Bayesian Modeling of Within-Herd Transmission Dynamics of Swine Influenza Virus

A DISSERTATION

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Dedication

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Abstract

Bayesian modeling of Within-Herd Transmission Dynamics of Swine Influenza virus

Influenza A virus (IAV), also known as swine influenza virus, commonly circulates in swine populations. IAV infection is a concern to swine producers, veterinarians and the general public, and is considered one of the top three respiratory diseases in terms of frequency of appearance in North American and the cause of an economically important respiratory disease in growing pig populations. On-farm assessment of health on an individual level for IAV infection involves the use of respiratory clinical signs (RCS) and behavioral observations by making inference from RCS to its causes using inductive reasoning as required for a Bayesian approach (BA). Therefore, the aims of this dissertation were to create Bayesian epidemiologic models using inductive reasoning with inverse probability, and to describe and better understand the within-herd transmission of IAV in wean-to-finish pig populations. A Bayesian approach is commonly used in veterinary medicine as it has been part of inductive reasoning regarding interventions, treatments and diagnoses. When veterinarians are managing patients or on-farm assessment of health, veterinarians start with their inferences from history and clinical signs to an underlying cause using inductive reasoning. The diagnostic test accuracies of RCS were 0.38 (95% Credible interval (CrI): 0.28-0.48 for Se) and 0.66 (95%CrI: 0.61-0.71 for Sp). The true IAV prevalence was estimated to be 0.24 (95%CrI: 0.16-0.30) and vaccination reduced such level of prevalence. By accounting for imperfect diagnostic test of RCS, the transmission rate was 1.40 day^{-1} (95% CrI: 0.42-5.52) and the reproductive number was 4.19 (95%CrI: 1.98-

26.29). Waning rate of maternal derive antibodies (MDA) rate for IAV H1N1 was estimated to be 0.016 day^{-1} (95%CI: 0.013, 0.019) and time to lack of MDA maternal immunity was 64.09 days (95%CI 60.77- 77.40). An epidemic can occur at any point in time during a wean-to-finish period with more than one epidemic peak with low MDA population and the sufficiency of initially infected pigs. IAV transmissibility was elucidated as strongly periodic (p-value < 0.001) with peak timing in mid-June. The absolute IAV intensity was 0.18. The relative IAV intensity was 2.41, implying that IAV disease intensity at the periodic peak was 2.41 times higher compared to that at the periodic nadir.

In conclusion, for a swine herd health management perspective, the use of RCS is able to potentially be used as on-farm assessment and measured for IAV transmissibility by inductive reasoning. Heterogeneity of MDA in wean-to-finish pig populations plays a crucial role in enhancing IAV transmission and waning MDA has interfered with vaccination to create more subclinical infections. Vaccination may be able to reduce the true IAV prevalence and has broader implications for the control and perhaps eradication of IAV. IAV transmissibility was elucidated as periodic. Better understanding waning of MDA, periodic IAV transmissibility, persistence and dynamics should result in a better design of the optimal control strategies and periodic IAV vaccination in growing pig populations.

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List of Abbreviations

β: Transmission rate

γ: Recovery rate

ψ: The rate of waning immunity

BA: Bayesian Approach

CI: Confidence Interval

CrI: Credible Interval

clog-log: Complementary log-log link function

IAV: Influenza A virus

MDA: Maternally-Derived Antibody

MSIR: Maternally immune-Susceptible-Infectious-Recovered Epidemic Model

NS: Nasal Swabs

OF: Oral Fluid

RCS: Respiratory Clinical Signs

R: The R statistical software package

R: The reproductive number

R_E: The effective reproductive number

R_C: The control reproductive number

R_0: The basic reproductive number

RRT-PCR: Reverse Real-Time Polymerase Chain Reaction

Se: Sensitivity

SIR: Susceptible-Infectious-Recovered Epidemic Model

Sp: Specificity

General introductions

The aims of the dissertation

The aims of the PhD dissertation were to create Bayesian epidemiologic models using inductive reasoning with inverse probability, and to describe and better understand the within-herd transmission of swine influenza virus, onward defined as influenza A virus (IAV), in wean-to-finish pig populations in order to make decisions for controlling, intervening and perhaps eliminating IAV from swine herds based on various conditions of the production system. This is done by the following 5 objectives:

Objective 1: To review the mechanistic of using Bayesians approach as inductive reasoning in veterinary medicine.

