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Bayesian estimation to test accuracy for influenza A infection via respiratory clinical signs in the absence of a gold standard

Nitipong Homwong[1,2*], Douglas Marthaler[1,3], Matteo Convertino[4,6,7], Montserrat Torremorell[1], Meggan E. Craft[1,6], Benjamin Hause[5] and John Deen[1]

[1]Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, Saint Paul, Minnesota, USA.
[2]Kasetsart University, Kamphaeng Saen, Nakhon Pathom, Thailand.
[3]Veterinary Diagnostic Laboratory, College of Veterinary Medicine, University of Minnesota, Saint Paul, Minnesota, USA.
[4]Division of Environmental Health Sciences and Public Health Informatics Program, School of Public Health, University of Minnesota, Minneapolis, Minnesota, USA.
[5]Veterinary Diagnostic Laboratory, Kansas State University, Manhattan, Kansas, USA.
[6]Institute on the Environment, University of Minnesota, Saint Paul, Minnesota, USA.
[7]Institute for Engineering in Medicine, University of Minnesota, Minneapolis, Minnesota, USA.

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Influenza A virus (IAV) infection in pigs is a concern to producers, veterinarians and the general public. This study presents models to estimate the sensitivities (Se) and specificities (Sp) of respiratory clinical signs (RCS), and real-time reverse transcription polymerase chain reaction (RRT-PCR) resulted from oral fluid (OF) and nasal swab (NS) samples in the absence of a gold standard. In addition, the models estimated an average prevalence of IAV infection in the Midwestern United States (US) growing pig populations. Bayesian model provided estimates under scenarios where IAV vaccination reduced only clinical manifestations, but not infection (basic model), or where vaccination reduced both. By the basic model, the Se and Sp of RCS from posterior distributions were 0.38 (95%Cridible interval (CrI): 0.28, 0.48) and 0.66 (95%CrI: 0.61, 0.71). The Se and Sp of of RRT-PCR were 0.84 (95%CrI: 0.87, 0.90) and 0.93 (95%CrI: 0.82, 0.97), and those of NS RRT-PCR were 0.79 (95%CrI: 0.71, 0.89) and 0.97 (95%CrI: 0.90, 0.99) respectively. The true prevalence estimate of IAV infection in the Midwestern US growing pig populations was 0.24 (95%CrI: 0.16, 0.30). In the second scenario, the Se and Sp of RCS were reduced by vaccination whereas those of NS and OF-RRT-PCR were not reduced by vaccination. Depending on the prior knowledge of vaccination, the model (in the second scenario) estimated that vaccination reduced the true prevalence of IAV in growing pigs, and thereby this has broader implications for the control and perhaps eradication of IAV in growing pigs.

Key words: Bayesian estimation, test accuracy, prevalence, influenza A virus, swine.

INTRODUCTION

Influenza A virus (IAV) is an enveloped-segmented, negative single-stranded RNA virus belonging to the family *Orthomyxoviridae,* including genera A, B, C, Togoviruses and Isavirus (Vincent et al., 2008). Most of

the United States (US) swine population is endemically infected with Influenza A virus (Allerson et al., 2013a; Romagosa et al., 2011; Torremorell et al., 2012). IAV is considered one of the top three respiratory diseases in growing pigs and causes productivity losses in sows (Holtkamp et al., 2007). IAV infection while coinfected with other respiratory pathogens can aggravate the porcine respiratory disease complex (PRDC) (Deblanc et al., 2012; Fablet et al., 2012; Rose et al., 2013; Vincent et al., 2008). Clinical signs of infection with IAV are characterized by fever, sneezing, coughing, rhinorrhea and lethargy, and sometimes, conjunctivitis and oculonasal discharge (Reeth et al., 2012). The estimated cost of disease for IAV infection in market pigs ranges from $3.23 to 10.31/head (Donovan, 2008; Dykhuis et al., 2012).

Important control measures for IAV in pigs include surveillance, monitoring, prevalence estimation, and risk factor studies (Greiner and Gardner, 2000). The test accuracy (sensitivity, Se and specificity, Sp) is commonly determined through a comparison with a "gold standard," which refers to a reference test with 100% Se and 100% Sp (Black and Craig, 2002) or with a reference test of known fixed values of Se and Sp under specified circumstances (Enøe et al., 2000).

However, a gold standard test is not always applicable, nor does it exist for all tests. In addition, for a diagnostic test to be considered accurate under the gold standard, its Se and Sp, along with the expected prevalence values must be fixed, which may be incorrect when the state of disease is dynamic, which can result in potential biases in the reported estimates (Enøe et al., 2000). Furthermore, in field settings, there is also the issue of uncertainty attributed to differences between sampling strategies and tested populations (Greiner and Gardner, 2000), which do not account for sampling methodology (Joseph et al., 1995), and the variability within and between herds (Davies, 2006; Enøe et al., 2000; Greiner and Gardner, 2000). Changes in Se and Sp estimates, as a result, may occur and should be taken into account by researchers.

Bayesian modeling, on the other hand, can fulfill such deficiencies by incorporating prior knowledge of test Se, Sp and unknown disease status (Enøe et al., 2000; Johnson et al., 2001). In addition, simultaneous posterior inferences about prevalence as well as Se and Sp of each diagnostic test are possible (Joseph et al., 1995). In the field of veterinary medicine, Bayesian modeling has been a popular method for estimating test accuracy for over fifteen years (Enøe et al., 2000; Paul et al., 2013; Praud et al., 2012; Toft et al., 2007). Test accuracy estimation is very important for the work of veterinarians and diagnosticians for surveilling and monitoring animal diseases. Currently, there is not a gold standard test with 100% Se and 100% Sp (perfect test) to compare for estimating the test accuracy of influenza A virus (IAV) via respiratory clinical signs (RCS), and nasal swabs (NS) and oral fluid (OF) RRT-PCRs in growing pigs.

In a context where a gold standard or a reference test is absent, as deemed in this case, the study thus focus on using full Bayesian model as the main analytic tool to estimate parameters of Se, Sp and true prevalence. Therefore, the objectives of this study were: in scenario 1: to estimate Se and Sp of RCS, and NS and OF RRT-PCRs; to estimate the true prevalence using both RCS and NS, and in scenario 2: to understand how vaccination affects estimates of the test accuracy and true prevalence.

MATERIALS AND METHODS

Data sets

This study utilized published data from two studies: a field study on active surveillance of swine influenza infection in growing pig populations in the Midwestern United States (US) (Corzo et al., 2013) and an experimental challenge study of IAV in swine (Romagosa et al., 2012) (Table 1).

In the first study, 16,170 nasal swabs were collected from 540 groups (30 nasal swabs per group), and RCS was observed in whole groups of growing pigs from 32 farms between 2009 and 2011 as part of an active Midwestern US surveillance program for IAV. A group was considered positive if at least one of the 30 nasal samples tested positive by RRT-PCR (Corzo et al., 2013a). RCS was observed for 3 min after pigs had been forced to stand up for at least a minute. If at least one pig in the group exhibited coughing, sneezing or nasal discharge, "presence" of respiratory clinical signs was documented. If no clinical signs were notable, "absent" was noted (Corzo et al., 2013; Rose et al., 2013; Vincent et al., 2008). In the second study, 105 pen-based samples of oral fluids were collected. A group was considered positive when at least one of the 10 nasal samples tested positive (Romagosa et al., 2012).

