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Full Length Research Paper

Prevalence and associated risk factors for *Mycobacterium avium* subsp. paratuberculosis in dairy cattle in Mexico

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The purpose of this study is to determine the seroprevalence and the associated risk factors to *Mycobacterium avium* subspecies paratuberculosis. A total of 4,487 serum samples were collected from cattle in 173 farms from different parts of Mexico. Information about potential risk factors and spatial location of the farms was obtained through a questionnaire to farm owners and by global positioning system (GPS) apparatus respectively. An enzyme-linked immunosorbent assay (ELISA) kit was used to detect the presence of antibodies against paratuberculosis (MAP). Maps showing areas of high risk of MAP and maps showing areas with environmental conditions for the presence of paratuberculosis were elaborated with Maxent. The overall prevalence of the disease was 5%. The higher seroprevalence was in family run systems (11.3%). The spatial analysis showed higher prevalence (6%) in the South Pacific region. Three factors had a significant relationship with prevalence of the disease: history of tuberculosis, grazing in open fields and belonging to the family run system.

Key words: *Mycobacterium*, paratuberculosis, dairy cattle, seroprevalence, Mexico.

INTRODUCTION

Paratuberculosis (PTB), or Johne's disease (JD), is a chronic, progressive, infectious granulomatous enteritis caused by *Mycobacterium avium* subspecies paratuberculosis (MAP), which affects ruminants, especially dairy cattle, and a variety of domestic species (Manning and Collins, 2001; Kudahl et al., 2007). Characteristic symptoms include: diarrhea, progressive weight loss and death in adult animals. MAP has been

reported to survive milk pasteurization (Grant et al., 2002), and has been related to Crohn's disease in humans (Timms et al., 2012). The main route of infection with MAP is the fecal-oral route, but it can also be transmitted through colostrum (Streeter et al., 1995), and milk from subclinical or clinical infected cows (Streeter et al., 1995; Sweeney et al., 1992; Taylor et al., 1981). In utero, infection has also been reported (Whittington and

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Windsor, 2009).

Clinical paratuberculosis has an important economic impact on the dairy industry; losses due to this disease in the dairy industry in the US have been estimated in 1.5 billion dollars annually (Merkal et al., 1987). No data about the economic impact of the disease is available in Mexico. The reasons for such losses are: low milk production (Benedictus et al., 1987), large calving intervals (Abbas et al., 1983), low slaughter weights (Whitlock et al., 1985), shorter life expectancy, loss of potential breeding value, infertility and increased incidence of mastitis (Buergelt and Duncan, 1978). Efforts to treat the disease or to develop a vaccine have not been successful. Until an effective cure or prevention is found, early diagnosis and scientific management practices alone will help in protecting the animals against this disease.

Johne's disease has a worldwide distribution. The prevalence in some countries is as high as 40%, as is the case for the United States of America (Sánchez-Villalobos et al., 2009), similar rates are reported in Canada (Sorensen et al., 2003). In cattle, it is endemic in The Netherlands, Austria, and Belgium, where the prevalence rate is 54, 7, and 41% respectively. In Europe, Sweden is the only country with a MAP-free status (Singh et al., 2008). In Australia, infection rates fluctuate between 9 and 22% (Sánchez-Villalobos et al., 2009) respectively. In the south west of England, the mean seroprevalence is 7.1% (Woodbine et al., 2009).

Latin America is not the exception; Argentina reports prevalence rates of 18.8% in dairy farms and 6.8% in beef farms. In Rio de Janeiro, Brazil, the prevalence is 33%. In Venezuela 72% of the herds is infected (Sánchez-Villalobos et al., 2009). In Mexico, reports from different studies, usually involving small sample sizes, show a prevalence of about 30%, especially in dairy cattle.

Therefore, the main objective of this study was to determine the seroprevalence of *M. avium* subspecies paratuberculosis in dairy cattle and the associated risk factors.

MATERIALS AND METHODS

The data

Data came from a large cross-sectional study in 173 farms conducted between January, 2010 and December, 2012. The study involved farms from different parts of Mexico which are under three main systems of milk production prevalent in the country: intensive, family-run and double-purpose farms. Intensive and family-run farms have Holstein-Friesian cattle, double-purpose farms have mainly *Bos indicus* breeds, which are primarily used for calving and, as a secondary purpose, milk production. The estimated sample size was about 3,500 animals, using a 10% hypothetical prevalence for brucellosis, 1% error and 95% confidence level. Even though the sample size estimated was 3,500 animals, the final number of samples collected was 4,487. A stratified multistage sampling design was used. Considering that the population of dairy cattle is located in specific regions, each region was considered as a

stratum in the first stage. In the second stage, states were selected within each stratum, and counties selected within each state. Counties were not randomly selected; instead, they were selected from a list of milk producing counties. Finally, due to the lack of a good sampling frame, convenience sampling was used to select herds and animals within herds. All sampling personnel were advised to select herds from different areas of each county which will form a representative sample. To reduce the total variance of sampling, the sampling fraction by stratum (region) was determined dividing the total number of samples by the total population (3,500/2,000,000=0.0019). Subsequently, to determine the number of animals sampled per stratum, the sample fraction was multiplied by the size of the population in each stratum.

Blood samples

Ten milliliters of blood were collected from each animal from the middle coccygeal vein with a 20-gauge, 1-in needle in a 10 ml serum-separator Vacutainer tube (Becton Dickinson and Company, Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, 07417 to 1885, USA). Antibodies against PTB were determined by Enzyme-link-immunosorbent-assay (ELISA) kit (IDEXX Laboratories, Inc., Westbrook, ME, USA), using the protoplasmatic strain 3065 MAP (Martinez et al., 2012).

Epidemiological information

In order to collect epidemiological information, a questionnaire was administered to the owners of the herds to identify farm management practices and herd performance. The questionnaire included open items (any answer possible) and closed items (possible answers provided in the questionnaire) related to general characteristics of farms, such as size, breed and production, as well as target questions referring to potential risk factors for MAP transmission.

Statistical analysis

The statistical analysis was carried out in three phases. First, a univariate descriptive analysis was performed throughout frequencies and descriptive statistics, followed by a bivariate analysis to identify those variables potentially associated with MAP prevalence. Finally, all variables with a p value ≤ 0.20 were considered for a multivariate logistic regression model to obtain adjusted odds ratios. Analysis was performed with Epi info tm7.1.0.6. (Centers for Disease Control and Prevention) and SPSS (SPSS Inc. 233 South Wacker Drive, 11th Floor, Chicago, IL 60606-6412 EE.UU).

Spatial information

All farms were spatially located using a spatial location apparatus (GPS). This information was used to estimate risk areas of the disease throughout geostatistical modeling (kriging). These analyses were performed with ArcView from ArcGIS 10 (ESRI, Redlands, CA).

Ecological niche modeling

In order to determine a relationship between environmental variables from BIOCLIM (<http://www.worldclim.org>) and the presence of MAP, an ecological niche modeling with maxent was performed. Maps showing predicted relative suitability for the

Table 1. Prevalence of paratuberculosis in cattle in Mexican dairy farms, by system of production.

System of production	Positive animals	Total	Prevalence (%)
Intensive	105	2303	4.6
Dual-purpose	63	1617	3.9
Family-run	64	565	11.3
Total	232	4485	5.2

Table 2. Prevalence of paratuberculosis in cattle in Mexican dairy farms by state.

State	Positive animals	Total	Average prevalence (%)	Number of herds	Prevalence range (%)
Intensive					
Aguascalientes	13	147	9	7	0-29
Chihuahua	13	714	2	15	0-7
Coahuila	11	202	5	6	0-12
Durango	3	222	1	7	0-10
Guanajuato	14	147	10	4	2-22
Hidalgo	36	379	9	16	0-50
Querétaro	15	492	3	17	0-17
Family-run					
Jalisco	64	565	11	24	0-42
Dual-purpose					
Chiapas	6	509	1	20	0-14
Sinaloa	18	319	6	10	0-18
Veracruz	39	791	5	47	0-25
Total	232	4487	5	173	0-50

presence of cases were elaborated. Twenty-five percent of the herds were randomly selected to test the model accuracy. Environmental data used by maxent were: temperature and precipitation, and the 19 environmental variables from BIOCLIM with 2.5 min of resolution, converted to a common projection.