Objective 2: To investigate the test accuracy of using respiratory clinical sign observation.

Estimates of this aim are used for Objective 3.

Objective 3: To estimate transmission rate and the reproductive number accounting for misclassification of diseases by using respiratory clinical sign observation as a measure of disease transmission.

Estimates of this aim are used for Objective 4.

Objective 4: To develop an epidemiological model that can describe the most likely events of being epidemic affected by waning maternal immunity using stochastic process.

Objective 5: To develop a seasonal modeling and investigate periodic IAV transmission dynamics.

As the main focus of the dissertation has been on IAV modeling with an application of Bayesian approach, it has been prioritized to not collect specific data for the purpose of Objectives 1. The empirical analyses of the dissertation (Objectives 2 and 5) are based on existing data sets. To achieve Objectives 3 and 4, observational and experimental settings are utilized. However, it has been chosen to obtain expert opinions for the purpose of the modeling part (Objective 2).

Outline of the dissertation

The dissertation contains 7 chapters, including three main sections. Back ground information includes Chapter 1 and 2. Applications and methods include Chapter 3, 4, 5 and 6. Lastly, conclusions are in Chapter 7 **(Figure 1)**.

Chapter 1 provides an overview of the principles and concepts of IAV transmission and transmission models. Bayesian approach and concepts that uses in veterinary medicine as similar as inductive reasoning with inverse probability are described in Chapter 2. Chapter 3 presents the general theory and an application behind Bayesian estimation of diagnostic test accuracy in an absence of the gold standard, in particular for respiratory clinical signs (RCS). As Bayesian approach used as inductive reasoning and it infers causal direction from RCS exhibition to causes of RCS, the use of RCS observation as the measurement of clinical disease using Susceptible-Infectious-Recovered (SIR) epidemic model for IAV transmission within a growing pig population is given in Chapter 4. Chapter 5 presents stochastic process as modeled as viral transmission dynamics within a herd in conjunction with waning maternal immunity using the Maternally immune-Susceptible-Infectious-Recovered (MSIR) epidemic model

to describe the spread of the clinical disease. The modeling of a periodic IAV transmission in growing pig populations using harmonic regression and Fourier spectral analysis is presented in Chapter 6. Chapter 7 discusses and links results in order to provide general conclusions, and finally, further directions related to the dissertation.

The dissertation chapters are the following titles:

Chapter 1: Swine influenza: inverse learning from transmission modeling

Chapter 2: Bayesian approach for inductive reasoning to clinical veterinary medicine: The math of experience

Chapter 3: Bayesian estimation to test accuracy for influenza A infection via respiratory clinical signs in the absence of a gold standard

Chapter 4: Bayesian estimation of the reproductive number for influenza A virus infection accounted for imperfect diagnostic test of respiratory clinical sign observation in growing pig populations