For the purpose of this study, from here onwards, the word "herd" is used to refer to any group of 3 to 30 week-old pigs housed in finishing farms located in the Midwestern US during the time of the study conducted, and any room of three week-old pigs housed in the research animal units at the University of Minnesota. Growing pigs in the same farm but from different visits were considered a distinctive "herd."

Bayesian model

Prior information

Beta probability densities were used as prior distributions for parameters: Se and Sp of RCS, Se and Sp of OF and NS RRT-PCR and the prevalence and the probability of a swine herd being endemic for IAV. Such beta prior distributions can be accomplished using past data, if available; by examining published values from previous studies; by drawing from expert opinion; alternatively, by combining all of these options (Joseph et al., 1995; Suess et al.,

*Corresponding author. E-mail: homwo001@umn.edu; Tel: +1-612-615-1588.

Table 1. Diagnostic test results from a field setting (RCS versus NS RRT-PCR) with unknown prevalence and from an experimental study (OF versus NS RRT-PCR) with known prevalence.

	Result	Field setting unknown prevalence			Experimental setting known prevalence		
		RCS		Total	OF RRT-PCR		Total
NS RRT-PCR		Present	Absent		Positive	Negative	
	Positive	43	74	117	37	9	46
	Negative	144	279	423	0	59	59
	Total	187	353	540	37	68	105

2002) All beta priors were assumed to be independent (Cowling et al., 1999).

1. The prior Se and Sp of RCS have not been published. Hence, the study employed non-informative priors.
2. The prior Se and Sp of OF RRT-PCR results were illustrated at a group-based level while the NS RRT-PCR results were demonstrated at an individual level and were elicited elsewhere (Goodell et al., 2013).
3. The prevalence of IAV infection in the US swine herd was 29% with a standard deviation of 13% (Choi et al., 2002; Olsen et al., 2000).
4. The probability of endemic IAV in a swine herd was based on past history, and was considered endemic throughout the year, implying that at least one swine herd is infected with IAV each month (Olsen et al., 2000).

All prior distributions were implemented in scenario 1 (the basic model). To understand the effects of vaccination on the test accuracy and the true prevalence, the second scenario was modeled, where vaccination protects against infection, and the prevalence was proportional to vaccine effectiveness. Vaccine effectiveness and the prevalence proportional to vaccine effectiveness were estimated using the experimental study data (Romagosa et al., 2012). Vaccine effectiveness was estimated by one minus the odds ratio (OR), where % Effectiveness = (1-OR)x100 (Weinberg and Szilagyi, 2010). The OR was estimated using a binomial regression model.

To convert the elicited prior values of Se and Sp (RCS, OR and NS RRT-PCR), and the prevalence to the prior Beta distributions, the *Parameter* Solver v3.0[1] was used by matching the closest fitting Beta probability distributions. *Parameter Solver* computed the Beta distribution parameters with 95% lower and upper percentiles of the distribution, and graphed the results of those Beta distributions (Table 2).

Sensitivity analysis of the prior distribution

Since the duration of infection affects the Se (Greiner and Gardner, 2000), this study categorized the priors into three groups based upon this study initial assumptions: if samples were taken within one week of infection, if samples were taken within two weeks of infection and if samples were taken without any information on the course of infection. The prior distributions were that the prior Se OF and NS RRT-PCR were between 0.77 and 0.92, and between 0.75 and 0.90, respectively. The prior Sp OF and NS RRT-PCR results were between 0.80 and 0.97, and between 0.80 and 0.99, respectively. The prior distribution were that the prior Se of OF and NS RRT-PCR results were between 0.08 and 1.00, and between 0.00 and 1.00 (Goodell et al., 2013). The non-informative prior Beta Se of OF and NS RRT-PCR results were employed. Because of the lack of information regarding variability and point estimate of the test Sp of NS and OF RRT-PCR, non-informative priors were used instead.

Assumptions

Due to the absence of a gold standard, two populations were used to estimate test accuracies. In the first population, this study tested two approaches: RCS relying on visual observation of clinical outcomes, which is a subjective measure, while NS RRT-PCR and RNA-based technique is an objective measure. Given such conditions, the conditional independence assumption was used for this modeling. Alternatively, the conditional dependent assumption between RCS and NS RRT-PCR was modeled to compare the previous assumption. In the second population, the study compared the NS and OF RRT-PCR results, which are both a RNA-based technique and an objective measure. Conditional dependence assumption was used for modeling the second population (Branscum et al., 2005; Enøe et al., 2000; Gardner et al., 2000). Other two assumptions were included in order to jointly model accuracy of NS RRT-PCR between two populations (field versus experiment). First, the test accuracy of NS RRT-PCR was assumed to be equal and second, assumed to be unequal across field and experiment populations (Bouwknegt et al., 2008; Branscum et al., 2005; Johnson et al., 2001). The 4 combined assumptions were made, and the models run to investigate a final model. The final model was selected using a deviance information criterion (DIC), which is described in the next section.

Bayesian computation

Bayesian Markov Chain Monte Carlo (MCMC) computation was performed using Gibbs sampler in JAGS 3.4.0 (Plummer, 2013) and constructed following previously described methods (Branscum et al., 2005; Geurden et al., 2008; Nérette et al., 2008; Toft et al., 2005). The detailed model structure is included (Table 4, Appendix A) as well as a conceptual model with Directed acyclic graph (Figure 1). The JAGS model codes were written in R v3.2.0 (R Core Team, 2015) The “rjugs” and “R2 jags” packages were used as an add-on for calling JAGS from R to perform Gibbs sampling (Plummer, 2015; Su and Yajima, 2015). The analysis of MCMC chains and graphics was performed by using the “CODA," “ggmcmc” and “ggplots2”packages (Hadley, 2009; Marin, 2013; Plummer et al., 2006).

In all analyses, 250,000 iterations with 3 chains of Gibbs samplers were run, where the first 5,000 iterations were discarded. Sampling thinning was applied by taking 5 samples from the posterior distribution of applicable parameters. The convergence of the three chains was assessed by visual inspection using

[1] Available at http://biostatistics.mdanderson.org/SoftwareDownload/

Table 2. Description of the prior distribution for Se and Sp of RCS, OF and NS RRT-PCR, and prevalence in field and experimental populations.