RESULTS

The overall herd seroprevalence of *M. paratuberculosis* in dairy cattle in Mexico was 48%, and the animal seroprevalence was 5% (range 0 to 50%). The highest animal prevalence was observed in the family-run system (11.3%), compared to the intensive (4.6%) and the double-purpose (3.9%) systems (Table 1). Results show that prevalence varies from state to state, from 1 to 11%, and in herds within a state, from 0 to 50% (Table 2). The highest prevalence was found in some herds in the State of Hidalgo (50%) which has a high density of dairy cattle (25000 dairy cattle) in 102 herds in the production complex "Tizayuca". This complex is characterized by the closeness of the farms, origin of replacement stock, national and international. According to herd size, prevalence was higher in herds of 200 to 300 cows (9.5%)

and in farms using raw milk to feed calves (7.7%).

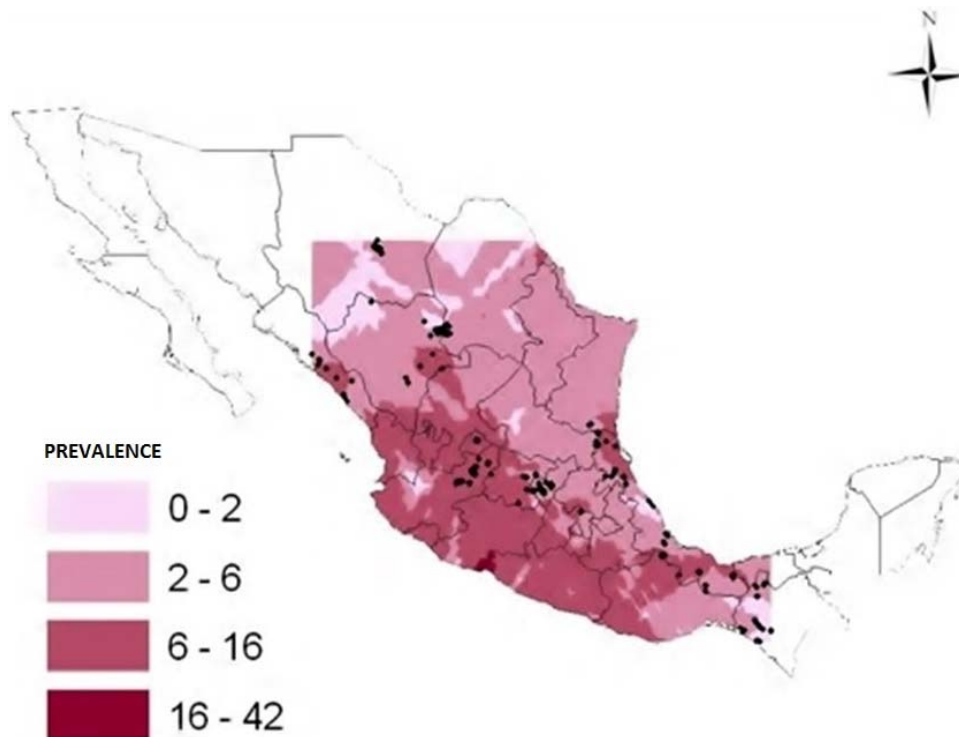
In order to identify risk factors associated to prevalence, a logistic regression analysis was performed. Very few factors showed significant correlation ($P < 0.05$); animals with positive serology to tuberculosis, $OR = 2.9$ ($CI_{95\%}$ 1.9 to 4.2), animals grazing in open fields, $OR = 2.1$ ($CI_{95\%}$ 1.1 to 4.2), and the production system: $OR = 2.4$ ($CI_{95\%}$ 1.2 to 4.8) for dual-purpose and $OR = 4.5$ ($CI_{95\%}$ 3.1 to 6.7) in family-run systems (Table 3).

The spatial analysis to establish risk areas of paratuberculosis in dairy cattle showed that the highest risk is in the South Pacific region (over 6%), where the family-run system is located. In the Gulf of Mexico and central north regions, the prevalence is rather low, 0 to 6% (Figure 1). It is worth mentioning that the Northwest and Southeast areas of the country were not sampled. Therefore, prediction does not cover the whole country.

Figure 2, shows the ecological niche modeling (maxent) map with predicted relative habitat suitability for the presence of MAP. Habitat suitability increases from blue to yellow to red. The climatic variable with the highest prediction value in the model was seasonal temperature. The zones with favorable climatic conditions

Table 3. Adjusted odds ratio for risk factors associated with the prevalence of dairy cattle paratuberculosis in Mexican dairy herds.

Risk factor	Category	P	OR	95% CI	
				Lower	Upper
History of tuberculosis	No	-	-	-	-
	Yes	0.000	2.9	1.9	4.2
Feeding	Concentrates	-	-	-	-
	Open field Grazing	0.026	2.1	1.1	4.2
Production system	Intensive	-	-	-	-
	Double purpose	0.017	2.4	1.2	4.8
	Family run	0.000	4.5	3.1	6.7

**Figure 1.** Predicted risk of paratuberculosis in dairy cattle in Mexico.

such as temperature and humidity for the survival of the organisms show highest incidence. These areas include Sinaloa, a region formed by parts of Jalisco, Guanajuato and Aguascalientes, and La Laguna, which includes parts of Coahuila and Durango, and some parts of Chiapas.

DISCUSSION

The average prevalence rate of paratuberculosis in cattle used for milk production in Mexico was 5%. This pre-

valence rate is similar to that obtained in some other parts of the world; 5% in the State of New York in the US (Obasanjo et al., 1997), 5.8% in England (Cetinkaya et al., 1997), but much lower than that reported in Michigan, also in the US (66%) (Johnson-Ifeorunlu and Kaneene, 1998) and Alberta, Canada, 9.1% (Scott et al., 2006). In Latin American and Caribbean countries, the prevalence rate ranges from 16% in cattle to 4.3 in sheep and goats (Fernández-Silva et al., 2014).

Earlier reports of the prevalence of paratuberculosis varies: Salman et al. (1990) reported a prevalence of

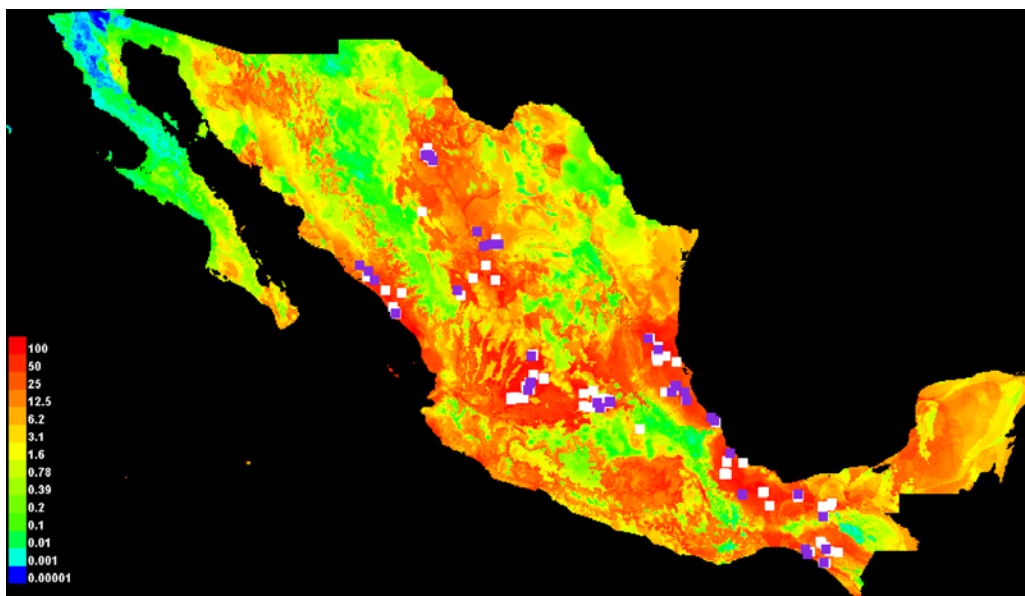


Figure 2. Predicted potential suitable areas for the presence of paratuberculosis in dairy cattle in Mexico. Areas in red indicate favorable conditions.

