Comparison of bacterial culture, PCR and a mix-ELISA for the detection of \textit{Salmonella} status in nursery and grow-to-finish pigs in Western Canada using a Bayesian approach

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Abstract
Bayesian and traditional statistical methods were used to estimate accuracy of bacterial culture, broth-enriched real-time-PCR for feces and a mix-ELISA (Svanovir\textsuperscript{®}) for serum to detect \textit{Salmonella} in nursery and grow-finish pigs on 10 farms in western Canada. In nursery pigs, one pooled pcn fecal sample and one blood sample were taken from each of 30 randomly selected pens. In grow-finish pigs, samples were similarly collected; an individual fecal sample was also taken from each pig bled. Only 8/247 ELISA-positive nursery pigs were detected; 80/247 pens were culture positive. Since there was no agreement between pen culture and ELISA results in the nursery pigs, further evaluation of test accuracy was not possible at this level. Among grow-to-finish pigs, agreement between culture and ELISA was fair ($\kappa = 0.26 - 0.38$). Agreement between culture and the RT-PCR was nearly perfect ($\kappa = 0.92 - 0.97$).

For grow-finish pigs, Bayesian posterior estimates for test sensitivity (Se) and specificity (Sp) were similar to traditional estimates at an ELISA cutoff of OD40\%. Depending on ELISA OD\%, sampling level and prior information specified in the model, estimates for culture and RT-PCR Se ranged from 65\% - 75\% (culture Sp assumed ~100\%). RT-PCR Sp was 98\%-99\%. Mean estimates for the ELISA Se/Sp were 47/87\% (OD≥20\%) and 31/92\% (OD≥40\%) at the pen level, and 61/86\% (OD≥20\%) and 38/96\% (OD≥40\%) for individual pigs. This study has demonstrated that a Bayesian approach to estimating the accuracy of tests for \textit{Salmonella} in pigs is a viable alternative to traditional methods of test evaluation.

Introduction
Traditionally, the evaluations of the sensitivity (Se) and specificity (Sp) of diagnostic tests are done by comparison to a gold standard, a test (or tests) which accurately determines the true disease state of an animal. As is often the case, though, evaluation of tests for detecting \textit{Salmonella} infection in pigs is complicated by the lack of a gold standard. A Bayesian approach to test evaluation offers an alternative approach in which none of the competing tests are treated as the imperfect gold standard and the diagnostic error rates are estimated for all studied tests (Hui and Zhou, 1998). In this approach, prior information (e.g. previous research, expert opinion) is combined with the observed data to obtain posterior distributions of test parameters. Knowledge of the true disease or infection status of the animal is not necessary, and instead this unknown information is incorporated into the model as a latent variable (Eneoe et al., 2000). The Bayesian approach is particularly useful for evaluating tests that are measuring chronic, persistent infections and for which no gold standard exists (Branscum et al., 2005). \textit{Salmonella} infections are prevalent in pigs, with most infections being sub-clinical. Infection may be cleared, or pigs may progress to a chronic carrier state with intermittent shedding. These chronic carriers then contribute to the persistence of \textit{Salmonella} within the herd. Within sub-clinically infected herds, there will be found a mix of both acute and chronic \textit{Salmonella} carrying or shedding pigs which may be either seropositive or seronegative. The study objective was to evaluate the diagnostic accuracy of a bacterial culture, real-time
PCR, and an ELISA for detecting Salmonella in western Canadian pigs under field conditions using Bayesian methods, and to compare the results of this approach to those obtained with traditional methods.

Materials and methods

Farm selection: To ensure Salmonella-positive samples were obtained, 10 farrow-to-finish farms were purposely selected based on presumed Salmonella status (7 positive, 3 negative). Herds were presumed positive at the time of herd selection if clinical salmonellosis was observed within the previous 12 months, if Salmonella species were identified during routine testing, or if replacement breeding stock were purchased from known Salmonella-positive farms; otherwise, herds were presumed negative. Sample collection: On each farm, individual fecal samples (10 g) were collected from 1 grow-finish pig in each of 30 randomly selected pens; from each of these pigs, blood was also collected. Pooled fecal samples (min 25 g/pen) were also collected from each of these pens. Pooled feces and 1 individual blood sample were likewise collected from 30 nursery pens on each farm.

Bacteriological Culture: Culture for Salmonella was performed by the Agri-Food Laboratories Branch, Alberta Agriculture and Rural Development, using a previously published protocol (Wilkins et al., 2009).

RT-PCR: RV and TT enrichment broths from each sample cultured as described above were mixed together then analyzed using a previously published RT-PCR assay with primers and hybridization probes to the Salmonella invA gene (Bohaychuk et al., 2007).

Serology: Serum samples were analyzed using a covalent mix-ELISA (Svanovir®, Svanova Biotech, Uppsala, Sweden) and was carried out according to the manufacturer’s instructions.