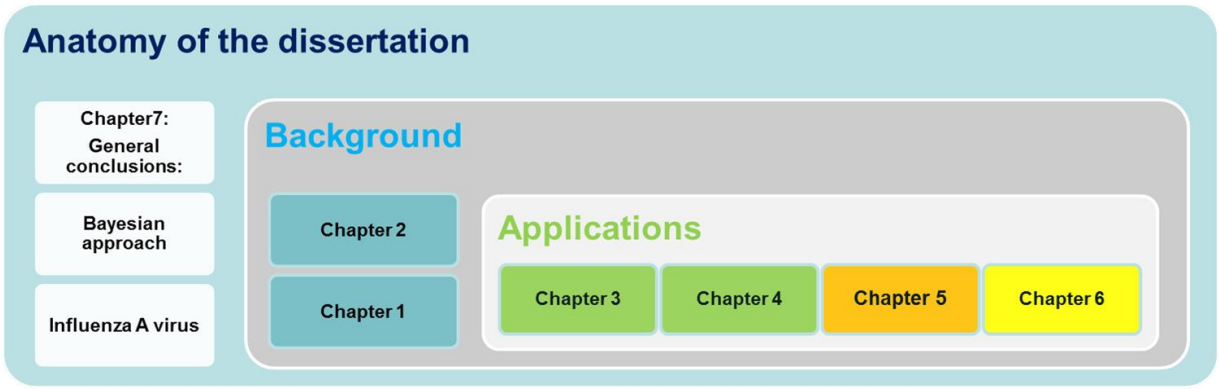
Chapter 5: Stochastic modeling effects of waning maternal immunity in growing pig population

Chapter 6: Periodic influenza A virus transmission in Midwestern United States growing pig populations: Harmonic regression and Fourier spectral analysis study

Chapter 7: General discussion and conclusions

Figure

Figure 0-1: Representation of outline and anatomy of the dissertation



Chapter 1 : Swine influenza: inverse learning from transmission modeling

Introduction

Influenza viruses belong to the family *Orthomyxoviridae*, which is composed of five genera: influenza A, B, C, Togovirus, and Isavirus (Vincent et al., 2008). Influenza B and C viruses are primarily human pathogens. Togovirus is a tick-born virus that occasionally infects mammals. Isavirus causes salmon infectious anemia (Pattison, 2008). Influenza A viruses (IAV) are zoonotic pathogens, cause economic losses to the swine industry and are the focus of this review.

Influenza A is an enveloped segmented, negative sense single-stranded RNA virus with a total of eight segmented genes encoding for 10 proteins. Segment 1 encodes Polymerase B2 (PB2); the segment 2 encodes Polymerase B1 (PB1); the segment 3 encodes Polymerase A (PA); the segment 4 encodes Hemagglutinin (HA); the segment 5 encodes Nucleoprotein (NP); the segment 6 encodes Neuraminidase (NA); the segment 7 encodes Matrix 1 (M1) and Matrix 2 (M2); and finally segment 8 encodes Non-structural 1 (NS1) and Non-structural 2 (NS2) (Webster et al., 1992). Viruses are classified into subtypes by the antigenic properties of intra membrane glycoproteins: HA and NA. HA and NA are highly variable while internal proteins, nucleoprotein (NP) and matrix protein (M), are more conserved. The HA is composed of 18 antigenically different (H1-H18) and NA is of 11 different (N1-N11), with H17N10 and H18N11 recently identified in bats (Worobey et al., 2014; Wu et al., 2014). Viruses are classified by species from which the isolate originate, location, arbitrary numerical designation, year and subtype, e.g., A/Swine/Iowa/15/1930 H1N1 (Palese and Shaw, 2007).

Influenza A viruses in swine populations

Influenza A viruses (IAV), known as swine influenza viruses, are commonly circulating in swine population. Evidence of IAV and its subtypes has been found endemically circulating among swine populations throughout the year and among many countries around the world (Romagosa et al., 2011; Torremorell et al., 2012; Allerson et al., 2013b). Most IAV prevalence studies have been performed via a detection of antibodies against specific subtypes of the virus.