46% in 8,100 dairy cattle from 110 premises in Baja California. Chávez et al. (2004) reported a low prevalence of 13% in fighting bulls in Veracruz (Morales, 1994) while prevalence rates in dairy cattle and dual purpose cattle in Guanajuato were 30 and 25%, respectively (Santillán et al., 2003). An earlier report by Miranda (2005) in the Tizayuca dairy complex in the state of Hidalgo, indicated a prevalence rate of 8.8%, which is much lower than that shown in this study. The variation in the prevalence rate could be attributed to the low sample size in the earlier study as compared to this study survey.

In general, the main differences between this results and those from other studies are the sample size and the number of herds included in the study. Most studies in Mexico are based on low sample sizes and smaller geographic areas, whereas this study involved 173 herds from 11 states, including cattle from three different milk production systems. The study did not randomize the sample, and included a more representative population in comparison with earlier studies.

In this study, the highest prevalence was found in family-run systems (11.3%). This could be a consequence of the minimal use of technical services, such as veterinary support and technology. In addition, in this system cows are kept in production for longer periods of time (4.5 to 7 years) as opposed to the intensive system; therefore, there is a higher chance for developing this chronic disease.

The low prevalence (4.6%) in the intensive production system could be the result of the high replacement rate in this population, the average life span of a cow in this system is about 3.5 years. Cattle in the dual purpose system had a prevalence of 3.9%. The low prevalence

rate in this area could be attributed to the low density of cattle per Km and also the tropical conditions prevailing in this area may be detrimental to the survival of the organism.

Results of the maxent model are shown in Figure 2. Habitat suitability for presence of *M. paratuberculosis* is observed in wide areas of the national territory, especially in the tropical, central and central north areas. However, maxent is a model that assumes random distribution of species, and in the present study the dairy herds are not randomly distributed. Therefore, this map should be taken with caution. The highest predicted habitat suitability in our study could be more the result of the location of human populations than environmental variables *per se*.

The ability to rapidly diagnose and identify the causative agent are critical for combating diseases and stopping epidemics (Wadhwa et al., 2012; Kaur et al., 2013). Recent technological developments have led to the proliferation of new and improved diagnostic tests that hold promise for a better management and control of infectious diseases (Wadhwa et al., 2014). New technologies such as microfluidics (Wadhwa et al., 2012) and “Lab-on- Chip” (Liu et al., 2011) are examples of promising new technologies with the potential to be used as a laboratory-free diagnostic devices for infectious diseases in animal husbandry.

This study indicated that the prevalence of MAP in dairy cattle in Mexico is low compared to previous reports either from Mexico or from our neighboring and European countries. Therefore, a practical recommendation for national authorities in animal health is to take actions now to reduce, and eventually eliminate, this disease from

dairy cattle population before it spreads and prevalence increase.

Conflicts of interest

The authors have none to declare.

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A Review

A Bayesian approach for inductive reasoning to clinical veterinary medicine: The math of experience

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A Bayesian approach (BA) is well-used in veterinary medicine as it has been used for inductive reasoning regarding interventions, treatments and diagnoses. The objectives of the current article were (1) to examine the state of BA used for inductive reasoning in veterinary medical problems and (2) to illustrate how veterinarians update states of knowledge (prior clinical experience) to a new state of knowledge (posterior clinical experience). When veterinarians are managing patients, they start with their inference from history and a clinical sign to an underlying cause using inductive reasoning. In updating from a prior clinical experience to a posterior clinical experience, the strength of evidence plays an important role. Nevertheless, if an experienced veterinarian uses his/her previous experience of a current patient's clinical signs, he/she may not move from the prior clinical experience to a posterior clinical experience and is less likely to change his/her treatment decisions. In comparison, for a novice veterinarian who would have less prior clinical experiences with given clinical signs, his/her prior clinical experience would easily be changed to a posterior clinical experience after taking history and physical examination. In brief, the more prior clinical experience a veterinarian has, the more rapid a diagnosis is made. The stronger the evidence, the more precise inference will be.

Key words: Bayesian, inference, reasoning, inductive, veterinarian.

INTRODUCTION

In clinical practice, experience is an unmeasured aspect in making a diagnosis. To make a diagnosis,

veterinarians imply the association from cause to effect. For example, if pigs were infected with influenza A virus

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(IAV), they may present with coughing as their primary clinical sign, or if dogs are exposed to canine parvovirus (CPV), they may present with bloody diarrhea. On the other hand, when managing most patients, veterinarians start their inference from a clinical sign to an underlying cause. The former reasoning (from cause to effect) pathway cannot be made since veterinarians rarely know the true cause of a disease. They have to reason in an opposite direction (from clinical sign to cause). In the statistical perspective, the former pathway of thinking is called “deductive reasoning” while the latter pathway (from clinical sign to cause) is called “inductive reasoning” (Cockcroft, 2008).

Since the 1970s, inductive reasoning has been employed in clinical veterinary medicine (Lorenz, 2009). It was originally called, “pattern recognition” and more recently “problem-oriented approach (POA)” and “evidence-based veterinary medicine (EBVM)”. In the 1980s, the term “evidence-based medicine” (EBM) was minted at McMaster Medical School in Canada (Rosenberg and Donald, 1995). EBM is defined as “the conscientious, explicit, and judicious use of the current best evidence in making decisions about the care of individual patients” (Sackett et al., 1996). EBM can be practiced in any situation where there is doubt about an aspect of clinical diagnosis, or prognosis (Rosenberg and Donald, 1995). In veterinary medicine, EBVM would be defined similarly as it uses the current best evidence to make clinical decisions concerning the care for animal patients. EBVM has been described as “just in time learning (as opposed to just in case learning), science into practice or from publication to patient” (Cockcroft, 2008).

The veterinarian uses all of the information collected from evidence, such as signalment, patient history, physical examination and laboratory results to answer the question, “What is the cause(s) of the problem that is associated with the clinical presentation (that is, disease effect)?” From a statistical point of view, the veterinarian is answering the question, “What is the probability of a potential cause?” For instance, the probability of classical swine fever (CSF) in coughing pigs in the United States (US) may be near zero, since CSF is no longer in the US and will therefore be excluded from the differential diagnosis. Similarly, the probability that dog with bloody diarrhea is infected with CPV is near zero due to the low prevalence of CPV in the US; therefore, CPV will be removed from the differential diagnosis. This type of probability is called “inverse probability”, which is different from the probability (direct probability) of having a sign if an animal is exposed to the agent (Holland, 1986).

Inverse probability is typically used as a basis for making inductively statistical inference and finding the “probability of causes” and future events derived from a past event (starting with the conclusion desired or desirable proposition and seeking for premises which make it true or probable) (Dale, 1999; Hald, 1998). It is

“inverse” because it involves inferring backwards from the present day to the past or “from effects to causes” (Fienberg, 2006). The term “inverse probability” is also known as the “Bayesian approach (BA)” (Aldrich, 2008; Bayes and Price, 1763; Fienberg, 2006; Stigler, 1986).

In clinical veterinary medicine, veterinarians always deal with a rapidly changing body of evidence obtained from physical examination, patient history and laboratory results. When new evidence is uncovered, a veterinarian’s clinical decisions may be changed as well as the lists of differential diagnoses will be reduced. A utility of BA for veterinary diagnostic test has been well-addressed elsewhere (Bonde et al., 2010; Branscum et al., 2005; Gardner, 2002; Greiner and Gardner, 2000; Paul et al., 2013; Toft et al., 2005). Therefore, the objectives of the current article were to examine the state of BA used for inductive reasoning in veterinary medical problems and to illustrate how veterinarians update states of knowledge (prior clinical experience) to a new state of knowledge (posterior clinical experience).