Statistical analyses: Data for grow-to-finisher pigs and nursery pigs were analyzed separately. Herds were divided into 2 populations based on herd size: population 1 consisted of 5 herds with < 250 breeding females per herd; population 2 consisted of 5 herds > 400 breeding females per herd. A 3-test, 2-population Bayesian model with dependence between culture and RT-PCR was specified using the WinBUGS software (http://www.mrc-bsu.cam.ac.uk/bugs/). Since this model lacks identifiability, informative prior information were required; these were the mean values and standard deviations (s.d.) generated through a systematic review of primary research evaluating these tests in various swine populations (Wilkins et al., 2009; unpublished data). Prior information for Sp was set at a value approaching 100%, as all isolates were confirmed by serotyping. Other prior information is indicated in Table 1. These values were converted to \( \beta \) distributions using the Beta Buster software (http://www.epi.ucdavis.edu/diagnostic tests/betabuster.html). The results of the 3-test, 2-population model with dependence between culture and RT-PCR were also compared to a 2-test, 2 population conditional independence model for culture and ELISA alone as well as to traditional estimates of Se and Sp.

Results

Agreement between tests:

Nursery pigs: The mean age of pigs in these pens was 6.6 weeks (range 3-11 weeks). Only 8/247 ELISA-positive nursery pigs were detected; 80/247 were culture positive. There was almost no agreement between the ELISA and either culture or RT-PCR (OD\( \geq \)20%: \( \kappa = 0.02 \); OD\( \geq \)40%: \( \kappa = 0.001 \)). Agreement between culture and RT-PCR was excellent (\( \kappa = 0.88 \)). With no agreement between either bacterial culture or RT-PCR and the ELISA in samples from nursery pigs, this dataset was not examined further.

Grow-finishers: The mean age of pigs in these pens was 16.5 weeks (range 8-27 weeks). Seventy-two percent (84/117) of Salmonella isolates from pooled feces and 74% (55/77) of isolates from individual feces belonged to serogroups B, C1 and D. Corresponding ELISA OD\( \geq 20\% \) results for B, C1 and D culture-positive samples were 50% (42/84) ELISA-positive for pen samples and 63% (34/54) for individual samples. ELISA OD\( \geq 40\% \) results were 31% (10/84) and 41% (22/54) ELISA-positive for pen and individual samples, respectively. The ELISA (at either OD\( \geq 20\% \) or OD\( \geq 40\% \)) was not more likely to detect seropositive pigs if the corresponding pooled or individual fecal sample was positive for serogroups B, C1 and D than if other serogroups were present (\( p = 0.18 \), \( p = 0.22 \)). There was only fair agreement between the ELISA and either culture or RT-PCR (OD\( \geq 20\% \); \( \kappa = 0.31 \); OD\( \geq 40\% \); \( \kappa = 0.26 \)) at the pen level. Only fair agreement was seen at the individual level also (OD\( \geq 20\% \); \( \kappa = 0.38 \); OD\( \geq 40\% \); \( \kappa = 0.36 \)). Agreement between culture and RT-PCR was almost perfect (\( \kappa = 0.97 \)).

Estimation of Se and Sp
Individual pigs were considered *Salmonella* positive if they were positive by any test. Division into 2 populations according to herd size resulted in different apparent prevalences for each population (Table 1). Bayesian prevalence estimates are also shown in Table 1. *P*-values for the goodness-of-fit statistic ranged from 0.10 to 0.36 for all models (Table 1), indicating that there was no substantial lack of fit in these models. A small but significant covariance between $Se_c$ and $Sp_c$ was detected. The resulting posterior (mean) estimates from each model at both ELISA OD≥20% and ≥40% for the pen data and individual data are shown in Table 1. The influence of posterior information is most noticeable in the 2-test Model D, whereby specification of an uninformative prior for $Se_c$ resulted in posterior $Se_c$ estimates 14-17% greater than the other models. Relative $Se_p$ and $Sp_p$, as compared to culture and estimated according to traditional methods, were excellent. The estimates of relative $Se_c$, $Se_p$ and $Sp_p$ were similar to results obtained from the Bayesian analysis when ELISA cutoff value was OD40%.

**Discussion**

The results from the Bayesian analysis were similar to results obtained from traditional estimates of $Se$ and $Sp$ at an ELISA cutoff value of OD40%; however, the Bayesian approach eliminated some of the variability in the estimates associated with the choice of ELISA cutoff value. While estimates for $Se_c$ obtained via the traditional approach varied by about 12% depending on ELISA cutoff value, the posterior Bayesian estimates for $Se_c$ within each model were constant regardless of cutoff value, and corresponded closely to traditional estimates at cutoff OD≥40%. This effect was also apparent in the posterior estimates for *Salmonella* prevalence in each population, where prevalences within models were constant regardless of cutoff value.