In the United States of America (USA), three mains of IAV subtypes are consistently circulating in the swine populations including H1N1, H1N2 and H3N2 (Ma et al., 2007; Vincent et al., 2008; Corzo et al., 2013a). A recent seroprevalence study tested HA antibodies from 2,375 pigs and showed that 27.7% were positive for classical swine H1 influenza exposure (Olsen et al., 2002). A second study tested 111,418 pig sera using hemagglutinin-inhibition (HI) and found that 22.8% had antibodies to H1N1 or H3N2. Of those positive samples, 66% were exposed to H1N1, and the remaining to H3N2 (Choi et al., 2002b). Recently, an active surveillance study on 16,170 nasal swabs tested for viruses by RRT-PCR from the Midwestern USA has reported the prevalence of H1N1, H1N2 and H3N2, to be 18.0%, 16.0% and 7.6%, respectively (Corzo et al., 2013a). Feral pigs are also susceptible to IAV. A multi-state study on 875 feral swine sampled from six states in the USA found that IAV seroprevalence was 1%, 5% and 14.4% in Mississippi, California and Texas respectively. All the samples tested positive for H3N2 while in Oklahoma, Florida and Missouri, no IAV exposure was detected (Hall et al., 2008). Another study investigated the prevalence of viruses in feral swine in North and South Carolina around regions of dense commercial pig farms. In North Carolina,

IAV seroprevalence was 90.7%. Among its subtypes, human H1N1 (73%), rH1N1 (7%), H1N1 (14%) and H3N2 (47%) were detected. In South Carolina, seroprevalence against IAV was not detected (Corn et al., 2009). In Hawaii, 19% of the feral swine samples were positive to IAV (Stephenson et al., 2015).

In Canada, prevalence studies in the Ontario province uncovered that 87.7% of sows and 47.2% of finishing pigs were positive to H1N1 (Poljak et al., 2008a). A similar study tested in only finisher pigs found a seroprevalence of 13.4% and 2.7% for H1N1 and H3N2, respectively. In the following year, the prevalence for H1N1 and for H3N2 increased to 14.9% and to 25.9% (Poljak et al., 2008b).

In the Netherlands, prevalence for H1N1was 30-44% and for H3N3 was 68% (Masurel et al., 1983). In Great Britain, seroprevalence for classical swine H1N1 virus (26%) and human H3N2 virus (39%) was estimated (Brown et al., 1995). A Europe-wide study, including Belgium, the Czech Republic, France, Germany, Italy, Ireland, Poland and Spain, tested a total of 4190 sow sera from 651 farms and concluded that all seven countries were seropositive to H1N1 subtype and the most widespread was followed by H1N2 and H3N2 subtypes (Van Reeth et al., 2008). In Spain, a herd-level seroprevalence study tested 98 commercial farms and showed that 92.9%, 64.3% and 92.9% tested positive to H1N1, H1N2 and H3N2 (Simon-Grifé et al., 2011). Besides, European feral pigs carry antibodies to IAV as similarly observed in the USA. For example in German, a study conducted found that 5.2% of wild pigs were positive antibodies to both H1N1 and H3N2 (Kaden et al., 2008).

In China, a meta- analysis of seroprevalence over a 10-year period (1999–2009) estimated that the seroprevalence was 31.1% and 28.6% for H1 and H3 respectively (Liu

et al., 2011). Another prevalence study conducted in Fujian province found antibodies against H1, H3 subtypes (Song et al., 2010).

Of 742 finishing pig samples from 53 different Korean farms tested, H1 and H3 exposures were detected at 51.2% and 43.7%, correspondingly (Jung et al., 2007). A second study in those regions reported seropositivity against H1N1, H1N2 and H3N2 at 45.5, 33.3 and 5%, respectively (Jung and Song, 2007). A different study on 6,418 growing-finishing pig sera detected 46.1% seropositivity to IAV. Among these, it was estimated that 41.5% of the samples had antibodies against H1, whereas only 3.7% had antibodies against H3. However, 0.9% of the samples tested positive for both subtypes (Pascua et al., 2008).

In Argentina, a study on 13 farrow-to-finish farms, reported detecting antibodies at rates of 89%, 73%, and 62% against H1N1, H3N2 and both subtypes, respectively (Pineyro et al., 2009).

