Assessment of the diagnostic accuracy of three commercial ELISAs for the detection of antibodies against *Salmonella* spp in pigs through Bayesian methods.

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Abstract

The diagnostic accuracy of three ELISA tests (Herdcheck® Swine Salmonella, SALMOTYPE® Pig Screen, and PrioCHECK® Salmonella) was analysed. A low correlation between herd prevalence according to bacteriology and herd seroprevalence using the three ELISAs was found. Their estimated sensitivity (Se) and specificity (Sp) using a Bayesian approach was variable, with Herdcheck® Swine Salmonella showing the best overall accuracy (Se= 0.88; Sp= 0.74). All of the ELISAs overlooked an important proportion of *Salmonella*-infected pigs.

Introduction

Currently the European Union (EU) is trying to set up Community targets for reducing the prevalence of *Salmonella* serovars with public health significance in pig herds (Regulation (EC) No 2160/2003). Thus, once the prevalence of *Salmonella* infection has been estimated for each country, member states will have to establish time limits within which the targets should be reached. It's anticipated that immunoassays such as ELISA will be important tools for the development and evaluation of the different programs for controlling this infection. Indeed, some countries are presently using serological results to classify herds, with respect to the risk they pose, based on the level of seroprevalence observed for slaughtered pigs (Mousing *et al.* 1997; Sandberg *et al* 2002)

Different ELISAs are available for the detection of antibodies against *Salmonella* spp in pigs. Given the epidemiology of this infection, and in particular the possibility of some animals to clear the infection during the finishing period, it is difficult to find agreement between bacteriological culture on mesenteric lymph nodes (MLN) and serology at slaughter (Nollet et al, 2005). This has given rise to the common statement that serology is not useful to determine the health status of an individual but that it should work at the herd level (Funk et al, 2005; Farzan et al, 2007). The problem may arise when serology is used to classify herds instead of detecting infection.

We present in this paper the results yielded by three ELISAs performed on meat juice, and assess through Bayesian approaches the Se and Sp of these tests. We also show the potential impact that these tests would have had should they have been used for herd classification.

Material and Methods

A total of 837 meat juice (from diaphragm muscle) and MLN (25 grams) samples were collected from 34 pig herds from the Aragon region (NW Spain) at the time of slaughtering (an average of 25 animals per herd).

Bacteriology was performed on MLN following the standard ISO 6579 (ISO, 2002). Briefly, MLN were removed from the intestinal package free of fat or connective tissue, decontaminated before analysis by dipping into absolute alcohol and further flaming of the external surface. Lymph nodes were then pooled

into a plastic bag, cut in small pieces with sterilized scissors and smashed with a Stomacher® (Seward Medical, London, UK). Then they were placed into 225 ml of buffered peptone water (BPW) for 18±2 hours at 37±1 °C. Three drops of incubated BPW were inoculated into a Modified Semi-solid Rappaport Vassiliadis (MSRV) medium plate and incubated for 24±3 h at 41.5±1 °C. If growth was observed at 24 or 48 hours a 1 µl loop of the growth area was plating on the surface of three selecting media (Xylosine Lysine Deoxycholate -XLD- Brilliant Green -BG- and Aes Laboratoire *Salmonella* Agar Plate -ASAP). Suspected colonies were confirmed biochemically and serotyping performed at the National Centre for Animal Salmonellosis in Madrid, Spain.

Three commercially available ELISA tests were used in this study, namely, Herdcheck® Swine Salmonella (Idexx Laboratories, US), hereafter test A; SALMOTYPE® Pig Screen (Labor Diagnostik Leipzig, Germany), or test B; and PrioCHECK® Salmonella (Prionics, Switzerland), or test C. All the tests were carried out according to manufacturers' instructions and the recommended cut-off values were used, except for test B where sera within the doubtful range were considered negative. According to the manufacturers all the tests were able to detect antibodies against the same serotypes, mostly those belonging to serogroups B, C1 and D.

The correlation between herd prevalence (from bacteriology) and herd seroprevalence for each of the test used was evaluated through Pearson correlation coefficient (MedCalc Software, Belgium) after transforming data to stabilize the variance using the arcsin of the square root of a proportion.

To estimate the Se and Sp of the three ELISA tests to detect pigs with and without antibodies against *Salmonella* spp, a Bayesian analysis, without a gold standard, of all three ELISA tests jointly, was performed. The (unknown) true state of interest was true seroconversion (yes, no), since all three tests aimed for detection of antibodies. In the model, the prevalence for true bacteriology for each herd is sampled from a distribution. The prevalence for true seroconversion follows indirectly through transition probabilities between the true states for bacteriology and seroconversion. Test results for bacteriology are related to true state for bacteriology, with a sensitivity and specificity, while tests results for the ELISA tests are related to true state for seroconversion, with a sensitivity and specificity. The specificity for bacteriology was taken to be 1. Joint ELISA test results, conditional upon true state for seroconversion, are modelled cf. Engel et al. (2006, 2008). Because the tests are based on similar principles, the model allows for conditional dependence between tests.