The Svanovir ELISA detects antibodies against serogroups B, C1, or D, and should detect immunological response to these serogroups, but in the current study this ELISA (at OD≥20%) only detected 50% of pens and 63% of pigs positive for these serogroups. Furthermore, the Svanovir ELISA detected similar proportions of pens and individuals which were culture-positive for serogroups other than B, C1 and D. As shown in this study, a large portion of pigs shedding *Salmonella* from serogroups B, C1 and D may not be detected by the Svanovir ELISA. For this reason, it is important to recognize and quantify the limitations of this test (or similar ELISAs) in the populations to which it is applied.

The RT-PCR used in this study has been previously evaluated in a variety of sample matrices and the results have been described elsewhere (Bohaychuk et al., 2007). The excellent agreement between the two tests was confirmed in the current analysis. Existing research indicates that PCR of pre-enriched samples tends to be quite sensitive; specificity, however, may be more variable. Identification of presumptive colonies of *Salmonella* is the most time, labor and cost intensive part of *Salmonella* culture; therefore, the use of PCR as a screening tool may improve time and cost effectiveness, particularly when prevalence is low.

Initially, the objective of this study was to evaluate culture, ELISA and RT-PCR in both nursery and in grow-to-finish pigs; however, there were not enough seropositive nursery pigs detected to allow the analysis to be done at this production level even though the prevalence based on culture positive was 31%. Even though a significant portion of nursery pigs were found to be shedding *Salmonella*, it is likely that most of the nursery pigs examined in our study were too young to have been able to mount a detectable immune response. Serology for *Salmonella* is not useful in nursery pigs, and, therefore, efforts to monitor *Salmonella* status in these animals would use bacterial culture, PCR or antigen-capture ELISA assays.

When considering the *Salmonella* status of pigs, pens or herds given the results of both culture (or RT-PCR) and ELISA, the latent class would be a mixture of exposure and infection. Sub-clinical *Salmonella* infection in pigs is not an acute infectious disease, but rather a persistent, chronic infection within herds which are comprised of individual pigs with various shedding/infection status (Schwartz, 1999). Within the context of the Bayesian model, the latent class becomes "ever exposed" (culture, RT-PCR or ELISA positive) or "never exposed" (culture, RT-PCR and ELISA negative), and reflects the information that the pig provides regarding the potential for contamination in its environment. This has important implications for *Salmonella* monitoring and control programs which focus on identifying high-risk farms, defined by within-herd *Salmonella* prevalence. The Bayesian approach may allow more precise estimates of herd prevalence by combining both ELISA and culture results. Bayesian methods provide the advantage over traditional methods by allowing estimation of test parameters without having to designate
either test as the gold standard and allowing incorporation of prior knowledge of test parameters and provide a viable alternative for comparing tests for *Salmonella* in pigs.

Table 1. Bayesian and traditional estimates of pen- and pig-level Se (%) and Sp of culture, Svanovir ELISA and RT-PCR used to detect *Salmonella* infection in grow-finish pigs

<table>
<thead>
<tr>
<th>Model</th>
<th>ELISA OD&lt;sup&gt;20%&lt;/sup&gt;:</th>
<th>ELISA OD&lt;sup&gt;60%&lt;/sup&gt;:</th>
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<tr>
<td></td>
<td>Se&lt;sub&gt;c&lt;/sub&gt;</td>
<td>Se&lt;sub&gt;p&lt;/sub&gt;</td>
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<tr>
<td>Pen:</td>
<td></td>
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<tr>
<td>A</td>
<td>70</td>
<td>69</td>
</tr>
<tr>
<td>B</td>
<td>89</td>
<td>50</td>
</tr>
<tr>
<td>C</td>
<td>61</td>
<td>49</td>
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</tbody>
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| Pig:  |                |                |                |                |                |                |                |                |                |                |                |                |
| A     | 65             | 67             | 61             | 98             | 86             | 25             | 48             | 65             | 66             | 38             | 98             | 96             | 24             | 47             |
| B     | 82             | 64             | 83             | 20             | 37             |                | 82             | 42             | 94             | 19             | 37             |                |                |                |
| C     | 51             | 62             | 80             | 24             | 41             |                | 63             | 40             | 92             | 36             | 47             |                |                |                |

Model A: 3-test model with conditional dependence between culture and PCR; prior information for Se<sub>c</sub>: mode 57%, with 95% certainty >37%; Sp<sub>c</sub>: ~100%; Se<sub>p</sub> mode 57%, with 95% certainty >37%; Sp<sub>p</sub>: mode 98%, with 95% certainty >90%. All other priors non-informative e.g. β (1,1).

Model B: 2-test, 2 population model (culture and ELISA only); prior information for Sp<sub>c</sub> and Sp<sub>p</sub> as above. All other priors non-informative e.g. β (1,1).

Model C: Traditional estimates of test Se and Sp (culture and ELISA only)

*<sup>a</sup>* prevalence in herds with <250 breeding females; *<sup>b</sup>* prevalence in herds with >400 breeding females

References:


