Assessment of the diagnostic accuracy of bacteriological culture and the *invA*-gen-based PCR for the detection of *Salmonella* organisms from caecal content from slaughtered pigs through Bayesian approaches

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Abstract

The goal of this study was to determine the accuracy of a culture technique and the *invA*-gen-based PCR, for the detection of *Salmonella* spp from caecal samples from slaughtered pigs. For this purpose a Bayesian approach was used. Two hundred and three pigs were used. Animals were grouped into 2 populations: 96 from small farms and 107 from large farms. Sensitivity was 56% (95% Credible Interval: 40-76) for culture and 91% (95%CI: 81-97) for PCR. The specificity of the PCR was 88% (95%CI: 80-95). According to these estimates, the percentage of pigs with *Salmonella* organisms in their faeces at slaughter in this population was at least 25.5%. It is concluded that bacteriology on caecal samples alone is a poor diagnostic method to carry out studies on the prevalence of salmonellosis in pigs, and that the sensitivity of this technique would be probably lower if the procedure is simplified or is carried out on non-stressed pig populations. PCR was considered a reliable screening method for the diagnosis of pig salmonellosis but prone to some misclassifications that should be considered if this technique is used.

Introduction

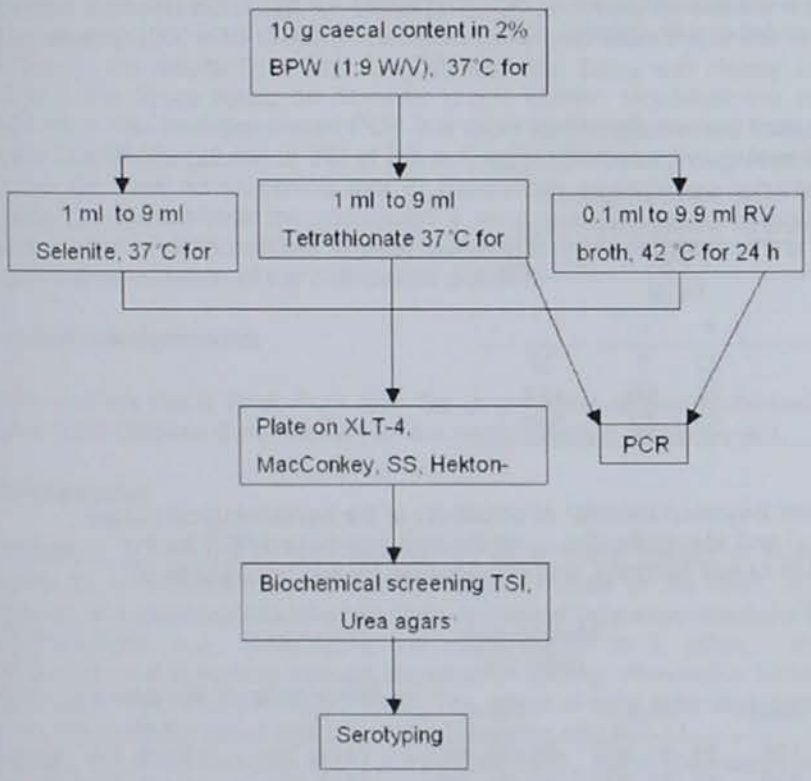
The accuracy of bacteriology for the isolation of *Salmonella* organisms from faeces from subclinically-infected pigs is a current matter of research. A large number of pre-enrichment, enrichment, and selective media have been described, and different combinations of them have been used for isolation of *Salmonella*, apparently showing large variations in terms of sensitivity (Hoorfar and Mortensen, 2000; Rostagno et al, 2005). Moreover, asymptomatic pig salmonellosis is characterized by intermittent shedding, thus culture may yield false negative results simply because the organism is absent from the sample collected. Thus, bacteriology is considered to have low sensitivity (Se). In contrast, its specificity (Sp) is 100%. Polimerase chain reaction (PCR) based on the *invA*-gen of *Salmonella* spp. is considered a rapid and reliable diagnostic test for the detection of *Salmonella* organisms (Rahn et al, 1992; Malorny et al, 2003). It appears to show high Se, although its Sp is sometimes questionable (Arnold et al, 2004). However, if the assessment of its accuracy is based on direct comparison to bacteriology it will yield biased estimates of Se and Sp. An alternative to estimate the accuracy of 2 tests in absence of a gold standard is the use of latent-class approaches. They allow for the estimation of Se and Sp of the tests by cross-classifying their results after applying them to individuals from 2 populations with different prevalences, and assuming constant Se and Sp of the tests across populations (Enøe et al, 2000). We estimate the accuracy of culture and PCR for the detection of *Salmonella* organisms on caecal samples from slaughtered pigs using a latent-class (Bayesian) approach.