Clinical signs of influenza A virus infection

Typical swine influenza outbreaks are characterized by respiratory clinical signs (RCS) such as dyspnea, rhinorrhea, nasal discharge, sneezing and coughing, and systematic signs such as a rapid onset with high fever, anorexia, lethargy, ocular discharge, and sometimes, conjunctivitis (Olsen et al., 2000; Van Reeth, 2007; Reeth et al., 2012). Influenza morbidity can reach up to 100%; however, mortality is generally low and recovery occurs within 7-10 days. Due to high fever and anorexia, swine tend to lose bodyweight affecting in elevating feed conversion ratio (FCR), and average daily weight gains (ADWG), and longer time to reach the market bodyweight (Er et al., 2014). Some

studies have suggested that low levels of exposure to viruses might prelude clinical signs (Van Reeth et al., 1999; Van Reeth, 2000; Van Reeth, 2007). Subclinical IAV infections are very common as reported that infection with one or more IAV subtypes can be detected in pigs without having RCS (Allerson et al., 2013a). Usually, IAV infections are frequently seen in only 20 to 30 % of pigs in a herd (Brown, 2000). Recent study from a field setting revealed that 34.6% of pigs in a population exhibited clinical signs. Among those pigs, 22.9% were positive to IAV. For no clinical signs exhibiting pigs, and pigs that did not exhibited clinical signs, 20.9% were positive to IAV (Corzo et al., 2013a). Using a cough-sneeze scoring system as a measurement of disease severity caused by influenza infection has been developed based upon clinical signs of respiratory diseases (**Table 1-1)**(Detmer et al., 2013). However, the use of RCS as a measurement of disease transmission may be less accurate in vaccinated herds as sick pigs might endure illness, leading to "hidden" respiratory signs (Homwong et al., 2015b). The lack of clinical signs could be resulted in the presence of maternal antibodies via colostrum, vaccination or previous exposure (Corzo et al., 2013a).

Clinical signs infected with IAV may be severe if co-infection occurs. The disease of the co-infection is called "Porcine respiratory disease complex (PRDC)." Co-infection between IAV and porcine respiratory and reproductive syndrome virus (PRRSv) resulted in fever and more exacerbated respiratory signs (Van Reeth et al., 1996). Experimental infection between IAV and *Mycoplasma hyopneumoniae s*howed that pigs infected with both agents coughed significantly more than pigs infected with only one agent (Thacker et al., 2001).

Pigs infected with the H1N1-2009 pandemic strain viruses showed relatively lower reproductive performance, agalactia, diarrhea, edematous eyelids, and conjunctivitis (Brown et al., 1995; Moreno et al., 2010; Sreta et al., 2010). Pigs infected with those viruses at younger ages showed more severe effects on FCR and ADWG than those infected at older ages (Er et al., 2014). In addition, such strain viral infection in adult females results in lower reproductive performance (Ma et al., 2010).

Economic impact caused by influenza A virus infection

Influenza infection is among the top three respiratory infection in nursery-finishing pigs, and among the topmost two infection in breeding herds, leading to productivity losses (Holtkamp et al., 2007). Co-infection with other respiratory pathogens can aggravate PRDC (Choi et al., 2002b; Vincent et al., 2008; Deblanc et al., 2012; Fablet et al., 2012; Rose et al., 2013). The estimated cost of a disease for IAV infection in market pigs ranges from $3.86-10.31/head, depending on whether or not a co-infection exists (Donovan, 2008; Dykhuis Haden et al., 2012). Furthermore, infected growing pigs add two weeks more to the time that it takes finishing pigs to reach a market weight, thereby influencing profitability (Olsen et al., 2000; Miller et al., 2001). Clinical outbreaks of an sow abortion have also been associated with H3N2 IAV infection (Olsen et al., 2000; Choi et al., 2002b).