Variable	Parameters[1]	Median	95% CrI[2]	SD	Distribution	Reference
RCS	η_c	0.50	0.03-0.98	0.08	Beta (1,1)	Non-informative
-	θ_c	0.50	0.03-0.98	0.08	Beta (1,1)	Non-informative
Time-of-sampling within 1 week of infection						
OF RRT-PCR	η_o	0.83	0.75-0.99	0.03	Beta (77.85,15.75)	Goodell et al.(2013)
-	η_o	0.95	0.80-0.97	0.03	Beta (39.97,4.34)	Non-informative
NS RRT-PCR	η_n	0.88	0.77-0.92	0.03	Beta (71.23,12.28)	Goodell et al. (2013)
-	θ_n	0.97	0.80-0.99	0.05	Beta (24.75,2.03)	Goodell et al. (2013)
Time-of-sampling within 2 weeks of infection						
OF RRT-PCR	η_o	0.68	0.08-1.00	0.28	Beta (1.22,0.60)	Goodell et al. (2013)
-	θ_o	0.50	0.03-0.98	0.08	Beta (1,1)	Non-informative
NS RRT-PCR	η_n	0.56	0.00-1.00	0.38	Beta (0.38,0.34)	Goodell et al. (2013)
-	θ_n	0.50	0.03-0.98	0.08	Beta (1,1)	Non-informative
Unknown course of infection						
OF RRT-PCR	η_o	0.50	0.03-0.98	0.08	Beta (1,1)	Non-informative
-	θ_o	0.50	0.03-0.98	0.08	Beta (1,1)	Non-informative
NS RRT-PCR	η_n	0.50	0.03-0.98	0.08	Beta (1,1)	Non-informative
-	θ_n	0.50	0.03-0.98	0.08	Beta (1,1)	Non-informative
Field prevalence	π_f	0.29	0.06-0.55	0.13	Beta (2.7,7.68)	Choi et al. (2002) Olsen et al. (2000)
Experimental prevalence	π_e	0.92	0.80-0.99	0.01	Beta (24.75,2.04)	Romagosa et al. (2012)
-	-	0.17	0.14-0.12	0.01	Beta (100.9,496.4)	

[1]η denoted sensitivities of RCS *c*, of OF *o* and of NS *n* RRT-PCR, [1]θ denoted specificities of RCS *c*, of OF *o* and of NS *n* RRT-PCR, [1]π denoted the true prevalence of field setting, *f* and of experimental setting *e*, [2]CrI denoted a credible interval.

Traceplots, Gelman-Rubin R-hat (Potential Scale Reduction Factor) and diagnostic Geweke z-score plots (Gelman and Rubin, 1992; Geweke, 1991). The analysis was repeated, and the results were virtually identical, with relatively low Monte Carlo errors (<5%). In addition, autocorrelation monitoring was assessed by the draws of the corresponding Markov chains. MCMC sample median was presented as a point estimate while the 2.5 and 97.5 percentiles were presented as 95% credible intervals (CrI).

Individual outliers and the reasonableness of the prior assumption were checked using Bayesian p-value (positive predictive check), which is the predictive probability of having an extreme value, and measure goodness of fit the model, which is close to 0.5 (0.06-0.94) as possible (Carlin and Louis, 2008; Geurden et al., 2008; Lunn et al., 2012). Model selection was based upon DIC, where the smaller DIC is preferred and a difference of 5 is substantially better. A DIC difference exceeding 10 is considered to be an event more of a significant better fit (Carlin and Louis, 2008; Spiegelhalter et al., 2002).

Sensitivity analyses in the final model were investigated for the prior distributions introduced as a reflection of uncertainty about knowing time-of-sampling (Garthwaite et al., 2005), by changing the prior Beta distributions of time-of-sampling, within 1 or 2 weeks, or no information regarding the course of infection as mentioned in the previous session (Prior information) accompanied with scenario 1 and 2 (Figure 2). In summary, after selecting the final model (based on DIC), the models were run six times in total.

RESULTS

Diagnostic test results with two populations from the field setting (RCS versus NS RRT-PCR) with unknown prevalence, and from the experimental study (OF versus NS RRT-PCR) with known prevalence was shown as 2x2 table. The vaccine effectiveness against infection was 98.62% (95%CI: 92.96-99.73%), which was estimated from the experimental setting. NS RRT-PCR was tested in both populations and accuracy of that test assumed to be equal was held. The assumption of conditional independence between RCS and NS RRT-PCR was modeled. The final model was selected using DIC of 42.2 (based on parsimony since it was the simplest model) (Table 3 and 4). Bayesian p-value of 0.88 for the final model supports the suitability of the assumptions.

As a basic scenario model (scenario 1), posterior estimates were calculated (Table 5). The Se and Sp of

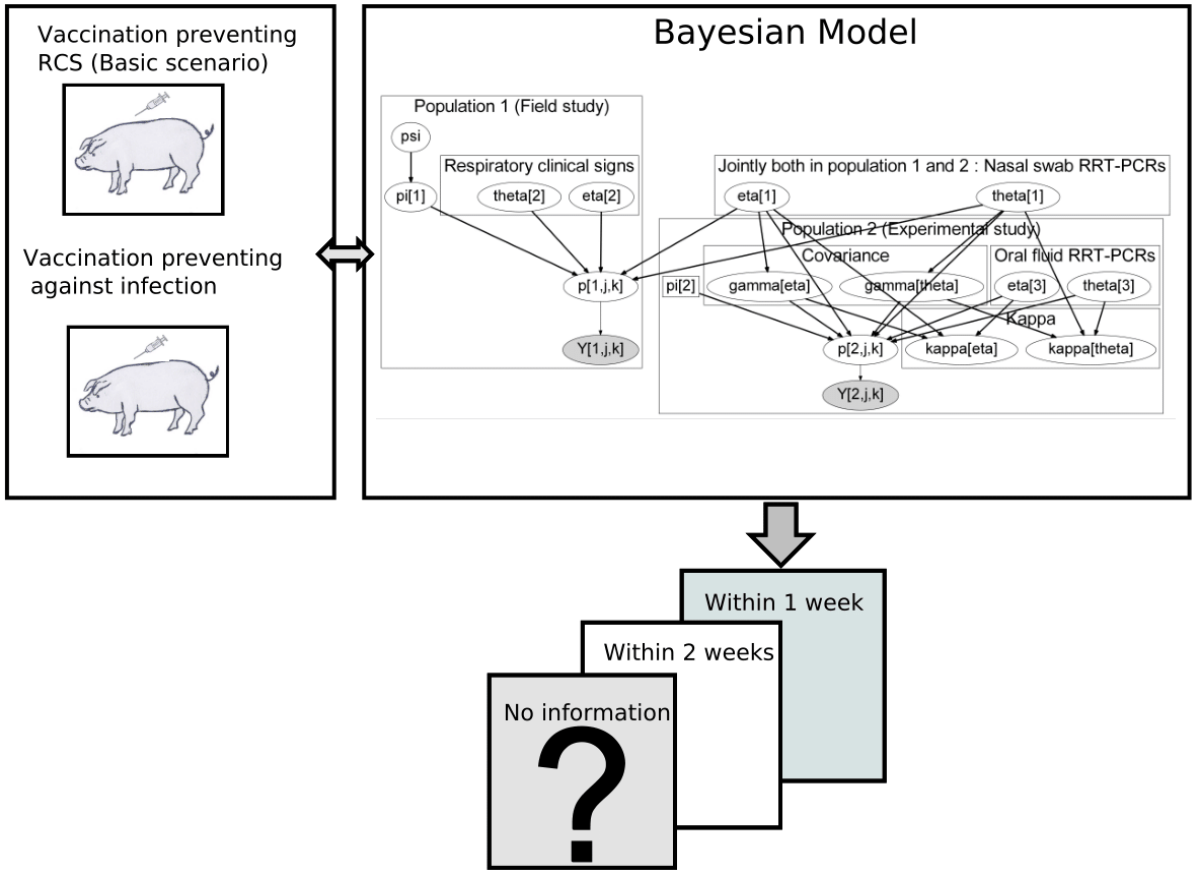

Figure 1. A conceptual model representing scenarios of (a) a vaccination that prevents RCS (Basic scenario) or (b) a vaccination that prevents against infection. For each scenario, three priors were implemented with regard of prior information of time-of-sampling within 1 or 2 weeks, or no information concerning the course of infection.