Material and methods

A total of 203 slaughtered pigs from 19 producers were sampled between September 2005 and March 2006 from 3 abattoirs in Saskatchewan, Canada. Ten pigs were randomly chosen from one of the producers delivering animals the day of sampling. Pigs were grouped into 2 populations: 96

from small farms (<2,000 hogs/year) (P_A), and 107 from large farms (>2,000 hogs/year) (P_B). Caecal content was collected and 10 grams submitted to qualitative *Salmonella* isolation following a culture method based on pre-enrichment with buffered peptone water (BPW), 3 selective enrichment media (tetrathionate –TT-, selenite, and Rappaport-Vassiliadis –RV- broths) and 4 selective, solid media (Xylose-Lysine-Tergitol-4, *Salmonella/Shigella*, Hekton-Enteric, and MacConkey). Isolates were further submitted to the National Laboratory for Bacteriology and Enteric Pathogens in Ottawa, for serotyping. Aliquots from the RV and TT broths were subjected to DNA extraction through a phenol-chloroform method, and a PCR targeting the *invA* gene of *Salmonella* spp was used following the method described by Malorny et al (2003) with slight modifications. A summary of the procedures is outlined in Figure 1.

Figure 1: Flow diagram of the methods used to isolate *Salmonella* in samples from caecal content from slaughtered pigs.



Priors for the Bayesian analysis were based on published literature. For Se of the culture (Se_{cul}) we used a mode of 75% with a 5th percentile as low as 40% (beta distribution defined by α= 5.29 and β=2.43); Sp of the culture (Sp_{cul}) was considered to be 100%; a reasonable prior for sensitivity of PCR (Se_{PCR}) was 85% with a 5th percentile as low as 65% (α=15.13 and β=3.49); same beta distribution was used for Sp of the PCR (Sp_{PCR}); a mode of 35% was used for P_A and P_B, with a minimum prevalence of 6.25% for P_A and 21.5% for P_B, thus the corresponding beta distributions were α=1.21, β=1.39 for P_A and α=9.31, β=16.44 for P_B. The conditional independence model for two tests, two populations was used (Branscum et al, 2005). To check for consistency of results and assess the influence of priors on the estimates the model was further repeated using non-informative priors (α=1, β=1). Posterior inferences were based on 100,000 iterations. Convergence was assessed by visual checking of the Kernel density and trace plots for each parameter, and by running multiple chains from dispersed starting values and further estimate the Gelman and Rubin statistic (Gelman and Rubin, 1992).

Results

Salmonella spp was isolated from 6.25% of the samples of P_A, and 21.4% of P_B. The cross-classification of results of the 2 tests for both populations is shown in Table 1. All culture-positive samples were PCR-positive except one. Out of 67 PCR-positive samples, only 28 (41.8%) were culture positive. Table 2 shows the posterior medians obtained with the different models used. When prior information was used for all parameters (model 1) posterior medians were 56% (95%CI=40, 76), 91% (95%=81, 97), 88% (95%=80, 95), 11% (95%CI=4, 20), and 40% (95%=28, 51%), for Se_{cul}, Se_{PCR}, Sp_{PCR}, P_A, P_B, respectively. Results were similar when non-informative priors were used for the Se_{cul} (model 2), with the estimate of Se_{cul} in this model decreasing to 52% (Table 2). When non-informative priors were also included for Se_{PCR} and Sp_{PCR} (model 3), the Se_{cul} dropped to 50% but Se_{PCR} and Sp_{PCR} estimates went up (95% and 92%, respectively). If all the priors, except those for Sp_{cul} were non informative (model 4), the Se_{cul} decreased even more (48%). When all priors used were non-informative, including those for Sp_{cul}, the results obtained were similar to those from model 4, with median Sp_{cul} being 99% (95%CI=96, 100) (results not shown).

