).
Serological test performed on meat juice is considered an alternative to the analysis of serum to estimate the *Salmonella* prevalence and several studies have documented a clear correlation between antibody levels in serum and in meat juice (Nielsen et al., 1998). However in our study a low concordance between the serological results obtained on serum and meat juice was found. The reason for this discrepancy is not completely clear but it is possible that factors such as stress or the state of hydration of animals may influence the meat juice results (Davies et al., 2003). Another possible reason for this could be found in the quality of the meat juice samples (for instance presence of small blood clots on the meat surface, blood vessels in the meat sample) that could influence the ELISA' s results (Feld N.C. et al., 2005).

Conclusion

Based on the results obtained in our study we can conclude that:

- the analysis of lymph nodes seems to be more sensitive for the detection of *Salmonella* than the analysis of faeces;
- as expected the agreement between bacteriology and serology is low, since these two methods measure different phenomena and are suitable to be used in different situations and with different purposes;
- we found a low concordance between serological results obtained analysing serum and meat juice;
- large differences exist between the serological results obtained using the three ELISA kits.

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