Evaluation of enzyme-linked immunosorbent assay (ELISA) tests and culture for determining *Salmonella* status of a pig herd

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SUMMARY

The purpose of this study was to evaluate *Salmonella* ELISA tests and a culture technique to determine *Salmonella* status using samples collected from commercial herds. Faecal samples from 15 finisher pigs on each of 40 swine farms were cultured for *Salmonella*. Sera from the same 600 pigs were analysed for the presence of antibodies to *Salmonella* by means of two different ELISA kits Salmotype (Salmotype® Labordiagnostik, Leipzig, Germany) and IDEXX (Herdchek* *Salmonella*; IDEXX Laboratories, Schiphol-Rijk, Noord-Holland, The Netherlands). The Salmotype ELISA test demonstrated a moderate ability to differentiate culture-positive pigs from culture-negative pigs while IDEXX was relatively poor in classifying those pigs correctly (The area under the curves were 0·79 and 0·65 for Salmotype and IDEXX respectively). The maximum value of sensitivity plus specificity was gained at the cut-off optical density (OD) $\geqslant 25\%$ for Salmotype (sensitivity 0·65, specificity 0·84) and at the OD $\geqslant 9\%$ for IDEXX (sensitivity 0·59, specificity 0·69). The maximum herd sensitivity and herd specificity was 0·64 (95% CI 0·49–0·79) and 0·72 (95% CI 0·59–0·86) for the Salmotype test and 0·73 (95% CI 0·59–0·87) and 0·62 (95% CI 0·47–0·77) for the IDEXX. Culture and the ELISA tests showed fair agreement at the herd level ($\kappa=0·3$, $P<0·05$). Likewise there was significant disagreement between the two ELISA tests at the pig level, but very good agreement between the two ELISA tests ($\kappa=0·8$, $P<0·0001$) at the herd level. The coating antigens in Salmotype and IDEXX represent only 48% of the antigens of *Salmonella* isolated in our study and need to be revised based upon the *Salmonella* serovar distribution in Ontario.

INTRODUCTION

Salmonella spp. are important foodborne pathogens associated with pork products [1–3]. In order to control and minimize this source of human infection, efforts should be made at all levels of the pork supply chain (pre- and post-harvest). Epidemiological investigations need to be performed to determine the prevalence of *Salmonella*, and subsequently to evaluate appropriate intervention policies and to determine control programmes. In order to estimate the prevalence of *Salmonella* and to evaluate the effectiveness of interventions as accurately as possible, it is necessary to investigate bacteriology and serology methods more thoroughly. Culture and serology are the two different approaches for determining *Salmonella* status at the individual or population level and the use of each technique depends on the research question. Culture and isolation of *Salmonella* from faecal samples has been the most frequently used approach

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to determine the *Salmonella* status at the pig and herd level [4, 5]. Bacterial culture can be used to isolate *Salmonella* from caecal contents during the shedding phase of infection and also from other tissues including caecal lymph nodes, carcass surface and pharynx in the slaughtering stage. In addition, serotype, phage-type, molecular markers, and the antimicrobial resistance pattern might be determined. Another advantage of this technique is that if *Salmonella* is grown via culture the farm is unquestionably positive. Even though the culture of faecal material is a useful method to determine infections during the shedding phase, the disadvantage of culture is that animals carrying *Salmonella* may not shed the bacteria at the time of sampling or the culturing technique may not be sensitive enough to pick up *Salmonella* if only small numbers are present [6–8]. Culture technique is also an important factor *per se*. The media, enrichment steps, and weight of the faecal sample influence the probability of isolating *Salmonella* from the faecal sample [5]. In addition, the technique is labour intensive and costly so that culturing large numbers of samples per farm to increase the likelihood of finding a positive sample becomes prohibitively expensive and may not be practical for routine applications, particularly for screening purposes.

The *Salmonella* (LPS-Mix-ELISA) lipopolysaccharide-mixed-enzyme linked immunosorbent assay was first developed in Denmark based on local *Salmonella* antigen distribution with particular emphasis on those serovars which were of importance in terms of food safety concerns [9]. Since then, the ELISA test has been used to detect antibody to *Salmonella* in the meat, plasma and serum from pigs in many countries [10–20]. The advantage of using a serological test such as an ELISA is that it is fast and relatively inexpensive. In addition, the test measures whether the pig has had previous exposure and is not dependent on whether the pig is shedding or not at the time of testing [14]. Although culture and the ELISA tests measure different stages of *Salmonella* infection, the test characteristics of the commercially available ELISA tests are reported by the manufacturer and elsewhere based on a comparison with culture techniques under experimental conditions. Therefore, there are issues still to be resolved concerning the commercially available *Salmonella* serological tests. In an international ring trial, there was variation between 12 laboratories using 'in-house' or commercially available ELISA kits in testing the same samples correctly [21]. The correlation between serology

and *Salmonella* shedding has been demonstrated using experimental trials [9] and in field studies conducted in the countries where the tests were designed [11]. However, the correlation between these serological tests and bacterial culture to determine *Salmonella* status on swine farms is largely unknown in Canada, particularly for Ontario in that the coating antigens used in the tests are generally based on European serovars.

The purpose of this study was to investigate the application of two ELISA tests; Salmotype (Salmotype® Labordiagnostik, Leipzig, Germany) and IDEXX (Herdchek* *Salmonella*; IDEXX Laboratories, Schiphol-Rijk, Noord-Holland, The Netherlands) under field conditions to determine *Salmonella status* in finisher pig herds.

MATERIALS AND METHODS

Sampling

Forty farms were selected and in each farm, 15 finisher pigs in the grower-finisher barn were chosen for the study (600 pigs). Rectal faecal samples from three pigs per pen were collected into sterile containers and stored on ice packs during transport to the laboratory. A 10-ml blood sample was taken from each pig that provided a faecal sample. Blood samples were centrifuged, sera separated and stored at -70 °C.

Culture

Samples were cultured using 25 g faeces and a double enrichment step [5]. Twenty-five grams of faeces were added to 225 ml of buffered peptone water (BPW) and incubated for 2 h at 37 °C. A 0·1-ml sample was added to 9·9 ml Rappaport–Vassiliadis broth (RVB) and incubated for 2 h at 41·5 °C. Cultures were stored at room temperature (20–22 °C) for 96 h after which 0·1 ml of the RVB culture was inoculated into 9·9 ml RVB and incubated at 41·5 °C for 24 h. Finally, a loopful of the delayed secondary enrichment (DSE) was plated out on xylose-lysine-tergitol-4 (XLT-4) agar and Brilliant Green (BG) agar and incubated at 37 °C for 72 h and examined every 24 h for growth. The presumptive *Salmonella* colonies were selected and tested on triple sugar iron (TSI), lysine decarboxylase, citrate, urease agars, and examined using an 'O' agglutination test. The isolates were submitted to the OIÉ Reference Laboratory for Salmonellosis

for serotyping. A herd was classified as positive when *Salmonella* was isolated from at least one faecal sample.

ELISA

Sera were analysed for the presence of antibodies against *Salmonella* at Biovet (Biovet Inc., Quebec, Canada) using two kits; IDEXX Herdchek* *Salmonella* (IDEXX Laboratories Inc.) and Salmotype (Salmotype Labordiagnostik). Both the Salmotype and IDEXX kits were designed to detect antibodies to *Salmonella* by the same immunological reaction between antigen and antibody. The coating antigens in these ELISAs include LPS of serogroup B, C1 and D (O-antigens 1, 4, 5, 6, 7 and 12).

Data analysis

Data were entered into an Excel spreadsheet (Microsoft Excel 2000, Microsoft Corp., Redmond, WA, USA) and then imported into STATA (Stata 8 Intercooled for Windows 9x; StataCorp., College Station, TX, USA) in which the data were analysed. The sensitivity of each ELISA test at different optical density (OD) cut-points was plotted *vs.* the specificity to generate receiver-operating characteristic (ROC) curves [22]. The HERDACC program [23] was used to identify the number of seropositive pigs per farm, which resulted in the optimal herd sensitivity and specificity [22]. These numbers were used to classify herds as sero-positive and seronegative. Herd seropositivity status was compared to herd culture in order to estimate the herd-level sensitivity and specificity of the serological tests.