Prior distributions were specified for two parameters of the distribution for prevalence of bacteriological true positives, sensitivity of bacteriology, two transition probabilities between true states for bacteriology and seroconversion, parameters for sensitivity and specificity of the ELISA tests and for conditional dependence between these tests. Priors were based on the literature or diffuse, i.e. wide and non-informative. Markov chain Monte Carlo was performed with the WinBUGS package (Spiegelhalter et al., 2003).

Results

In 235 (28.1%) out of the 837 MLN samples analysed, *Salmonella* spp. was isolated. The relative sensitivity (rSe) and specificity (rSp) of each of the ELISA tests, as compared to culture results, are shown in Table 1.

A total of 176 (74.9%) *Salmonella* isolates belonged to the serogroups supposedly detected by the three ELISA tests (B, C1 and D). The ability of these tests to detect animals infected with the these *Salmonella* serogroups was variable, with test A detecting 122 (69.3%), test B 108 (61.4%), and test C only 50 (28.4%) of them.

The squared correlation coefficient (r^2) between herd prevalence and herd seroprevalence was low for the three ELISA tests: 0.2 ($P \leq 0.01$), 0.08 ($P=0.01$) and 0.21 ($P \leq 0.01$), for tests A, B and C respectively. In

addition, a marked discrepancy was observed in the herd classification when either bacteriology or serology was used to determine the status of the pig herds. Discrepancies were also found among the serological tests (Table 2).

Results of the Bayesian analysis are shown in Table 3. Accuracy of the three tests was moderate, with test B showing relatively high Se but low Sp, test C with low Se but high Sp and test A in between the other two tests. Overall, test A appeared to have the highest accuracy as measured by the Youden's index (J).

Discussion

As expected, serology did not correlate well with bacteriology at the individual level (Nollet et al, 2005), even in those animals infected with the *Salmonella* serogroups (B, C1 and D) targeted by these ELISA tests. The low, although significant, Pearson correlation coefficients observed between culture and serological results at the herd level indicated that only a small proportion of the observed prevalence could be explained by serology, which seemed to be related to the individual discrepancies observed. In fact, the relative Se and Sp (with regard to bacteriology) were low for the three ELISA tests. The best, but still modest, overall relative accuracy was shown by test A (Youden's index -J- = 0.20), which was in line with the results obtained from the Bayesian analysis without use of a gold standard. The overall accuracy of test A to detect antibodies against *Salmonella* spp was higher than that for the other two ELISA tests. This result suggests that test A may work better for the diagnosis of *Salmonella* spp. exposure under the conditions for Spanish finishing pig farms.

This study clearly shows that different ELISA tests may yield different test results. Although all of them may be useful to detect infected herds, they may introduce important bias when used for herd classification. When control programs were put in place in Spain, the use of a given ELISA test may have financial consequences for the farmers, especially if different serological tests with variable Se and Sp are allowed. Standardization of commercially available serological tests is thus advisable.

In any case, the study shows that it is necessary to develop improved tests for the diagnosis of this infection, because an important proportion of *Salmonella*-infected pigs is overlooked by the tests.

Table 1. Apparent antibody prevalence (AP_{ab}), relative sensitivity (rSe) and specificity (rSp) with regard to bacteriology, and Youden's index for the three ELISA tests analysed.

Test		Bacteriology +	Bacteriology -	AP_{ab}	rSe	rSp	J*
A	+	155	275	0.51	0.66	0.54	0.20
	-	80	327				
B	+	133	256	0.46	0.57	0.57	0.14
	-	102	346				
C	+	62	74	0.16	0.26	0.88	0.14
	-	173	528				
TOTAL		235	602				

*J=Se+Sp-1

Table 2. Number and percentage of herds within each health status category according to the different tests used.

	Herd status Negative herds	≤20% positive animals	>20% and ≤40% positive animals	>40% positive animals
Bacteriology	3 (8.8%)	11 (32.3%)	10 (29.4%)	10 (29.4%)
Serology				
Test A	0 (0%)	7 (20.6%)	7 (20.6%)	20 (58.8%)
Test B	0 (0%)	10 (29.4%)	5 (14.7%)	19 (55.9%)
Test C	9 (26.5%)	16 (47%)	4 (11.8%)	5 (14.7%)

Table 3. Estimates (posterior medians) and 95% credibility intervals (95%CrI) for the sensitivity (Se) and specificity (Sp) and the Youden's index (J) of the three ELISA tests studied for the diagnosis of pig salmonellosis on meat juice.

Test	Se (95% CrI)	Sp (95% CrI)	J*
A	0.88 (0.68, 0.92)	0.74 (0.64, 0.91)	0.62
B	0.96 (0.81, 0.99)	0.43 (0.34, 0.54)	0.39
C	0.40 (0.28, 0.55)	0.99 (0.96, 0.99)	0.39

*J=Se+Sp-1

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