Table 1. Cross-classification of the results of the bacteriological culture and *invA*-gen-based PCR for the detection of *Salmonella* organisms in caecal samples from two populations of slaughtered pigs.

		P_A		P_B		TOTAL
		PCR		PCR		
		+	-	+	-	
Culture	+	6	0	22	1	29
	-	11	79	28	56	174
TOTAL		17	79	50	57	203

Table 2. Results from different Bayesian models[a] of sensitivity of the bacteriological culture (Se_{cul}), and sensitivity (Se_{PCR}) and specificity (Sp_{PCR}) of the *invA* gen-based PCR for the detection of *Salmonella* spp in caecal samples, and prevalences for two populations of slaughtered pigs (P_A and P_B).

	Median (%) (95% CI)					
	Se_{cul}	Sp_{cul}	Se_{PCR}	Sp_{PCR}	Prevalence P_A	Prevalence P_B
Model 1[b]	56 (40, 76)	100	91 (81, 97)	88 (80, 95)	11 (4, 20)	40 (28, 51)
Model 2[c]	52 (36, 73)	100	91 (81, 97)	89 (81, 96)	11 (5, 21)	41 (29, 52)
Model 3[d]	50 (35, 71)	100	95 (83, 99)	92 (82, 99)	13 (5, 23)	42 (30, 53)
Model 4[e]	48 (34, 70)	100	94 (82, 99)	93 (82, 99)	13 (5, 23)	45 (31, 57)

95%CI: 95% credibility intervals;
[a]Models 1 to 4 were based on the assumption of conditional independence between Se_{cul} and Se_{PCR}. Sp_{cul} was considered 100%.
[b]Model 1 uses the following informative priors: Se_{cul}: beta(5.29, 2.43); Se_{PCR}: beta(15.13, 3.49); Sp_{PCR}: beta(15.13, 3.49); prevalence P_A: beta(1.21,1.39); prevalence P_B: beta (9.31, 16.44);
[c]Model 2 uses non-informative (1, 1) priors for Se_{cul};
[d]Model 3 uses non-informative (1, 1) priors for Se_{cul}, Se_{PCR} and Sp_{PCR};
[e]Model 4 uses non-informative (1, 1) priors for Se_{cul}, Se_{PCR}, Sp_{PCR}, and prevalences.

Discussion

This study would confirm the low Se_{cul} (56%) Since we used an important amount of caecal content (10g) which was cultured on 3 different broth media for selective enrichment (after a pre-

enrichment with BPW), and further plated on 4 selective, solid media, we think that the ability of this culture technique to recover *Salmonella* organisms should have been enhanced considerably. Should other simpler culture techniques were used then a likely lower Se_{cul} would be expected. Se_{cul} is also affected by the number of organisms shed by animals, which is positively influenced by stressors such as handling, commingling of pigs, transportation, food deprivation or lairage (Williams and Newell, 1970; Craven and Hurst, 1982). The Se of this culture method may have been even lower if performed on faecal samples from pigs at the farm. According to our estimate of Se_{cul} the expected percentage of pigs shedding *Salmonella* in their faeces at slaughter in this population was at least 25.5%. By contrast, PCR has been considered a technique of high Se for the diagnosis of *Salmonella* infection in faecal samples after enrichment in proper media (Malorny et al, 2003; Myint et al, 2006), but of questioned Sp (Arnold et al, 2004). PCR positive results from samples from which *Salmonella* cannot be isolated can be due to nonviable *Salmonella* organisms (i.e. due to the presence of inhibitors of *Salmonella* growth in the faeces, previous treatment of the animal with antimicrobials, or because the animal has consumed nonviable organisms from the environment), or as a result of cross-reactivity with *Salmonella*-like bacterium (Ward et al, 2005). Although the results from this study showed that Se_{PCR} was clearly superior to that of the culture (91%), the Sp_{PCR} would be however much inferior, misclassifying around 12% of the negative samples. The *invA*-gen-based PCR is a rapid diagnostic method that, despite the lower Sp shown, detected 96.5% (28 out of 29) of the culture positive samples. It seems reasonable to think that it could be used as screening tool in *Salmonella* surveillance schemes. Bacteriology (on caecal samples) alone should be considered a very poor diagnostic method to carry out studies on the prevalence of salmonellosis in pigs, but useful as a confirmatory test and for further identification and characterization of the *Salmonella* isolates.

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