Agreement between tests

Agreement between the two ELISA tests and culture was evaluated at both the pig and herd levels. A κ (Cohen's kappa) statistic was used to find the extent of agreement between ELISA and culture methods beyond what would have been expected by chance. McNemar's χ^2 was also used to find whether there was a difference between the positive proportion of the two tests. A non-significant McNemar's χ^2 test indicates that the two positive proportions do not differ whereas a significant McNemar's χ^2 test means that there is disagreement between the tests and therefore assessment of kappa would not be valuable [22]. The common interpretations of

Table 1. *The serotype, serogroup and antigenic formula of 48* Salmonella *isolates*

Serovar	Antigenic formula	Sero-group	No. of isolates (%)
Typhimurium	4:i:2 or 4,5:i:2	B	14 (29·2)
I:Rough-O:d:l,w	-d:l,w*		9 (18·8)
Havana	23:fg:-	G2	5 (10·4)
Infantis	6,7:r:5	C1	5 (10·4)
London	10:l,v:6	E1	5 (10·4)
Brandenburg	4:l,v:z15	B	2 (4·2)
Mbandaka	6,7:z10:z15	C1	2 (4·2)
Cerro	18:z4,z23:-	K	2 (4·2)
S. enterica spp. I:17:-:-	17:-:-	J	2 (4·2)
S. enterica spp. I:18:-:-	18:-:-	K	1 (2·1)
S. enterica spp. IV:21:-:-	21:-:-	L	1 (2·1)

* 'O' antigen is not present.

kappa are as follows: <0·2 slight agreement; 0·2–0·4 fair agreement; 0·4–0·6 moderate agreement; 0·6–0·8; substantial agreement, and >0·8 almost perfect agreement [22].

RESULTS

Serotypes

The serovars, serogroup and antigenic formula of the isolates are shown in Table 1. The isolates belonged to serogroups B, C1, E1, G2, J, K, and L with the O antigens 4, 5, 6, 7, 10, 17, 18 and 23 (Table 1).

Test characteristics

The area under the curves were 0·79 [95% confidence interval (CI) 0·70–0·88] and 0·65 (95% CI 0·50–0·80) for Salmotype and IDEXX respectively. It indicates that Salmotype has moderate ability to differentiate culture-positive pigs from culture-negative pigs compared to IDEXX which is less accurate in discriminating these pigs.

The maximum value of sensitivity plus specificity (Youdan index) was gained at the cut-off OD ⩾25% for Salmotype (sensitivity 0·65, specificity 0·84) and at the OD ⩾9% for IDEXX (sensitivity 0·59, specificity 0·69) (Figs 1 and 2). As OD cut-offs of 10%, 20%, and 40% are more commonly used in the literature in order to identify seropositive and seronegative pigs, we decided to use cut-points of OD ⩾20% and OD ⩾10% for Salmotype and IDEXX respectively. Thus, the individual test specificity for Salmotype

Table 2. *Individual test characteristics of Salmotype and IDEXX ELISA tests at different cut-points of optical density (OD)*

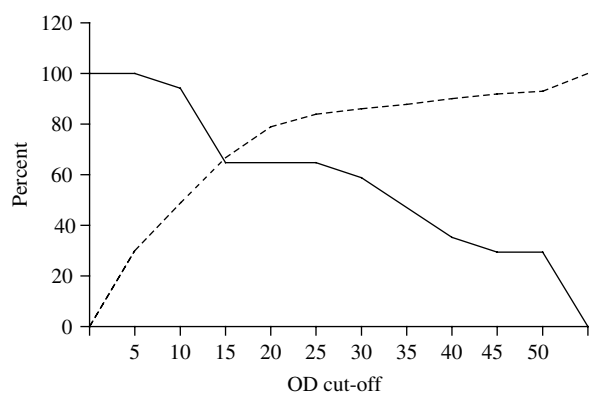| OD cut-off	Salmotype		IDEXX	
	Test sensitivity (95% CI)	Test specificity (95% CI)	Test sensitivity (95% CI)	Test specificity (95% CI)
OD ⩾10	0·94 (0·92–0·96)	0·49 (0·45–0·53)	0·53 (0·49–0·57)	0·72 (0·69–0·76)
OD ⩾20	0·65 (0·61–0·69)	0·79 (0·75–0·82)	0·41 (0·37–0·45)	0·86 (0·83–0·89)
OD ⩾30	0·59 (0·55–0·63)	0·86 (0·83–0·89)	0·29 (0·26–0·33)	0·91 (0·88–0·93)
OD ⩾40	0·35 (0·31–0·39)	0·90 (0·87–0·92)	0·29 (0·26–0·33)	0·93 (0·91–0·95)

CI, confidence interval.

Fig. 1. Plot of sensitivity (——) and specificity (- - -) at different optical density (OD) cut-offs for Salmotype (Salmotype® Labordiagnostik). The ELISA test was compared to culture as the gold standard and the sensitivity of the test at different cut-offs is plotted *vs*. specificity.
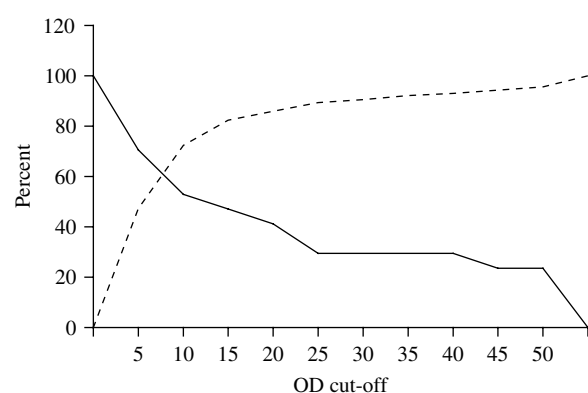

Fig. 2. Plot of sensitivity (——) and specificity (- - -) at different optical density (OD) cut-offs for IDEXX (Herdchek* Salmonella, IDEXX Laboratories). The ELISA test was compared to culture as the gold standard and the sensitivity of the test at different cut-offs is plotted *vs*. specificity.

dropped by 5% while the individual test sensitivity did not change (Table 2). On the other hand, using a cut-point of OD ⩾10% for IDEXX, the individual test sensitivity went down by 6% while the test specificity increased by 3% (Table 2). At these cut-points, the maximum value of herd sensitivity plus herd specificity was obtained while a herd was classified as positive with at least five and four seropositive pigs for Salmotype and IDEXX respectively (Table 3). The herd sensitivity and herd specificity was 0·64 (95% CI 0·49–0·79) and 0·72 (95% CI 0·59–0·86) for Salmotype and 0·73 (95% CI 0·59–0·87) and 0·62 (95% CI 0·47–0·77) for IDEXX (Table 3).

Agreement between the tests

The positive proportions obtained by culture and the two ELISA tests (OD ⩾20% for Salmotype and OD ⩾10% for IDEXX) differed at the pig level ($P < 0.0001$) (Table 4) suggesting disagreement between the individual ELISA tests and culture at the

pig level. Cohen's κ was 0·3 ($P < 0.05$) for both ELISA tests while a herd was classified as positive with at least five and four seropositive pigs for Salmotype and IDEXX respectively indicating fair agreement between the culture and the ELISA tests at the herd level (Table 5).

The two positive pig proportions obtained by two serological tests differed ($P < 0.0001$) indicating a significant disagreement between Salmotype and IDEXX at the pig level (Table 4). At the herd level, on the other hand, the positive proportions obtained by the two ELISAs did not differ and very good agreement ($\kappa = 0.84$) was observed between the two tests at this level ($P < 0.0001$) (Table 5).

DISCUSSION

Cut-points and test characteristics

We compared two serological tests, using bacterial culture as the gold standard in order to achieve the

Table 3. *Herd characteristics of Salmotype and IDEXX ELISA tests at different cut-points of seropositive pig* (as the herd cut-points)

No. of seropositive pigs	Salmotype*		IDEXX†	
	Herd sensitivity (95% CI)	Herd specificity (95% CI)	Herd sensitivity (95% CI)	Herd specificity (95% CI)
1	1·0 (1·0–1·0)	0·10 (0·09–0·19)	1·0 (1·0–1·0)	0·28 (0·14–0·41)
2	0·73 (0·59–0·87)	0·55 (0·40–0·71)	0·82 (0·70–0·94)	0·41 (0·26–0·57)
3	0·64 (0·49–0·79)	0·62 (0·47–0·77)	0·73 (0·59–0·87)	0·55 (0·40–0·71)
4	0·64 (0·49–0·79)	0·66 (0·51–0·80)	0·73 (0·59–0·87)	0·62 (0·47–0·77)
5	0·64 (0·49–0·79)	0·72 (0·59–0·86)	0·64 (0·49–0·79)	0·69 (0·55–0·83)

CI, Confidence interval.
* Salmotype: with OD ⩾20% as pig seropositive.
† IDEXX: with OD ⩾10% as pig seropositive.

Table 4. *Summary of agreement of ELISA* (*Salmotype* and IDEXX†*) *with culture at the pig level*

Tests	Agreement (95% CI)	Kappa (95% CI)
Salmotype and IDEXX	0·83 (0·80–0·86)	0·56 (0·06 to 0·63)‡
Culture and Salmotype	0·78 (0·75–0·82)	0·1 (−0·08 to 0·12)
Culture and IDEXX	0·72 (0·68–0·75)	0·05 (−0·08 to 0·01)

CI, Confidence interval.
* Salmotype: with OD ⩾20% as pig seropositive.
† IDEXX: with OD ⩾10% as pig seropositive.
‡ Despite apparent agreement, a significant McNemar's χ^2 test ($P < 0.0001$) indicating that the positive proportions obtained by the two ELISA tests differ and therefore assessment of kappa was not valuable.

Table 5. *Summary of agreement of ELISA* (*Salmotype* and IDEXX†*) *with culture at the herd level*

Tests	Agreement (95% CI)	Kappa (95% CI)
Salmotype and IDEXX	0·93 (0·80–0·98)	0·80 (0·24 to 0·92)
Culture‡ and Salmotype	0·70 (0·54–0·83)	0·32 (−0·10 to 0·58)
Culture and IDEXX	0·68 (0·51–0·81)	0·29 (−0·13 to 0·53)

CI, Confidence interval.
* Salmotype: with OD ⩾20% as pig seropositive and at least five seropositive pigs as herd seropositive.
† IDEXX: with OD ⩾10% as pig seropositive and at least five seropositive pigs as herd seropositive.
‡ At least one culture-positive pig indicated a positive herd.

test diagnostic characteristics. However, serology and bacteriology results cannot be easily compared [7] since at some stages the pigs may not be shedding bacteria but have antibodies to *Salmonella* as a result of a previous exposure. In addition, *Salmonella* infection has been demonstrated to be very dynamic and therefore culture and seroprevalence of *Salmonella* on swine farms may not be constant over time [16].

Both Salmotype and IDEXX tests were shown to have a poor ability to identify culture-positive and culture-negative pigs. The ability of these tests to identify positive pigs is dependent on where the cut-off of OD is established, which *Salmonella* antigens are used as coating antigens in the tests, and what isotype of immunoglobulin is used as the conjugated secondary antibody (i.e. anti-Ig or anti-IgM). The ability of an ELISA to identify culture-positive and

culture-negative pigs is also dependent on whether the culture-negative pigs have had exposure to *Salmonella*.

We used the OD cut-points for Salmotype and IDEXX as 20 and 10 respectively, at which the maximum value of sensitivity plus specificity (Youden index) was achieved [22]. However, this approach may not always be suitable and depends on the objective of the study. For example, by using a cut-point of OD ⩾10% for Salmotype, false negatives could be minimized to 6% compared to OD ⩾20% with 35% false negatives. On the other hand, by choosing a cut-point of OD ⩾40% the false positives could be minimized to a level of 10%.

Apparent false-negative serological results compared to culture results have been previously observed [7]. The false-negative results could be either interpreted biologically or considered as a result of technological problems. Isolation of *Salmonella* indicates

a true positive status due to the high specificity of culture. Therefore part of 35% and 47% false-negative pigs by Salmotype and IDEXX might be a result of the choice of antigens used in coating the ELISA plate. Some of the *Salmonella* serovars isolated from Ontario farms may not be included in this test. Salmotype and IDEXX include the coating antigens which represent 90% of serovars isolatedin Denmark and The Netherlands and are considered the most important zoonosis in those countries [9, 17, 24]. The coating antigens in Salmotype and IDEXX may in fact represent only 48% of the antigens of *Salmonella* isolated in our study (Table 1).

Recent infection can be taken as another biological interpretation for false-negative results. The IgM is the first immunoglobulin to appear after *Salmonella* infection [13] and 'anti-IgG' secondary conjugated antibody, which is included in IDEXX cannot bind to IgM resulting in more false negatives. The Salmotype test, on the other hand, includes 'anti-immunoglobulin' secondary antibody and, therefore, can detect both IgM and IgG.

False-positive results obtained by Salmotype (21%) and IDEXX (28%), on the other hand, may demonstrate the 'true' status of *Salmonella* infection in pigs that are carrier pigs or lower subclinical infection. Pigs may harbour infections but not shed *Salmonella* for extended periods [6, 13]. The pigs tested in the present study were all close to market weight or about 6 months of age, so that is very likely that many of these animals would have been infected at earlier stages and might have antibodies present but no longer shed *Salmonella* in their faeces.

In addition, the choice of tissue sampled may influence the profile of serovars [25]. A serovar might not be isolated from faeces and yet be present in another tissue resulting in the presence of antibody. One can speculate that the agreement between culture and serology might change if younger pigs are tested. It is also possible that false-positive serological results could be caused by cross-reaction to antibodies produced against other bacteria such as *Yersinia enterocolitica* [14, 21].

'False-negative' culture results is another reason for apparent false-positive serological results. The sensitivity of culture to detect *Salmonella* at the farm level has been reported to be 50–60% [14] and even lower at the pig level, therefore seropositive pigs might even shed bacteria which is not detected by culture.

Agreement between the ELISA tests and bacterial culture

We found no agreement between serology results and culture at the pig level that is expected and can be related to false-positive and false-negative serological results, as described previously. Despite this apparent disagreement at the pig level, a fair agreement was observed between serology and culture at the herd level. This agreement can be attributed to the fact that a culture-positive farm was defined as a farm with at least one positive pig. For example, a farm which was classified as positive by one culture-positive pig which tested negative serologically, may have tested serologically positive on the basis of seropositive pigs which did not shed *Salmonella*. Therefore, culture and serology would agree at the herd level but disagree at the pig level in this example.

Second, a specific farm may be positive in culture for serovars other than serovars included in the ELISA test. *Salmonella*-positive herds with multiple serovars have been reported in other studies [15, 24]. In a multi-serovar positive farm with one *S.* Typhimurium-positive pig and one *S.* Havana-positive pig, for example, ELISA could detect antibody to *S.* Typhimurium (carrying O:4,5 antigen) but not to *S.* Havana (carrying O:23 antigen) while the farm tests positive and therefore despite disagreement at the pig level, ELISA and culture agreed at the herd level.

Despite disagreement at the pig level, there was excellent agreement between Salmotype and IDEXX at the herd level. The pigs that tested positive by Salmotype were not necessarily positive by IDEXX and also the pigs that tested negative by Salmotype were not necessarily negative by IDEXX. However, the farm was considered positive based on the number of pigs that tested positive regardless of the individual identification of the pigs.

CONCLUSION

Culture and ELISA tests measure different stages of *Salmonella* infection and therefore cannot be easily compared. However, serological tests need to be revised based upon the *Salmonella* serovar distribution in Ontario. It is possible to find seronegative pigs that are positive based on bacterial culture and culture-negative pigs that test positive serologically. Therefore, choosing either a culture or an ELISA method depends on what question is going to be addressed.

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DECLARATION OF INTEREST

None.

REFERENCES

1. **Berends BR, et al.** Impact on human health of *Salmonella* spp. on pork in The Netherlands and the anticipated effects of some currently proposed control strategies. *International Journal of Food Microbiology* 1998; **44**: 219–229.

2. **Borch E, Nesbakken T, Christensen H.** Hazard identification in swine slaughter with respect to foodborne bacteria. *International Journal of Food Microbiology* 1996; **30**: 9–25.

3. **Nielsen B, Wegener HC.** Public health and pork and pork products: regional perspectives of Denmark. *Review of Science and Technology* 1997; **16**: 513–524.

4. **Christensen JD, et al.** Herd prevalence of *Salmonella* spp. in Danish pig herds after implementation of the Danish *Salmonella* Control Program with reference to a pre-implementation study. *Veterinary Microbiology* 2002; **88**: 175–188.

5. **Davies PR, et al.** Comparison of methods for isolating *Salmonella* bacteria from faeces of naturally infected pigs. *Journal of Applied Microbiology* 2000; **89**: 169–177.