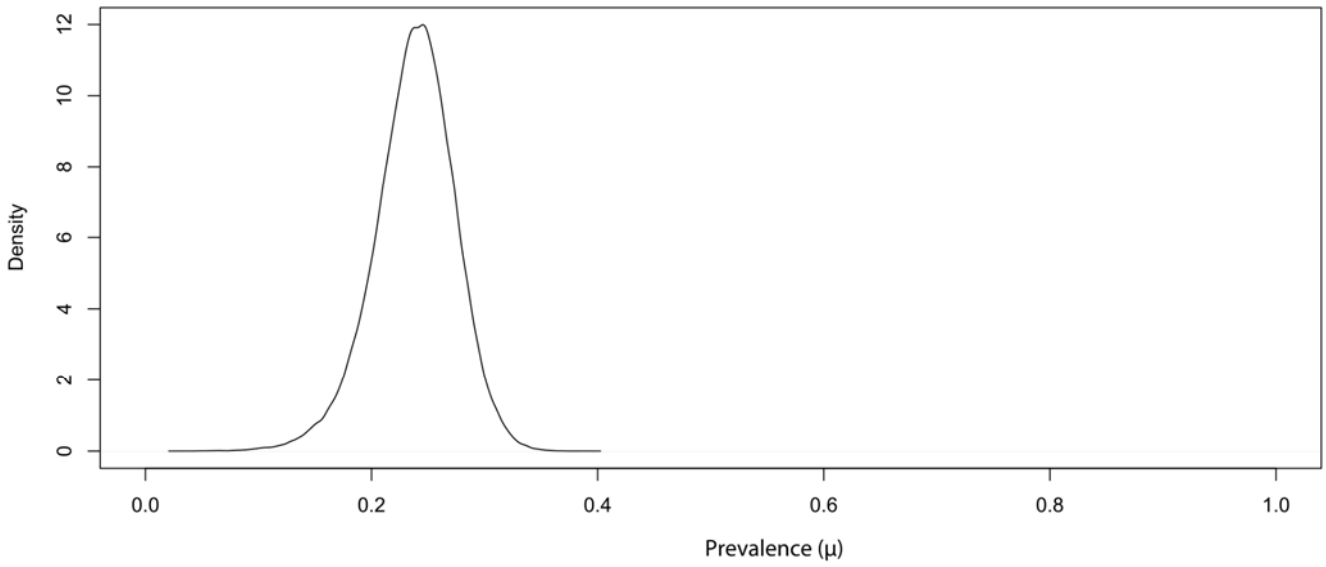

Figure 2. A probability distribution represents herd prevalence of IAV infection in the Midwestern US growing pig populations, the x-axis representing herd-level prevalence. The probability distribution was generated from three MCMC chains with 250,000 with 5000 discarded each chain.

Table 3. Deviance information criterions (DIC) for four assumptions.

Assumptions				
Conditional independent of RCS and NS RRT-PCR	**Accuracy of NS RRT-PCR was assumed to be equal across two populations**	**DIC[1]**	**pD**	**Deviance**
Yes	Yes	42.2	7.5	34.67
No	Yes	42.8	7.9	34.89
Yes	No	43.4	8.4	35.05
No	No	43.7	8.5	35.18

[1]DIC= Deviance+ pD.

Table 4. Final Bayesian model was selected by Deviance information criteria to estimate Se and Sp of RCS and OF as well as NS RRT-PCR and prevalence. The conditional independence assumption for accuracy of RCS and NS RRT-PCR was modeled and accuracy of RCS and NS RRT-PCR was assumed to be equal across two populations held.

Population *i*	Probability[1]	Structure of the model[2]
	Conditionally independent[3]	
1	p_{111}	$\pi_1\eta_1\eta_2+(1-\pi_1)(1-\theta_1)(1-\theta_2)$
	p_{112}	$\pi_1\eta_1(1-\eta_2)+(1-\pi_1)(1-\theta_1)\theta_2$
	p_{121}	$\pi_1(1-\eta_1)\eta_2+(1-\pi_1)\theta_1(1-\theta_2)$
	p_{122}	$\pi_1(1-\eta_1)(1-\eta_2)+(1-\pi_1)\theta_1\theta_2$
	Conditionally dependent	
2	p_{211}	$\pi_2[\eta_1\eta_3+\gamma_\eta]+(1-\pi_1)[(1-\theta_1)(1-\theta_3)+\gamma_\theta]$
	p_{212}	$\pi_2[\eta_1(1-\eta_3)-\gamma_\eta]+(1-\pi_2)[(1-\theta_1)\theta_3-\gamma_\theta]$
	p_{221}	$\pi_2[(1-\eta_1)\eta_3-\gamma_\eta]+(1-\pi_2)[\theta_1(1-\theta_3)-\gamma_\theta]$
	p_{222}	$\pi_2[(1-\eta_1)(1-\eta_3)+\gamma_\eta]+(1-\pi_2)[\theta_1\theta_3+\gamma_\theta]$

[1]p_{i11} is the probability of both tests 1and 2 positive in population *I*, [1]p_{i12} is the probability of test 1 positive with test 2 negative in population *I*, [1]p_{i21} is the probability of test 1 negative with test 2 positive in population *I*, [1]p_{i22} is the probability of both test 1 and 2 negative in population *I*, [2]π_1 is the true prevalence of influenza infection in field setting (unknown), [2]π_2 is the prevalence of influenza infection in experimental study (known), [2]η_1 and θ_1 represents the Se and Sp of NS RRT-PCR test, [2]η_2 and θ_2 represents the Se and Sp of RCS, [2]η_3 and θ_3 represents the Se and Sp of OF RRT-PCR test, [2]γ_η is the covariance (conditional covariance positive) between two sensitivity of the test (NS RRT-PCR versus OF RRT-PCR), [2]γ_θ is the covariance (conditional covariance negative) between two specificity of the test (NS RRT-PCR versus OF RRT-PCR),[3]Conditional covariance assumptions of the tests given the latent true disease status.