Consumers fear of pandemic 2009 H1N1 transmission via pork consumption also contributed to economic loss as a result of a sharp drop in demand for domestic pork (Johnson, 2009).n international trade banned on pork and pork products from the USA during such year and also contributed to major losses (Johnson, 2009). Within a four-

month period, the USA swine markets lost about $200 million arose from the 2009 pandemic viruses (Attavanich et al., 2011).

Misclassification of clinical signs and diagnostic test results

Since RCS are non-specific to a respiratory disease pathogen, clinical diagnoses may not sufficiently be accurate. However, a clinical representation can aid in choosing an appropriate set of diagnostic tests to confirm a respiratory disease pathogen. A diagnosis based on an imperfect test such as clinical signs can lead to "misclassification of a disease outcome." Misclassification can be divided into two categories, non-differential and differential misclassification.

One commonly used Real-time reverse transcription polymerase chain reaction (RRT-PCR) for the detection of influenza A virus in mammals targets the conserved matrix gene (M gene) and RRT-PCR can be performed within hours and at a relatively lower cost than viral isolation (Choi et al., 2002a; Richt et al., 2004). Additionally, RRT-PCR has been developed to HA and NA genes on the same samples. However, the misclassification of the disease outcome using RRT-PCR has raised several questions such as; dose a cutoff value classify herd status incorrectly? Could a cutoff value be moved to minimize the misclassification rate and maximize the values of test sensitivity (Se) and specificity (Sp)?

In epidemiologic studies, it is important to minimize herd status misclassification (Christensen and Gardner, 2000). The cutoff value influences Se and Sp. **Figure 1-1** represents hypothetical distributions of diagnostic positive and negative results being

tested and shows an overlapping area, suggesting that an optimal cutoff value (τ) that minimizes the misclassification rate can be selected.

Ct values < 35 have been used to classify infected samples as test positive while Ct values between 35 and 40 as low positive or suspect and Ct > 40 as test negative (Corzo et al., 2013b). Another interpretation of Ct cutoff value is that suspect samples are classified as test negative (i.e. Ct<35 positive and Ct≥ 35 negative) (Romagosa et al., 2012). Such RRT-PCR cutoff provides an accuracy of 100% Sp but lower Se (assuming the first cutoff value is true). On the other hand, lowering the test positive classification to a Ct of 29.66 leads an accuracy of 100% Se **(Figure 1-2)**. However, a decision that all suspect samples be considered uninfected would provide 100% Se while compromising Sp (test results contained true negative and false positive). The decision that all suspect samples be considered positive would reduce Se. It would remain at 100% Sp. Thus, based on arbitrary cutoff, accuracy on the RRT-PCR test will vary. The current analysis used a Bayesian two-component mixture model for the submitted diagnostic IAV data and found that the optimal Ct cutoff to minimize misclassification was at 35.6 **(Figure 1-2)**. This optimal cutoff maximizes the discriminatory power of RRT-PCR in an absence of a gold standard. Recent studies on *Mycobacterium avium* and *Staphylococcus aureus* have also used a similar Bayesian approach to minimize a misclassification rate of quantitative RT-PCR test results (Jafarzadeh et al., 2010; Mahmmod et al., 2013).

Transmission model

Ecological transmission models

Transmission is defined as the process by which the virus is shed from one animal and infects the next, causing immunological responses (Van Reeth, 2007). The main routes of IAV transmission in pigs have been documented as nose-to-nose contact, fomite and aerosol transmission (Brown, 2000; Tellier, 2009; Allerson et al., 2013c). Interspecies transmission is generally determined by the viral gene constellations. IAV has eight separate gene segments. The segmented genes allow IAV from different animal species to mix and create a new virus. Three IAV transmission models may be significant to IAV evolution such as mixing-vessel model, cross-species model, and variant-viruses model (H3N2v) (Nelson and Vincent, 2015).