6. **Schwartz KJ.** Salmonellosis. In: Straw, BE, D'Allaire S, Mengeling WL, Taylor DJ, eds. *Diseases of Swine*, 8th edn. Ames, Iowa: Iowa State University Press, 1999, pp. 535–551.

7. **van Winsen RL, et al.** Monitoring of transmission of *Salmonella* enterica serovars in pigs using bacteriological and serological detection methods. *Veterinary Microbiology* 2001; **80**: 267–274.

8. **Kranker S, Alban L, Boes J, Dahl J.** Longitudinal study of *Salmonella* Typhimurium infection in three Danish farrow-to-finish swine herds. *Journal of Clinical Microbiology* 2003; **41**: 2282–2288.

9. **Nielsen B, et al.** The serological response to *Salmonella* serovars typhimurium and infantis in experimentally infected pigs. The time course followed with an indirect anti-LPS ELISA and bacteriological examinations. *Veterinary Microbiology* 1995; **47**: 205–218.

10. **Mousing JP, et al.** Nation-wide *Salmonella* enterica surveillance and control in Danish slaughter swine herds. *Preventive Veterinary Medicine* 1997; **29**: 247–261.

11. **Sorensen LL, et al.** The correlation between *Salmonella* serology and isolation of *Salmonella* in Danish pigs at slaughter. *Veterinary Microbiology* 2004; **101**: 131–141.

12. **Alban L, Stege H, Dahl J.** The new classification system for slaughter-pig herds in the Danish *Salmonella* surveillance-and-control program. *Preventive Veterinary Medicine* 2002; **53**: 133–146.

13. **Beloeil PA, et al.** Impact of the *Salmonella* status of market-age pigs and the pre-slaughter process on *Salmonella* caecal contamination at slaughter. *Veterinary Research* 2004; **35**: 513–530.

14. **Lo Fo Wong DM, et al.** Recovery of *Salmonella* enterica from seropositive finishing pig herds. *Veterinary Microbiology* 2003; **97**: 201–214.

15. **Proux K, et al.** Development of a complete ELISA using *Salmonella* lipopolysaccharides of various serogroups allowing to detect all infected pigs. *Veterinary Research* 2000; **31**: 481–490.

16. **Rostagno MH, Hurd HS, Mckean JD.** Bacteriological and serological *Salmonella* prevalence in finishing pigs. In: *Proceedings of the 18th International Pig Veterinary Society (IPVS) Congress*, 2004; **2**: 649.

17. **van der Wolf PJ, et al.** *Salmonella* seroprevalence at the population and herd level in pigs in The Netherlands. *Veterinary Microbiology* 2001; **80**: 171–184.

18. **Blaha T.** The present state of the German QS S*almonella* monitoring and reduction programme. *Deutsche Tierarztliche Wochenschrift* 2004; **8**: 324–326.

19. **Leontides LS, Grafanakis E, Genigeorgis C.** Factors associated with the serological prevalence of *Salmonella* enterica in Greek finishing swineherds. *Epidemiology and Infection* 2003; **131**: 599–606.

20. **Lo Fo Wong DMA, et al.** Herd-level risk factors for subclinical *Salmonella* infection in European finishing-pig herds. *Preventive Veterinary Medicine* 2004; **62**: 253–266.

21. **van der Heijden HM.** First international ring trial of ELISAs for *Salmonella*-antibody detection in swine. *Berliner und Munchener Tierarztliche Wochenschrift (Berlin)* 2001; **114**: 389–392.

22. **Dohoo I, Wayne M, Stryhn H.** *Veterinary Epidemiologic Research*. Canada: AVC Inc., University of Prince Edward Island, 2003, pp. 85–120.

23. **Jordan D, McEwen SA.** Herd-level test performance based on uncertain estimates of individual test performance, individual true prevalence and herd true prevalence. *Preventive Veterinary Medicine* 1998; **36**: 187–209.

24. **van der Wolf PJ, et al.** *Salmonella* infections in finishing pigs in The Netherlands: bacteriological herd prevalence, serogroup and antibiotic resistance of isolates and risk factors for infection. *Veterinary Microbiology* 1999; **67**: 263–275.

25. **Davies PR, et al.** Observational evidence for differential translocation of *Salmonella* serovars to mesenteric lymph nodes of swine. In: *Proceedings of the 18th International Pig Veterinary Society (IPVS) Congress*, 2004; **2**: 652.