RCS were 0.38 and 0.66. The Se and Sp of OF RRT-PCR results were 0.84 and 0.93 while Se and Sp for the NS RRT-PCR results were 0.79 and 0.97. A posterior median estimate of the true IAV prevalence was 0.24 in the Midwest US growing pig populations (based on 16,170 of NS RRT-PCR and 540 groups of RCS for the filed setting data) and the true prevalence estimate was not influenced by the prior information (Figure 2). The Se posterior correlation medians between OF and NS RRT-PCR were 0.68, assuming conditional dependence. The Sp posterior correlation median between OF and NS RRT-PCT was 0.70, assuming conditional dependence. The posterior positive predictive kappa estimates of the OF and NS RRT-PCR tests were approximately 0.72, which indicated high agreement between the OF and NS RRT-PCR (Table 5).

To estimate the effects of vaccination on the test accuracy and the true prevalence, scenario 2 was constructed assuming vaccination prevents IAV infection, and sequentially both prior prevalence and RCS characteristics would be reduced. Posterior estimates were computed, and the Se of RCS was 0.3 (Table 6). The Sp accuracy estimate was not improved among time-of-sampling. The Se of NS RRT-PCR test was moderately decreased by time-of-sampling (0.97, 0.95, and 0.81). Similarly, the Sp of NS RRT-PCR test was

Table 5. Description of the first sceincario[3] of the posterior distributions for the test sensitivity, specificity, prevalence, correlation and kappa.

Variable	Parameters[1]	Sensitivity analysis (Time-of-sampling)								
		Within 1 week of infection			Within 2 week of infection			Unknown course of infection		
		Median	SD	95% CrI	Median	SD	95% CrI	Median	SD	95% CrI
RCS	η_c	0.38	0.05	0.28, 0.48	0.36	0.24	0.03, 0.93	0.36	0.24	0.04, 0.93
-	θ_c	0.66	0.03	0.61, 0.71	0.82	0.07	0.69, 0.97	0.82	0.07	0.70, 0.97
OF RRT-PCR	η_o	0.84	0.03	0.78, 0.90	0.37	0.05	0.27, 0.48	0.37	0.05	0.28, 0.47
-	θ_o	0.93	0.04	0.82, 0.97	0.81	0.22	0.16, 0.99	0.80	0.22	0.15, 0.99
NS RRT-PCR	η_n	0.79	0.04	0.71, 0.89	0.44	0.05	0.35, 0.58	0.44	0.05	0.35, 0.54
-	θ_n	0.97	0.03	0.90, 0.99	0.68	0.04	0.61, 0.78	0.68	0.04	0.62, 0.78
Prevalence	π_f	0.24	0.04	0.16, 0.30	0.25	0.12	0.06, 0.54	0.23	0.13	0.57, 0.54
Correlations[2]	ρ_η	0.68	0.15	0.34, 0.93	0.18	0.50	-0.73, 0.84	0.18	0.73	-0.75, 0.83
-	ρ_θ	0.70	0.15	0.33, 0.93	0.82	0.06	0.70, 0.93	0.82	0.06	0.70, 0.93
Kappa[2]	κ_η	0.72	0.20	0.35, 0.92	0.81	0.07	0.67, 0.93	0.81	0.06	0.67, 0.93
-	κ_θ	0.41	0.23	-0.20, 0.83	0.13	0.33	-0.51, 0.83	0.13	0.33	-0.51, 0.83

[1]η denoted sensitivities of RCS *c*, of OF *o* and of NS *n* RRT-PCR, [1]θ denoted specificities of RCS *c*, of OF *o* and of NS *n* RRT-PCR,[1]π_f denoted the true prevalence in a field setting, [1]ρ denoted correlations of sensitivity *η* and specificity θ between OF and NS RRT-PCR tests, [1]κ denoted kappa statistics for sensitivity *η* and specificity θ, [2]Calculated from tests between OF and NS RRT-PCR tests in population 2 with conditionally dependent model, [3]Model was run under the scenario that vaccination protects RCS but does not protect against infection, [3]The prior prevalence distribution of the experimental study was followed π_e~Beta(24.75, 2.04).

Table 6. Description of the second sceincario[3]of the posterior distributions for the test sensitivity, specificity, prevalence, correlation and kappa.

Variable	Parameters[1]	Sensitivity analysis (Time-of-sampling)								
		Within 1 week of infection			Within 2 week of infection			Unknown course of infection		
		Median	SD	95% CrI	Median	SD	95% CrI	Median	SD	95% CrI
RCS	η_c	0.30	0.18	0.05, 0.80	0.30	0.19	0.05, 0.83	0.31	0.21	0.03, 0.88
-	θ_c	0.79	0.02	0.75, 0.83	0.79	0.02	0.75, 0.84	0.79	0.05	0.71, 0.91
OF RRT-PCR	η_o	0.95	0.09	0.65, 0.99	0.83	0.19	0.32, 0.99	0.57	0.27	0.05, 0.96
-	θ_o	0.79	0.05	0.70, 0.89	0.77	0.05	0.67, 0.87	0.67	0.06	0.61, 0.85
NS RRT-PCR	η_n	0.97	0.04	0.79, 0.99	0.95	0.12	0.60, 0.99	0.81	0.25	0.18, 0.99
-	θ_n	0.71	0.04	0.65, 0.80	0.70	0.04	0.64, 0.79	0.68	0.05	0.55, 0.77
Prevalence	π	0.09	0.05	0.02, 0.21	0.09	0.05	0.02, 0.22	0.11	0.11	0.02, 0.44
Correlations[2]	ρ_η	0.74	0.10	0.53, 0.91	0.79	0.10	0.57, 0.97	0.83	0.09	0.63, 0.97
-	ρ_θ	0.20	0.24	-0.02, 0.82	0.18	0.26	-0.11, 0.82	0.31	0.61	-0.40, 0.89
Kappa[2]	κ_η	0.14	0.24	-0.02, 0.82	0.12	0.25	-0.06, 0.82	0.25	0.37	-0.25, 0.89
-	κ_θ	0.73	0.11	0.48, 0.91	0.78	0.12	0.52, 0.96	0.83	0.10	0.60, 0.98

[1]η denoted Se of RCS *c*, of OF *o* and of NS *n* RRT-PCR, [1]θ denoted specificities of RCS *c*, of OF *o* and of NS *n* RRT-PCR,[1]π_f denoted the true prevalence in a field setting, [1]ρ denoted correlations of sensitivity *η* and specificity θ between OF and NS RRT-PCR tests, [1]κ denoted kappa statistics for sensitivity *η* and specificity θ, [2]Calculated from tests between OF and NS RRT-PCR tests in population 2 with conditionally dependent model, [3]Model was run under the scenario that vaccination protects against an infection for IAV, [3]The prior prevalence distribution of the experimental study was followed π_e~Beta (100.9, 496.4).

delicately decreased (0.71, 0.70, and 0.68). Posterior median estimates of the true prevalence were approximately at 0.10 and strongly influenced by the level of infection changed by vaccination. The posterior

correlation of the test Se of OF and NS RRT-PCR was 0.80. The posterior correlation of the test Sp of OF and NS RRT-PCR was 0.20. The posterior predictive kappa estimates between the OF and NS RRT-PCR tests were incongruous (0.14, 0.12 and 0.25). The posterior predictive kappa estimates were substantial (0.73, 0.78) and uncovered a high level of agreement (0.83), which indicated high agreement between the OF and NS-RRT-PCR (Table 6).