A BAYESIAN APPROACH

A Bayesian approach is a statistical method of the conditional distribution of parameters and unobserved evidence, given the observed evidence (Gelman, 2008). It is considered as the natural statistical framework for both EBM and EBVM in order to make decisions that incorporate an integrated summary of the available evidence and associated uncertainty (Ashby and Smith, 2000). It is a more natural formalization of the normal scientific process of evaluating evidence (Dunson, 2001), integrating and synthesizing EBM in a systematic way (Ashby and Smith, 2000). It provides a common framework for problem solving and improving communication and understanding between owners and their animals from different backgrounds (prior experience) (Rosenberg and Donald, 1995). It is used to integrate individual clinical expertise (prior clinical experience) with the best available external clinical evidence from systematic research (Sackett et al., 1996). It is a synthesizing of the available external clinical evidence using Bayesian meta-analysis (Ashby and Smith, 2000). In addition, it can gauge the strength of prior clinical experience by evaluating whether evidence can dominate the prior experience or not (Greenland, 2006). It has been shown that a major change of prior clinical experience would require solid clinical evidence and then clinicians will logically update their prior clinical experience to updated clinical experience (Higgins et al., 2014).

A Bayesian approach was independently developed by Tomas Bayes and Pierre-Simon Laplace over 300 years ago (Aldrich, 2008; Bayes and Price, 1763; Fienberg, 2006; Stigler, 1986). However, the fundamentals of BA have been followed by the Laplace-Jeffreys objective

school, with additional modern refinements (Berger, 2006). The influence of BA can be seen in mathematics, statistics, computer science, bioinformatics, economics, physics, ecosystem, parasitology, and epidemiology as well as in human and veterinary medicine (Ashby and Smith, 2000; Basáñez et al., 2004; Dowd and Meyer, 2003; Fienberg, 2006; Gardner, 2002). A classic example of applying BA in order to make inductive reasoning from an effect to a cause is during 1855 to 1865 in London, England, where John Snow had used BA as his inductive reasoning to scientifically convince audiences that a source of cholera transmission was from a private water supplier company (Koch and Denike, 2006).

Components of a Bayesian approach

A Bayesian approach comprises three mathematical terms: (i) evidence¹, “ $p(x)$ ” (a.k.a. the marginal likelihood, or the probability of evidence), (ii) the prior experience², “ $p(\theta)$ ” and (iii) strength of evidence³, “ $p(x|\theta)$ ” (a.k.a. likelihood of evidence given a hypothesis). The posterior experience, “ $p(\theta|x)$ ”⁴ (a.k.a. updated posterior experience from prior experience after having seen the evidence) is equal to the product of prior experience times strength of evidence divided by the evidence. Mathematically, it is written as:

$$p(\theta|x) = \frac{p(x|\theta) \cdot p(\theta)}{p(x)}$$

Simply, the posterior experience is proportional to the strength of the new evidence times prior experience (Higgins et al., 2012b, 2014). To further illustrate this, we will use an example of coughing pigs, where the posterior experience was reversely inferred from the strength of the new evidence and the prior experience.

Example: Coughing pigs

A veterinarian observes coughing pigs (evidence) and wishes to make a diagnosis (inference) by asking the question, “Is coughing in pigs caused by IAV infection?” He/she needs to inductively infer the cause from a posterior clinical experience (posterior probability) as shown in Figure 1.

However, the coughing could be caused by multiple pathogens including classical swine fever, metastrongylus, *mycoplasma hyopneumoniae*, porcine respiratory coronavirus, classical swine fever virus, porcine circovirus type 2 virus, porcine reproductive,

respiratory syndrome (PRRSv) virus, or IAV, etc. (Zimmerman et al., 2012) (Figure 2).

From the Bayesian notation, the theorem is applied to the inference of coughing in pigs caused by IAV infection written as:

$$p(\text{flu}|\text{coughing}) = \frac{p(\text{coughing}|\text{flu}) \cdot p(\text{flu})}{p(\text{coughing}|\text{flu}) \cdot p(\text{flu}) + p(\text{coughing}|\sim\text{flu}) \cdot p(\sim\text{flu})}$$

The denominator is called the “probability of evidence” of coughing event (marginal likelihood). The Bayesian terms, notations and definitions were detailed in Table 1.

Figure 3 numerically illustrates BA (inverse probability) pathway for diagnosing coughing pigs. Based on previous experience (prior clinical experience), the veterinarian may expect that 29% of coughing cases are caused by IAV infection, even though multiple swine pathogens can cause some degree of coughing (Choi et al., 2002; Olsen et al., 2000). While the prior knowledge may or may not be accurate, the prior clinical experience is useful to estimate such a percentage when there is lack of clinical information and a need to make a decision for clinical intervention (Higgins et al., 2012a). A Bayesian approach allows the veterinarian to update the probability of IAV infection by obtaining new information given his previous knowledge and the strength of evidence, $p(\text{coughing}|\text{flu})$. If the probability of IAV infected pigs having coughing as a clinical sign, $p(\text{coughing}|\text{flu})$, is for example 0.3, the posterior clinical experience, $p(\text{flu}|\text{coughing})$, is 0.17. The calculation is illustrated in Figure 3. As BA measures a degree of prior clinical experience (hypothesis), from such posterior experience, it is implied from his/her clinical experience that there is a probability of 0.17 that those coughing pigs have IAV infection. Therefore, he/she has less confidence (low probability) concerning his/her prior clinical experience after he/she has had new evidence. In other words, if weak evidence is found, the prior experience stands; when moderate evidence is found, the prior clinical experience and the new evidence can be combined, modifying the moderate posterior clinical experience. If strong evidence is uncovered to discredit the prior clinical experience, this modifies the prior clinical experience, which changes intervention strategies (strong posterior clinical experience). A major change of prior clinical experience would require solid clinical evidence to update prior clinical experience to posterior clinical experience (Higgins et al., 2014). However, it is unlikely to be sufficient to warrant an intervention when using only prior clinical experience for implementing an intervention.

There is first-rate and fallacious evidence for guiding decisions of intervention. For any evidence, we also need to estimate the probability that first-rate evidence is obtained from clinical examination or diagnostic results. Thus, the definition of the observed first-rate evidence is

¹ “ x ” is data that has been observed.

² “ θ ” is a prior clinical experience about a disease.

³ “ $x|\theta$ ” is data that have been observed based on a prior clinical experience.

⁴ “ $\theta|x$ ” is a potential disease after having seen the data.

Table 1. Representation of the Bayesian terms, notations and definitions related to an example of influenza A virus (IAV) infection.

Bayesian term	Notation	Definition
Prior clinical experience	$p(\text{flu})$	Probability that pigs have IAV infection (prevalence)
The strength of evidence	$p(\text{coughing} \text{flu})$	Probability that IAV infected pigs are coughing as a clinical sign
-	$p(\text{coughing} \sim\text{flu})$	Probability that pigs negative to IAV infection have coughing as a clinical sign
-	$p(\sim\text{flu})$	Probability that pigs have no IAV infection
Posterior clinical experience	$p(\text{flu} \text{coughing})$	Probability that coughing is caused by IAV infection



Figure 1. The representation of inductive inference from posterior clinical experience (posterior probability).

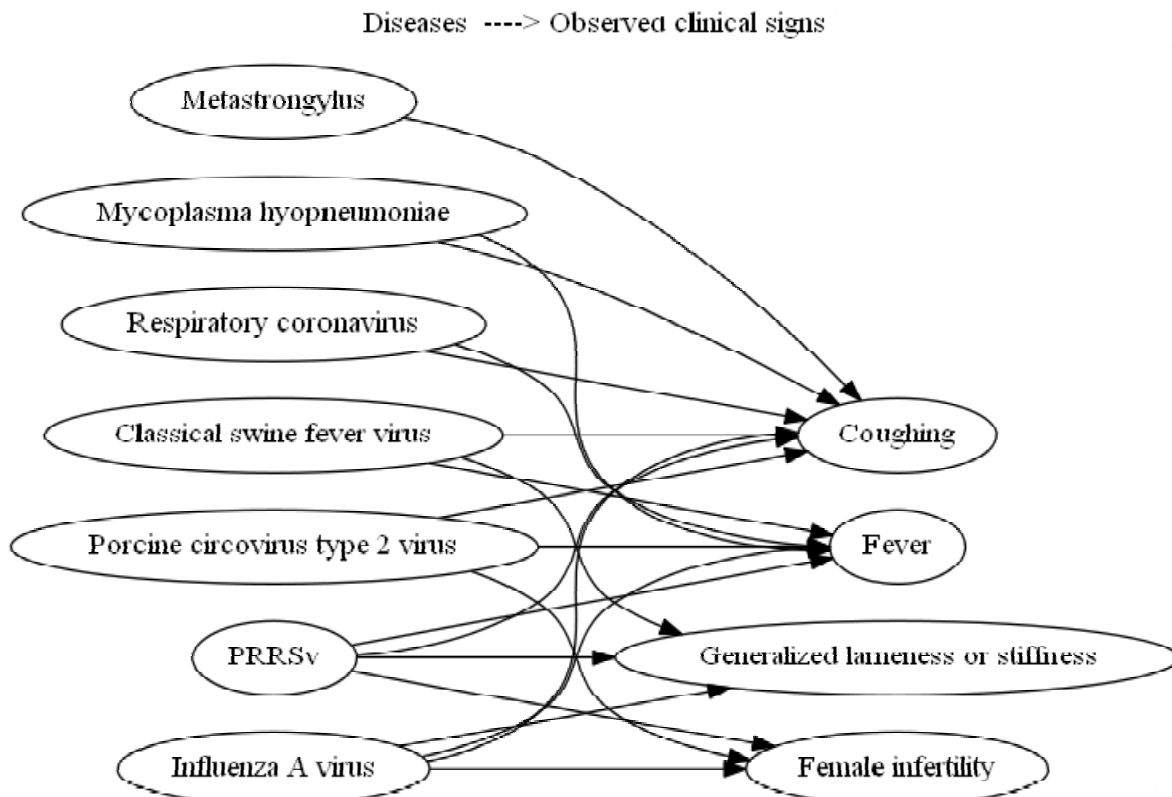


Figure 2. The processes of deduction (from diseases to observed clinical signs) and induction (from observed clinical signs to diseases) used in veterinary inference with an example of partially-selected swine diseases.

useful in the context of diagnosing disease and making an intervention decision. A simple approach may be to

increase the sample size to strengthen the evidence given the prior experience. Consider the following

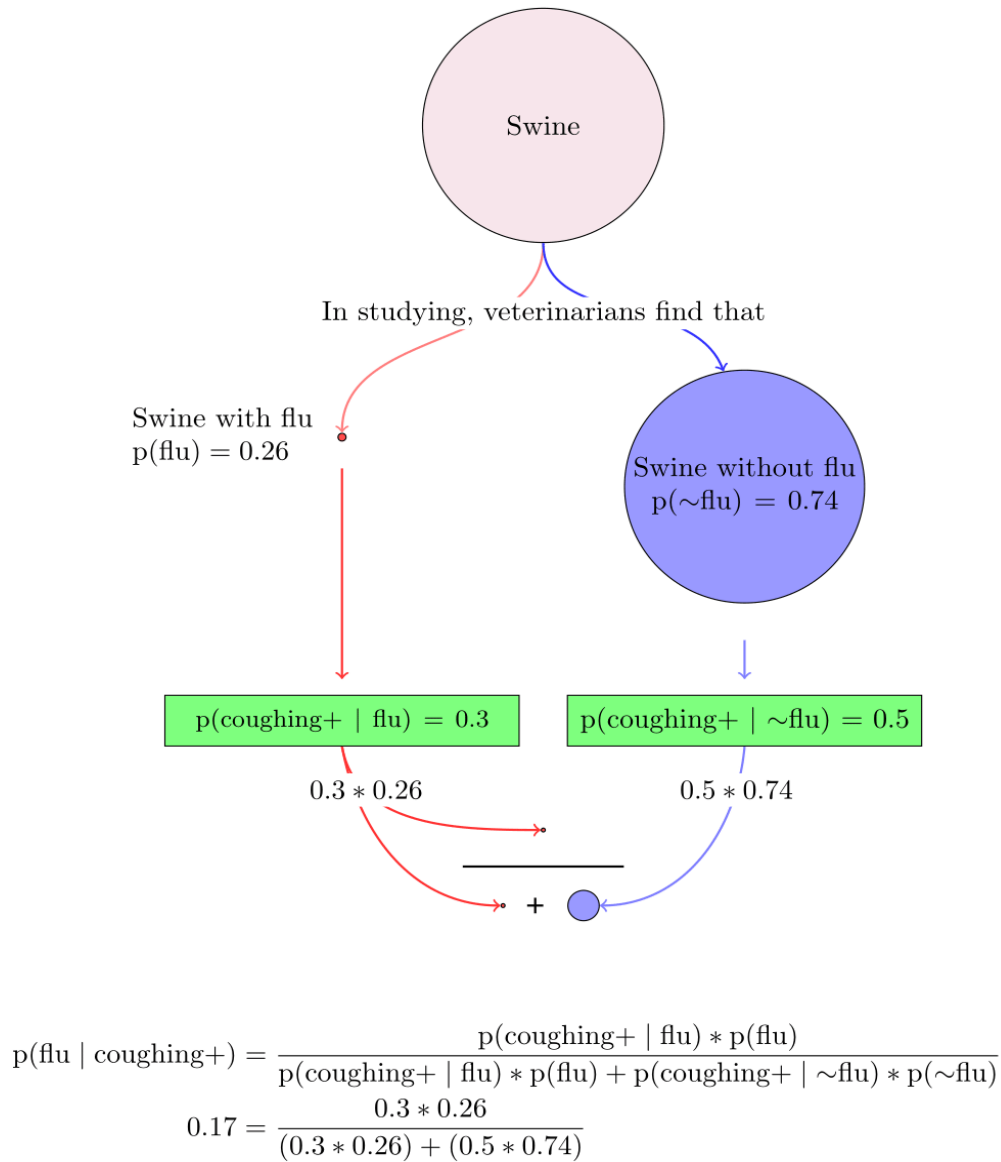


Figure 3. The calculation using Bayes’ theorem for inductive inference processes from coughing to influenza A virus infection.

examples; an inexperienced veterinarian is monitoring a healthy sow herd (negative sow herd) for H1N1-IAV using an ELISA test kit with the test specificity = 99.7% (95% CI: 99.5–100%). Randomly, 5 sows were tested at once and one is positive, “p(x)”. Given the prior clinical experience “p(θ)” and inductive thinking, the one positive is questioned. He/she is unsure if one positive sample represents 20% (1/5), “p(θ|x)” being the strength of evidence of prevalence of H1N1-IAV given what is previously known (that is, the prevalence of IAV was 29% with 13% SD (Choi et al., 2002; Olsen et al., 2000), by prior clinical experience of H1N1-IAV prevalence in US swine herd) (Figure 4). If 10 more samples were analyzed with 2 positive samples, or 20 samples with 3

positive samples, strength of evidence “p(x|θ)” will increase and then can create the posterior clinical experience p(θ|x).” Based on the first-rate evidence, the prior clinical experience will change from 29% to the posterior clinical experience of 20% prevalence (the most likely H1N-IAV prevalence), and thereby the veterinarian has learned something new. With a sample size of 20, the confidence in the posterior clinical experience and the precision about the estimate relatively increases as compared to the prior clinical experience, represented by narrower credible interval (confidence interval used in BA) is as shown in Figure 5. By choosing narrower credible intervals, inference and decision-making are better served, compared to chance (Poole, 2001). More

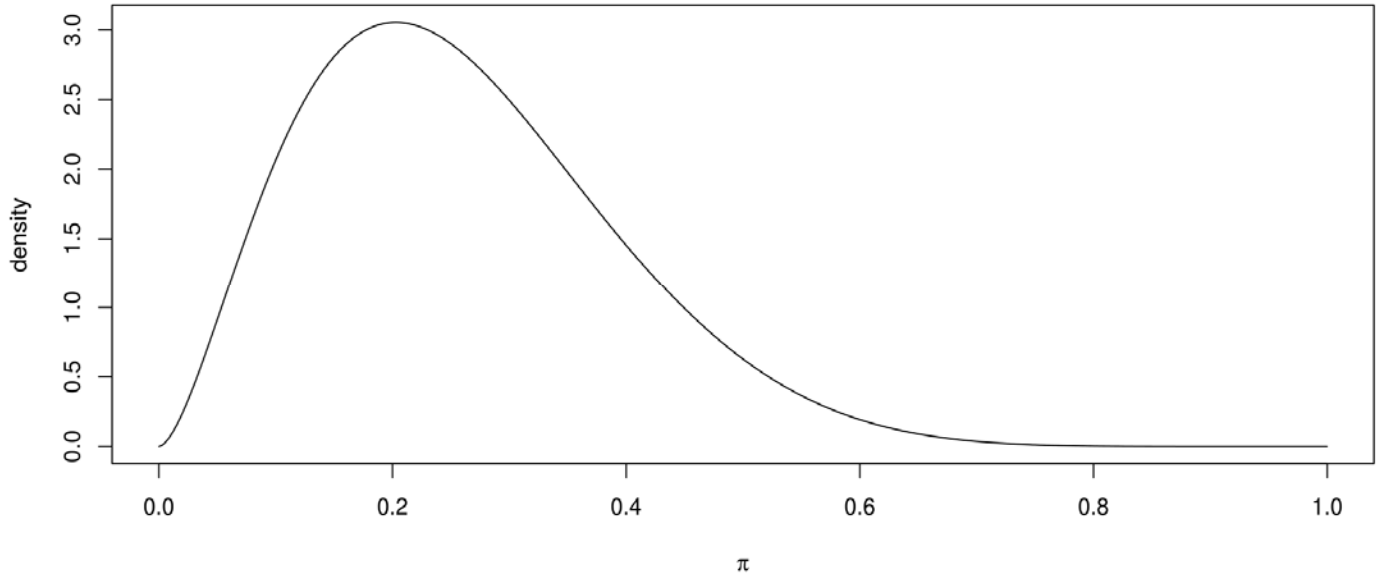


Figure 4. The distribution for prior clinical experience of an inexperienced veterinarian regarding prevalence of H1N1- influenza A virus in the United States swine herd (a horizontal axis is the prevalence with 29% most likely and standard deviation of 13%).

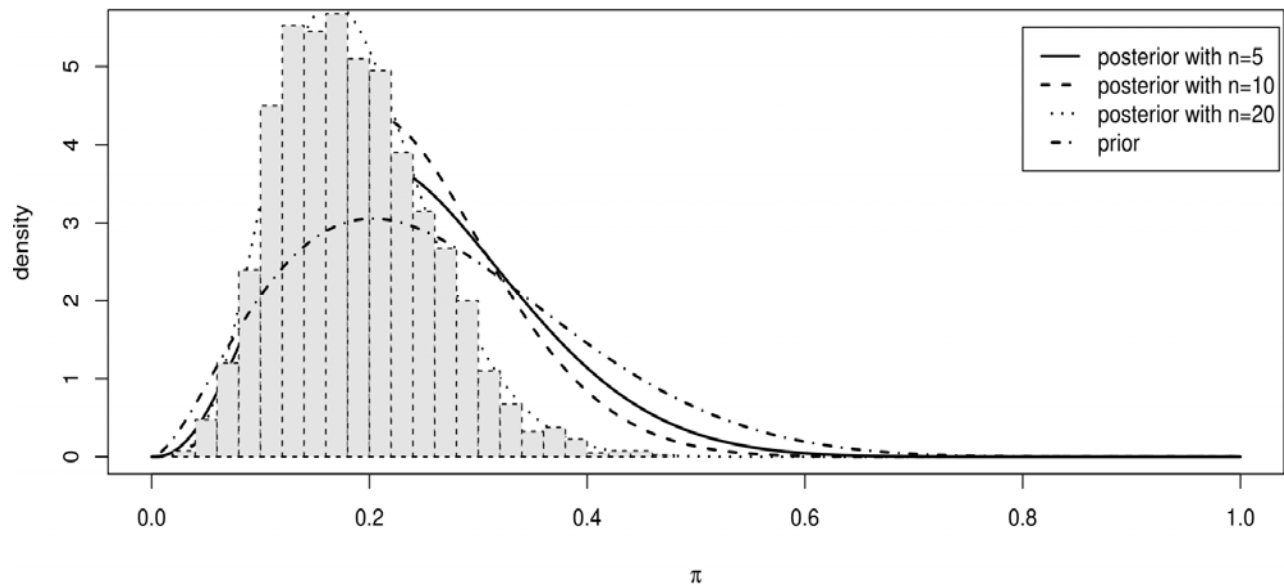


Figure 5. The represent of increasing strength of evidence as probabilistic graph using Beta-binomial model with 1, 2 and 3 positive samples out of 5, 10, 20 total samples, respectively.

precision (narrower credible interval) in the posterior clinical experience is the sum of precisions in the two sources of information (the strength of evidence and the prior clinical experience). The combined strength of these two sources of information lead to increasing precisions in understanding of evidence (Carlin and Louis, 2008). With more prior clinical experience, the veterinarian's decision regarding clinical intervention or treatment will

be more precise. Similarly, as the veterinarian finds stronger evidence, his/her decision regarding clinical intervention or treatment will also be more precise.

Based on numerical example (Figure 3), it is important to note that the inferences from deductive and inductive reasoning are not equal (Poole, 2001). The inference from deductive reasoning, " $p(x|\theta)$ ", had the probability of 0.30 that IAV infected pigs would be coughing as an

observed clinical sign. On the other hand, that from inductive reasoning, “ $p(\theta|x)$ ”, had the probability of 0.17 that coughing in pigs was caused by IAV infection. It is important to elucidate that performing statistical inference as deductive reasoning (frequentist) and as inductive reasoning (Bayesian) can end up with different conclusions. This is because the methods are answering different questions of making inference, and also both depict the opposite direction of causal models (Fienberg, 2006). However, if very strong evidence “ $p(x|\theta)$ ” has been found or theoretically when samples sizes is large (as $n \rightarrow \infty$) no matter what direction of a causal model is being made, both inductive and deductive inference will be identical (Geyer, 2012).

Example: Dog with pancytopenia

A young vaccinated dog is admitted to the small animal teaching hospital with a problem of pancytopenia, a decrease in the number of platelets, and red and white blood cells. The veterinarian investigates pancytopenia from signalment to identify a probable cause of pancytopenia. However, if the veterinarian uses deductive reasoning of making an inference, he has to use a number of tests to check each body system, which might cause pancytopenia. In contrast, if the veterinarian applies BA of making an inference, he/she will start from his/her prior clinical experience and then update that posterior clinical experience by accumulating new evidence (information) from history taking, physical examination and diagnostic results.

Starting from the prior clinical experience, a veterinarian would ask whether the dog has been showing diarrhea or vomiting. If the patient's history revealed no exposure to radiation, toxins or medications that could reduce the numbers of platelets, and red and white blood cells, from history taking and prior clinical experience, he/she would then update his/her posterior clinical experience (posterior distribution) using BA. The cause of pancytopenia may be infectious including parvovirus, canine distemper or ehrlichia infection. He/she would like to have stronger evidence (than from taking patient's history) to update his/her prior clinical knowledge of infectious diseases causing pancytopenia and also would like to coalesce evidence from the past concerning whether the patient has been showing diarrhea or vomiting. He/she continues to investigate more evidence using signalment, history and physical examination in order to increase the precision of the inference.

The broad category of infectious diseases is narrowed down to which one of the three infectious diseases would be a primary cause of pancytopenia with some certain probability. It is found that the patient has not had diarrhea or vomiting, the most likely cause of pancytopenia would be chronic ehrlichiosis with some

degree of certainty. To have stronger evidence, the patient's serum is tested using a specific diagnostic test-ImmunoComb® Canine Ehrlichia Antibody Test Kit (Biogal Galed Lab., Israel). If the diagnostic test was positive, following Bayesian reasoning, a feasible cause of pancytopenia of the young dog patient may be chronic ehrlichiosis with some certain probability relying on veterinarian's prior clinical experience of knowing *Ehrlichia canis* prevalence (Davies and Shell, 2002; Singla et al., 2011).

As a veterinary diagnostician, one prefers to make an inference of the serological positive result if such a result is truly positive and truly caused by a chronic *E. canis* infection. The true positive result is simply measured by the sensitivity of the ELISA kit. However, making inference that the serological positive result is truly caused by *E. canis* infection requires BA (3 points). In statistical terms, what is the probability that the serological positive test result would really be caused by *E. canis* infection? This is the mathematical way of incorporating the serological evidence accompanied with prior clinical experience concerning the previous prevalence of *E. canis*. One then updates the estimate of how likely is the serological positive test caused by *E. canis* infection (posterior clinical experience). This result is known as the predictive value of the test. Subsequently, the veterinarian updates the posterior clinical experience by making inference of how likely is pancytopenia caused by *E. canis* infection.

HETEROGENEITY OF PRIOR CLINICAL EXPERIENCE

Veterinarians' prior clinical experiences are heterogeneous. They range from being pessimistic to being enthusiastic (Higgins et al., 2014). Therefore, their clinical (posterior) expectations would be different. The strength of evidence needed concerning clinical expectations for them to agree with each other would also be different. Thus, two veterinarians that are different in experiences may provide a different decision for giving treatment options. The evidence will provide a factual basis for the decision, which will dictate the patient's care (Rosenberg and Donald, 1995).

When we consider the BA notation, BA has three terms in itself: prior clinical experience “ $p(\theta)$ ”, evidence “ $p(x)$ ” and strength of evidence “ $p(x|\theta)$ ”. If two veterinarians disagree about a treatment option, they are disagreeing based on one of these three terms. If a veterinarian uncovers the same evidence, for example, the positive serologic test of *E. canis*, an experienced veterinarian with strong belief in his/her prior clinical experience may think the result is a false positive because he has seen similar cases (based on his prior clinical experience). Given his prior clinical experience, stronger evidence is needed, “ $p(x|\theta)$ ”, to update his posterior clinical experience. For a veterinarian with little experience, serological evidence may be sufficient given the lack of

his/her prior clinical experience to update his posterior clinical experience. One, then, treats the patient with Doxycycline. No matter the result of the treatment to the dog patient (improving, stable or worsening pancytopenia), the veterinarian will learn from this experience. A Bayesian approach, however, is a mathematical way to learn from past experience and measure the strength of evidence given a prior clinical experience " $p(x|\theta)$ " (Ashby and Smith, 2000).

If experienced and inexperienced veterinarians understand BA, they can focus on the area of disagreement (the prior clinical experience or weak evidence) and resolve the disagreement quicker. If a veterinarian is not using BA for diagnosing the patient, the patient may be subject to additional medical tests and unnecessary procedures. For instance, the patient may be evaluated for drugs and toxins depressing bone-marrow activity, or tested for parvovirus or canine distemper viral infection (Davies and Shell, 2002).

Evidence

What kind of evidence is useful and where does the strength of evidence originate? Strength of evidence may come from the number of patients (sample size) since as the samples size increases (as numerically showed previously), the Bayesian point and interval estimates will be driven more by the observed data and less by the prior clinical experience (Dunson, 2001). However, a Bayesian approach does not require a large number of samples but sequential analysis (the number of bits of information from the same patient) (Berger, 2006). For example, the strength of evidence increases as veterinarians make inferences based on new evidence obtained from history taking, physical examination and then the diagnostic serological test result. A Bayesian approach is remarkable not only in that it tells us what is and is not good evidence, but it helps us to quantify how strong the evidence is. A Bayesian approach tells us how much veterinarians should update their clinical experience or how much they should change their expectation when new evidence becomes available.

A Bayesian approach distinguishes between weak evidence and strong evidence. If the posterior clinical experience is very different from the prior clinical experience, something has been learned, and if posterior clinical experience is the same as the prior clinical experience, strength of evidence (useful information content) is low. In many circumstances, a veterinarian finds very strong evidence.

Evidence that is sufficiently strong will permit a novice to make a discussion concerning clinical interventions or treatments with the confidence and precision as similar as an experienced veterinarian. Statistically speaking, this type of circumstance is called "a likelihood dominates a prior" (Carlin and Louis, 2008). Often, ones tend to believe results that support their preconceptions and

disbelieve contradicting results (Gelman, 2008). Veterinary clinical decisions need to be supported by evidence because the evidence lets veterinarians decide whether an intervention or treatment can be reliable (Rosenberg and Donald, 1995). Therefore, appraising evidence is crucial.

CONCLUSION

In this article, particular attention has been paid to examine the state of BA used for inductive reasoning in veterinary medical problems and to illustrate how veterinarians update states of knowledge, not focusing on a utility of BA in veterinary diagnostic test. A Bayesian approach is considered to be the natural framework of thinking in veterinary medicine. Pattern recognition and problem-based approach are based on this kind of thinking, although some veterinarians may not realize that they are using BA when making an inductive reasoning (inverse probability).

Animals are not able to speak and provide limited information to a veterinarian. The veterinarian has to gather information from signalment, history, physical examination and laboratory results. In making decisions to treat a particular disease, there are relevant quantities or outcomes the veterinarian has observed or recorded and other relevant quantities or outcomes the veterinarian has not yet observed or recorded, and all are therefore uncertain.

We have demonstrated that veterinarian's physical examinations and history taking are the way of gathering information incorporating the prior clinical experience out-flowing to posterior clinical experience to make a clinical decision. Also, we have emphasized that veterinarian, whether they know it or not, are always using BA to update their posterior clinical experience by starting from their prior clinical experience. Some veterinarians may have a different prior clinical knowledge based on their previous experience. However, as evidence strengthens, their posterior clinical experiences are updated to meet clinical agreement.

No matter whether we call this learning process of solving problems from the present to the past, in reality, the data is meaningless by itself without having gone through thought processes (statistical modeling, or reasoning) incorporating previously observed information (prior experience) to synthesize a conclusion (posterior clinical experience).

However, the conclusion could be changed if we have more information and evidence. Veterinarian's diagnoses are based on evidence, and the best diagnosis should also be based on evidence and previous experience. The more prior experience a veterinarian has, the faster the diagnosis is made. The stronger the evidence, the more precise the veterinarian's inference will be. Veterinary education needs a more formal recognition and utilization of BA in the veterinary curriculum.

Conflict of interest

The authors declare they have no conflict of interest.

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Full Length Research Paper

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

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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%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 coinfecting 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.,

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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
	Present	Absent		Positive	Negative	
NS RRT-PCR						
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 = $(1 - OR) \times 100$ (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* v3.0¹ 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 OF and NS RRT-PCR were between 0.77 and 0.92, and between 0.75 and 0.90, respectively. The prior Sp of 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 “rjags” 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 , ²CrI 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

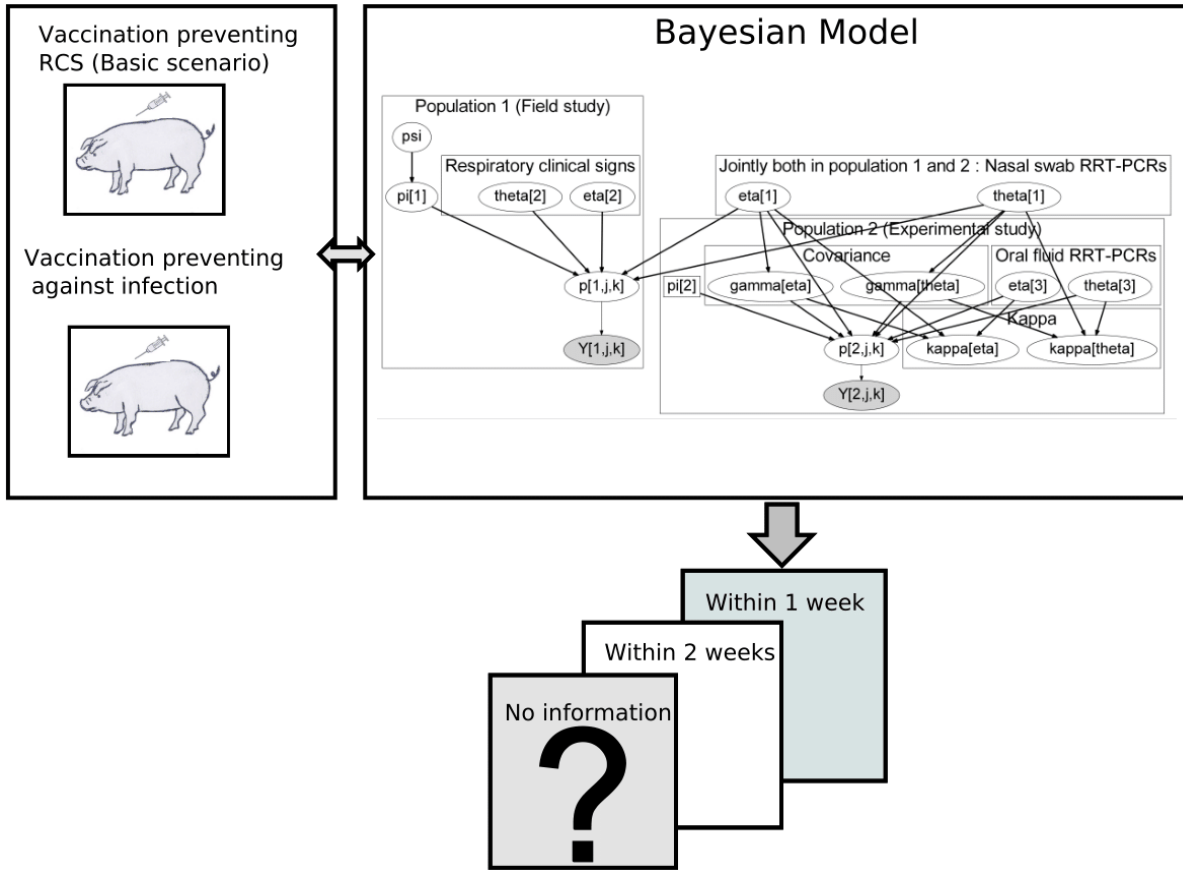


Figure 1. A conceptual model representing scenarios of (a) a vaccination that prevents RCS (Basic scenario) or (b) a vaccination that prevents against infection. For each scenario, three priors were implemented with regard of prior information of time-of-sampling within 1 or 2 weeks, or no information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

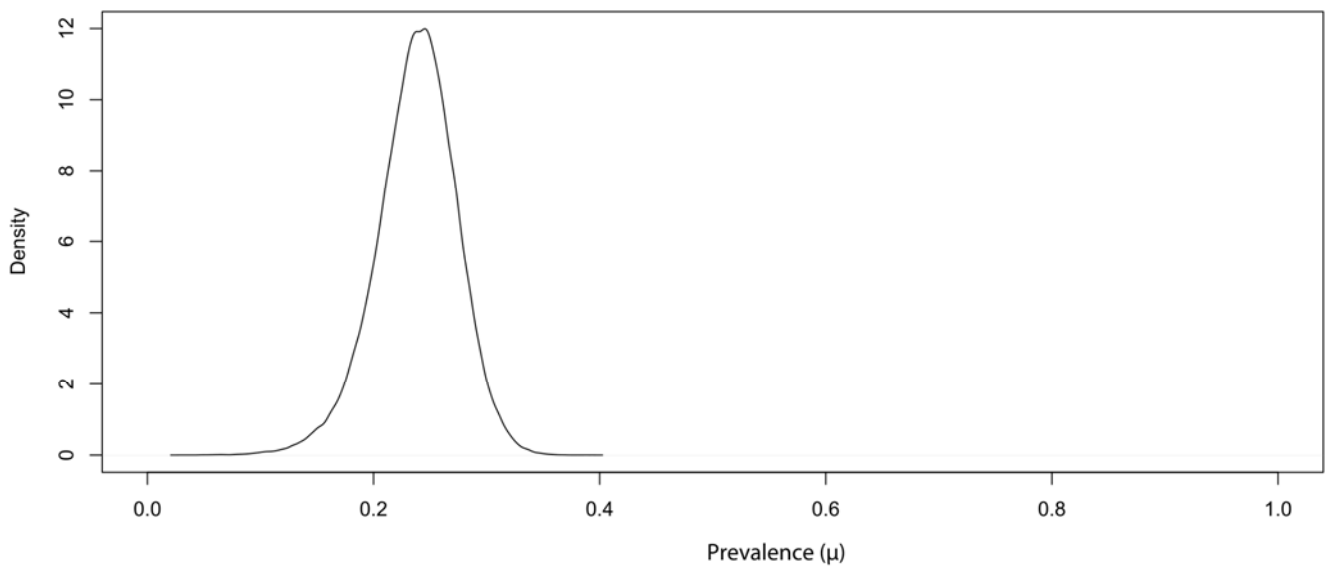


Figure 2. A probability distribution represents herd prevalence of IAV infection in the Midwestern US growing pig populations, the x-axis representing herd-level prevalence. The probability distribution was generated from three MCMC chains with 250,000 with 5000 discarded each chain.

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

Assumptions		DIC ¹	pD	Deviance
Conditional independent of RCS and NS RRT-PCR	Accuracy of NS RRT-PCR was assumed to be equal across two populations			
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

¹DIC= 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>i</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}	$\pi_2 [\eta_1 \eta_3 + \gamma_{\eta}] + (1 - \pi_2)[(1 - \theta_1)(1 - \theta_3) + \gamma_{\theta}]$
	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 1 and 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), ³Conditional 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 field 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 scenario³ 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 , ²Calculated from tests between OF and NS RRT-PCR tests in population 2 with conditionally dependent model, ³Model was run under the scenario that vaccination protects RCS but does not protect against infection, ³The prior prevalence distribution of the experimental study was followed $\pi_e \sim \text{Beta}(24.75, 2.04)$.

Table 6. Description of the second scenario³ 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 , ²Calculated from tests between OF and NS RRT-PCR tests in population 2 with conditionally dependent model, ³Model was run under the scenario that vaccination protects against an infection for IAV, ³The prior prevalence distribution of the experimental study was followed $\pi_e \sim \text{Beta}(100.9, 496.4)$.

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 through 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.

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Appendix A

Bayesian model, namely $y_{ijk} \sim \text{multinomial}(n_i, (p_{i11}, p_{i12}, p_{i21}, p_{i22}))$, 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 described 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: $k_\eta = \frac{2\gamma_\eta}{\eta_1(1-\eta_2)+\eta_2(1-\eta_1)}$, $k_\theta = \frac{2\gamma_\theta}{\theta_1(1-\theta_2)+\theta_2(1-\theta_1)}$ (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 (η) and Sp (θ) 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 $\pi_i[1]$ is the prior prevalence of infection in the field study population. The $\pi_i[2]$ is the prior prevalence of infection in the experimental study population. The η_i and θ_i 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. 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:

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

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.



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