With sensitivity analysis, the priors of the test accuracy (varied by time-of-sampling) were reviewed from Goodell et al. (2013), and used for non-informative priors**.** The accuracy of RCS and the prevalence, correlation, and kappa, were not changed by time-of-sampling assumption. The accuracy of OF and NS-RRT-PCR was slightly reduced from one week to two weeks, but two weeks was similar to no information. Thus, any imprecision arising in the prior distributions associated with fitting parametric distribution was not a major concern.

DISCUSSION

IAV infection in pigs is a major concern to producers, veterinarian and general public. Especially, IAV infection by other pathogens in growing pigs plays a crucial role in the porcine respiratory complex. Having accurate, rapid, easy, and practical on-farm tests is necessary for epidemiological and monitoring purposes. To the best of this study knowledge, this is the first report that estimates the Se and Sp of RCS associated with IAV infection in growing pigs using Bayesian model. The current Se and Sp estimates of RCS were 0.38 (95% CrI: 0.28, 0.48) and 0.66 (95% CrI: 0.61, 0.71), indicating RCS is not a reliable test for detecting IAV infections.

These results are consistent with a previous study by Allerson et al. (2013b), which found that influenza virus can be detected in pigs without having RCS. In this case, RCS creates false-negative results (Se=0.38). The absence of RCS at the individual level cannot rule out IAV infection. However, at the population level, Se may be improved, but may still provide false-negative results. In addition, infection with other non-influenza respiratory pathogens could generate false-positive RCS results (Sp=0.66). Being a subjective observation, the accuracy of RCS may differ between observers, but this could be minimized by training (Baadsgaard and Jørgensen, 2003).

Times of IAV infection and sampling are major factors affecting the test accuracy. To assess such accuracy, the sensitivity analysis of the prior distributions was conducted to investigate deviations of the test accuracy. This reflects the assumptions of time-of-sampling affecting the test accuracy but not in other estimates such as the prevalence, correlation, and kappa. For example, the Se of OF RRT-PCR test largely decreased (0.84, 0.37 and 0.37) while the Sp was slightly lower (0.93, 0.81 and 0.80). This finding indicates that the Se decreased dramatically, while the Sp decreased slightly in relation to time-of-samples (within 1 and 2 weeks of infection, and unknown course of infection, respectively). The determination of appropriate sampling time (providing the highest accuracy) may be difficult in practice. Regardless of test limitations, sampling at several sites during the same period of time should be performed to increase Se. As sampling variability may occur, different sampling methods may affect the test accuracy and the prevalence estimate.

Therefore, the IAV prevalence estimates may be inconsistent during a period of sampling. Likewise, with a method of sampling, Allerson et al. (2013a) indicated that the prevalence estimated by targeted sampling of pigs displaying RCS may be slightly overestimated compared to simple random sampling (Allerson et al., 2013b). One benefit of targeted sampling includes being able to conduct a herd diagnosis with fewer samples, making it a more cost-effective way to improve Se without decreasing the Sp (Christensen and Gardner, 2000).

In veterinary medicine, the conditionally dependent model should be considered first when modeling, and failing to allow models to be conditionally dependent will introduce bias in the estimate should be considered first when conducting analysis (Gardner et al., 2000; Toft et al., 2005). Based on those researches, the study four model assumptions were followed. For example, the conditional independence and dependence between RCS and NS RRT-PCR were modeled. The test accuracy of NS RRT-PCR was assumed to be equal across two populations (field and experiment settings). By using a DIC selection criterion, the study modeled the two tests as conditionally independent (RCS versus NS RRT-PCR) and conditionally dependent (OF versus NS RRT-PCR). The models allow correlations between OF and NS RRT-PCR tests to be positive or negative. In addition, the test accuracy of NS RRT-PCR was equal across field and experiment settings.

The current Se and Sp estimates of NS RRT-PCR were 0.79 (95%CrI: 0.71, 0.89) and 0.97 (95%CrI: 0.90, 0.99), respectively within the first week of infection. However, after one week of infection, the accuracy of NS RRT-PCR dropped to 0.44, which is quite low. This result could have happened because of a reduction in transmission of nose-to-nose contact after one week of infection. Even though IAV virus can be found in nasal secretion of positive pigs (Corzo et al., 2013b), a previous report showed that pigs can shed virus thought nasal secretion for 5 to 7 days (Mohan et al., 1981), which can be resulted in reducing the accuracy of NS RRT-PCR. The Se and Sp estimates of NS RRT-PCR may be lower than expected because the estimates obtained from experimental studies may overestimate the particular test performance compared with the field setting (Davies, 2006).

On the other hand, the test accuracy obtained from a field setting may underestimate the test performance since some of the variables cannot be controlled. For instance, viral titers in samples can affect the estimates differently. The test accuracy should not be extrapolated only from the experimental setting and then applied in the field settings. Since both experimental and field setting data was used, the study current estimates were strengthened, which result in more accurate estimates.

In the field setting, the status of IAV infection in Midwestern US growing pig populations was unknown. The true prevalence of IAV infection was estimated at 0.24 (95%CrI: 0.16, 0.30) using Bayesian model, which incorporated prior knowledge regarding the prevalence of IAV infection (Choi et al., 2002; Olsen et al., 2000; Poljak et al., 2008).This estimate was consistent regardless of sampling time and consistent with previous research, which reported that the sero-prevalence of IAV in HI test was 0.22 (Choi et al., 2002). This study contained a large sample size, consisting of 111,418 samples submitted to the University of Minnesota Veterinary Diagnostic Laboratory.

However, the study prevalence estimate was based on 16,170 of NS-RRT-PCR and 540 groups of RCS. In Canada, the IAV prevalence was reported as 0.47 in finishing pigs in the province of Ontario (Poljak et al., 2008). A similar study conducted in the same province reported that in 2004 the prevalence for H1N1 and H3N2 was 0.13 and 0.27 respectively. The following year, the prevalence for H1N1 increased to 0.15. The increase for H3N2, on the other hand, was more dramatic since the estimate was 0.26 (Poljak et al., 2008). The prevalence of IAV infection in the Midwestern US growing pig populations seems to be similar to findings from Choi and colleagues' study in 2002 and ours in 2011, where a year of samples was taken.

Based upon this study estimates, the study speculate that inspection of RCS would have lower utility compared to pen-based oral fluid testing within first week of infection. However, in the second week of infection, the Se of OF RRT-PCR decreased to 0.37 while the Sp of RCS increased to 0.82 (Table 5), which seems comparable to weeks 2 and 3. A similar characteristic was also found in scenario 2 (Table 6). If RCS is used for monitoring IAV infection in swine herds, it will create more false-negative results in an endemic herd. As et al. (2013) reported, positive growing pigs may not exhibit RCS (Allerson et al., 2013b). To implement RCS as a monitoring system in a swine herd, more studies are needed to evaluate the frequency of this observation, including a minimum number of pigs observed, and the economic costs associated with testing to justify having RCS observations and to obtain the precise and improved estimates of Se and Sp. The advantages of RCS as a monitoring system, along with other diagnostic tests for a group-based population, are low-cost and can be easily used on a farm.

However, RCS may be less accurate in vaccinated herds as sick pigs might endure illness, leading to "hidden" respiratory subclinical signs. In the cases of an acute infection, a change in the behavior because of fever or lethargy can reduce their likelihood to exhibit RCS and may increase Se. Such behavior needs to be further investigated to improve the precise estimate.

Conclusion

Bayesian model was employed to estimate the Se and Sp of IAV infection using RCS and NS and OF RRT-PCR applied to the Midwestern US growing pig populations. Observation of RCS is easy, affordable and safer for personnel as compared with the collection of NS and OF. However, the accuracy of RCS in the first week was lower than OF and NS RRT-PCR, but in the second week, the accuracy of RCS increased and was comparable to OR and NS RRT-PCR. RCS may potentially be used as measurement to estimate true prevalence of IAV infection (given its imperfect accuracy test) but may not be sufficient to be used as a diagnostic tool. The accuracy of RCS was reduced by vaccination but the accuracy of NS and OF-RRT-PCR was insignificantly reduced by vaccination.

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Conflict of interest

The authors declare they have no conflict of interest.

REFERENCES

Allerson M, Davies P, Gramer M, Torremorell M (2013a). Infection Dynamics of Pandemic 2009 H1N1 Influenza Virus in a Two-Site Swine Herd. Transbound. Emerg. Dis. 61(6):490.499.

Allerson M, Deen J, Detmer S, Gramer M, Joo HS, Romagosa A, Torremorell M (2013b). The impact of maternally derived immunity on influenza A virus transmission in neonatal pig populations. Vaccine 31:500-505.

Baadsgaard NP, Jørgensen E (2003). A Bayesian approach to the accuracy of clinical observations. Prev. Vet. Med. 59(4):189-206.

Black MA, Craig BA (2002). Estimating disease prevalence in the absence of a gold standard. Stat. Med. 21(18):2653-2669.

Bouwknegt M, Engel B, Herremans M, Widdowson M, Worm H, Koopmans M, Frankena K, de Roda Husman A, De Jong M, Van Der Poel W (2008). Bayesian estimation of hepatitis E virus seroprevalence for populations with different exposure levels to swine in The Netherlands. Epidemiol. Infect. 136(04):567-576.

Branscum AJ, Gardner IA, Johnson WO (2005). Estimation of diagnostic-test sensitivity and specificity through Bayesian modeling. Prev. Vet. Med. 68(2):145-163.

Carlin BP, Louis TA (2008). The Bayesian appoach. Bayesian methods

for data analysis. Chapman & Hall/CRC Press. New York, USA pp. 15-104.
Choi YK, Goyal SM, Joo H (2002). Prevalence of swine influenza virus subtypes on swine farms in the United States. Arch. Virol. 147(6):1209-1220.
Christensen J, Gardner IA (2000). Herd-level interpretation of test results for epidemiologic studies of animal diseases. Prev. Vet. Med. 45(1):83-106.
Corzo CA, Culhane M, Juleen K, Stigger-Rosser E, Ducatez MF, Webby RJ, Lowe JF (2013a). Active Surveillance for Influenza A Virus among Swine, Midwestern United States, 2009–2011. Emerg. Infect. Dis. 19(6):954-960.
Corzo CA, Romagosa A, Dee SA, Gramer MR, Morrison RB, Torremorell M (2013b). Relationship between airborne detection of influenza A virus and the number of infected pigs. Vet. J. 196:171-175.
Cowling DW, Gardner IA, Johnson WO (1999). Comparison of methods for estimation of individual-level prevalence based on pooled samples. Prev. Vet. Med. 39(3):211-225.
Davies P (2006). Principle of diagnostic testing of animal populations. In. AASV Ann. Meeting Proc. pp. 345-350.
Deblanc C, Robert F, Pinard T, Gorin S, Quéguiner S, Gautier-Bouchardon A, Ferré S, Garraud J, Cariolet R, Brack M (2012). Pre-infection of pigs with Mycoplasma hyopneumoniae induces oxidative stress that influences outcomes of a subsequent infection with a swine influenza virus of H1N1 subtype. Vet. Microbiol. 162:643-651.
Dendukuri N, Joseph L (2001). Bayesian approaches to modeling the conditional dependence between multiple diagnostic tests. Biometrics 57(1):158-167.
Donovan TS (2008). Influenza isolate selection methodology for timely autogenous vaccine use. In. the American Association of Swine. Vet. Ann. Meeting pp. 557-561.
Dykhuis HC, Painter T, Fangman T, Holtkamp D (2012). Assessing production parameters and economic impact of swine influenza, PRRS and Mycoplasma hyopneumoniae on finishing pigs in a large production system. In. the American Association of Swine. Vet. Ann. Meeting pp. 75-76.
Enøe C, Georgiadis MP, Johnson WO (2000). Estimation of sensitivity and specificity of diagnostic tests and disease prevalence when the true disease state is unknown. Prev. Vet. Med. 45(1–2):61-81.
Fablet C, Marois-Créhan C, Simon G, Grasland B, Jestin A, Kobisch M, Madec F, Rose N (2012). Infectious agents associated with respiratory diseases in 125 farrow-to-finish pig herds: a cross-sectional study. Vet. Microbiol. 157(1):152-163.
Gardner IA, Stryhn H, Lind P, Collins MT (2000). Conditional dependence between tests affects the diagnosis and surveillance of animal diseases. Prev. Vet. Med. 45(1):107-122.
Gelman A, Rubin DB (1992). Inference from iterative simulation using multiple sequences. Stat. Sci. pp. 457-472.
Geurden T, Berkvens D, Casaert S, Vercruysse J, Claerebout E (2008). A Bayesian evaluation of three diagnostic assays for the detection of Giardia duodenalis in symptomatic and asymptomatic dogs. Vet. Parasitol. 157(1):14-20.
Geweke J (1991). Evaluating the accuracy of sampling-based approaches to the calculation of posterior moments. Federal Reserve Bank of Minneapolis, Research Department.
Goodell CK, Prickett J, Kittawornrat A, Zhou F, Rauh R, Nelson W, O'Connell C, Burrell A, Wang C, Yoon K-J (2013). Probability of detecting influenza A virus subtypes H1N1 and H3N2 in individual pig nasal swabs and pen-based oral fluid specimens over time. Vet. Microbiol. 166(3):450-460.
Greiner M, Gardner I (2000a). Epidemiologic issues in the validation of veterinary diagnostic tests. Prev. Vet. Med. 45(1):3-22.
Greiner M, Gardner IA (2000b). Application of diagnostic tests in veterinary epidemiologic studies. Prev. Vet. Med. 45(1):43-59.
Hadley W (2009). ggplot2: Elegant graphics for data analysis. Springer. New York.
Holtkamp D, Rotto H, Garcia R (2007). The economic cost of major health challenges in large US swine production systems. In. the American Association of Swine Veterinarians Annual Meeting. Orlando, FL, USA pp. 85-89.
Johnson WO, Gastwirth JL, Pearson LM (2001). Screening without a "gold standard": the Hui-Walter paradigm revisited. Am. J. Epidemiol. 153(9):921-924.
Joseph L, Gyorkos TW, Coupal L (1995). Bayesian estimation of disease prevalence and the parameters of diagnostic tests in the absence of a gold standard. Am. J. Epidemiol. 141(3):263-272.
Lunn D, Jackson C, Spiegelhalter DJ, Best N, Thomas A (2012). Model checking and comaprison. The BUGS book: A practical introduction to Bayesian analysis. CRC Press. Boca Raton, FL.
Marin X (2013). ggmcmc: Graphical tools for analyzing Markov Chain Monte Carlo simulations from Bayesian inference. Available at: http://xavier-fim.net/packages/ggmcmc.
Mohan R, Saif Y, Erickson G, Gustafson G, Easterday B (1981). Serologic and epidemiologic evidence of infection in turkeys with an agent related to the swine influenza virus. Avian Dis. pp. 11-16.
Nérette P, Stryhn H, Dohoo I, Hammell L (2008). Using pseudogold standards and latent-class analysis in combination to evaluate the accuracy of three diagnostic tests. Prev. Vet. Med. 85(3):207-225.
Olsen C, Carey S, Hinshaw L, Karasin A (2000). Virologic and serologic surveillance for human, swine and avian influenza virus infections among pigs in the north-central United States. Arch. Virol. 145(7):1399-1419.
Paul S, Toft N, Agerholm JS, Christoffersen AB, Agger JF (2013). Bayesian estimation of sensitivity and specificity of Coxiella burnetii antibody ELISA tests in bovine blood and milk. Prev. Vet. Med. 109(3):258-263.
Plummer M (2013). JAGS Version 3.4.0 user manual.
Plummer M (2015). rjags: Bayesian graphical Models using MCMC. R package version 3-14. Available at: http://CRAN.R-project.org/package=rjags.
Plummer M, Best N, Cowles K, Vines K (2006). CODA: Convergence diagnosis and output analysis for MCMC. R News 6(1):7-11.
Poljak Z, Dewey CE, Martin SW, Christensen J, Carman S, Friendship RM (2008a). Prevalence of and risk factors for influenza in southern Ontario swine herds in 2001 and 2003. Can. J. Vet. Res. 72(1):7.
Poljak Z, Friendship RM, Carman S, McNab WB, Dewey CE (2008b). Investigation of exposure to swine influenza viruses in Ontario (Canada) finisher herds in 2004 and 2005. Prev. Vet. Med. 83(1):24-40.
Praud A, Gimenez O, Zanella G, Dufour B, Pozzi N, Antras V, Meyer L, Garin-Bastuji B (2012). Estimation of sensitivity and specificity of five serological tests for the diagnosis of porcine brucellosis. Prev. Vet. Med. 104(1):94-100.
R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing.

Appendix A

Bayesian model, namely y_{ijk}~ *multinomial*(n_i, (p_{i11}, p_{i12}, p_{i21}, p_{i22})), was constructed to estimate Se and Sp of RCS, NS and OF RRT-PCR tests with a sample size of n_i in population *i* for *i*=1, 2. The conditional independent model was constructed for RCS versus NS RRT-PCR tests. The NS versus OF RRT-PCR tests comparison was modeled as conditionally dependent. With the conditionally dependent model, the conditional covariance between the two (NS versus OF RRT-PCR tests) test Se, γ_η, and Sp, γ_θ, were modeled as descripted elsewhere (Dendukuri and Joseph, 2001). The corresponding correlations, ρ_η, ρ_θ, were calculated. The unobserved stochastic nodes are referred to as the parameters of the model. Furthermore, we modeled the kappa statistic by using equations from elsewhere which are represented by: $k_\eta = \frac{2*\gamma_\eta}{\eta_2(1-\eta_2)+\eta_3(1-\eta_3)}$, $k_\theta = \frac{2*\gamma_\theta}{\theta_2(1-\theta_2)+\theta_3(1-\theta_3)}$ (Gardner et al., 2000), where k_η, k_θ , were predictors for infected and non-infected populations respectively.

Appendix B

A conceptual model with directed acyclic graph **(Figure 1)** represents Bayesian model. The model estimates Se (eta) and Sp (theta) of RCS, NS and OF RRT-PCR tests. Ellipses are stochastic nodes. Grey and white nodes are observed variables and model parameters respectively. Rectangles are constant process of the experimental design. Dark and light arrows present deterministic and stochastic dependencies, respectively. There were 2 populations (*i*=1, 2) that are the populations in the field study and in the experimental study. The Y[i,j,k] are realizations of observed positive/negative counts in population *i* for test 1(j=1:positive, 2:negative) and test 2 (k=1:positive, 2:negative). p[i,j,k] represents the probability of a test positive/negative in population *i* where p[i,1,1] is the probability of both tests 1 and 2 positive. p[i,1,2] is the probability of test 1 positive with test 2 negative. p[i,2,1] is the probability of test 1 negative with test 2 positive. p[i,2,2] is the probability of both test 1 and 2 negative in population *i*. The pi[1] is the prior prevalence of infection in the field study population. The pi[2] is the prior prevalence of infection in the experimental study population. The eta[] and theta[] are Se and Sp. The gamma[eta] is the correlation between Se of NS versus OF RRT-PCR tests and gamma[theta] and the correlation between Sp of NS versus OF RRT-PCR tests. The kappa[eta] is the kappa statistic between Se of NS versus OF RRT-PCR tests and kappa[theta] is the kappa statistic between Sp of NS versus OF RRT-PCR tests. Psi (ψ) is the probability of influenza A being endemic (Figure 1).

Appendix C

With conditional dependent assumption, Se of the OF and NS RRT-PCR are conditionally dependent with γ_η (conditional covariance positive) and Sp of those are conditionally dependent with γ_θ (conditional covariance negative). In the Table 4, γ_η and γ_θ must be range between zero and one since it is elements of the probability. It can be expressed as:

$\max[-(1-\eta_1)(1-\eta_3), -\eta_1\eta_3] \le \gamma_\eta \le \min[\eta_1(1-\eta_3), \eta_1(1-\eta_3)]$ and, $\max[-(1-\theta_1)(1-\theta_3), -\theta_1\theta_3] \le \gamma_\theta \le \min[\theta_1(1-\theta_3), \theta_1(1-\theta_3)]$.

Where η_1 and θ_1 represents the Se and Sp of NS RRT-PCR test and η_3 and θ_3 represents the Se and Sp of OF RRT-PCR test.