1. "Mixing-vessel" transmission model

Due to characteristics of swine receptors being compatible to both avian and human IAV, swine is hypothesized to serve as a "mixing vessel" resulting in a novel reasserted progeny and consequently, producing IAV subtypes that can be potentially transmitted to humans (Webster et al., 1995; Olsen et al., 2000; Myers et al., 2006; Ma et al., 2007; Vincent et al., 2008). This ecological model has been historically suggested to result in transmission of virus from pigs to humans producing **pandemic viruses**. Recent literature identified that humans-to-swine transmission occurs more frequently than swine-to-human transmission. Theoretically, humans could also be considered a "mixing-vessel." Thus, humans also play a central role in seeding swine IAV globally (Nelson and Vincent, 2015).

2. Cross-species transmission model

IAV can transmit across different host species, including avian, swine, humans, equine, and canine (Myers et al., 2006; Song et al., 2008; Bowman et al., 2014). The extent of genetic diversity among IAV can provide such an opportunity for transmission**.** Avian species are considered "the viral donor" to other species. Avian-to-human transmission accounts, at least, for H1N1, H2N2, H3N2, H2N3 and H7N9. Avian-to-equine transmission is evidenced by H3N8 and H7N7 outbreaks. Avian-to-canine transmission of H3N2 and avian-to-swine movement of H1N1 and H3N2 also documented the cross-species transmission. However, such transmission rarely occurs (Nelson and Vincent, 2015). Cross-transmission among mammalian species can also occur. For instance, that of equine-to-canine spread of H3N8 and humans-to-swine H1N1, H1N2 and H3N2 suggested this possibility. This cross-species transmission model results in circulating **endemic viruses** in a new host (Nelson and Vincent, 2015). Until 1998, H1N1 circulated widely in the USA swine herds. However, in 1998, H3N2 viruses from humans were introduced into the swine population and caused widespread disease among pigs. The H1N2 was generated from reassortant viruses contain genes derived from swine-H1 and human-N2 (Vincent et al., 2008). Nowadays, H1N1, H1N2 and H3N2 can be detected in USA swine herds (Ma et al., 2007; Vincent et al., 2008; Corzo et al., 2013a). The three subtypes in swine are reverse-transmitted to humans, namely H1N1v, H1N2v and H3N2v. Evidence of cross-transmission of H2N3 between humans-to-swine was described in 2007 elsewhere (Ma et al., 2007). In 2013 in China, the first fatal human case infected with the novel influenza A/H7N9 from avian species was reported (Chowell et al., 2013).

3. Variant-viruses transmission model (H3N2v)

Most IAV subtypes circulating among swine populations do not infect humans. However, sporadic human infections with swine influenza viruses have occurred. When human infection with swine influenza viruses that are known to be genetically similar to viruses circulating in swine occurs, these viruses are called “variant viruses," denoted by the letter “v” at the end of the virus subtype designation.

H1N1pdm transmission from humans to swine generates H1N1pdm infected in swine populations. Reassortment between H1N1pdm and co-circulating triple-reassortant H3N2 viruses in swine, generated a novel reasserting H3N2v with seven triple-reassortant H3N2 virus segments in which Matrix (M) segment of H3N2v is of H1N1pdm origin (Bowman et al., 2014). Transmission of H3N2v from swine to human populations were first detected in humans during 2012, resulting in one adult fatality and more than 300 cases with symptoms similar to that of seasonal influenza (Bowman et al., 2014; Nelson and Vincent, 2015). In some cases, H3N2v also has spread between human populations but there has been limited evidence for viral transmission from person-to-person (Bowman et al., 2014).

Mathematical transmission models

Epidemiology involving in modeling of infectious diseases has grown more since early twentieth centuries. There are two significant subfields of such area that have been coined and continuously developed such as computational epidemiology and mathematical epidemiology (Brauer, 2009; Gorder, 2010; Marathe and Vullikanti, 2013). The main focus of computational epidemiology is to deal with information and

computation in its most general setting of infectious diseases using a programming language (i.e. R, Python, Matlab, or C++ languages, etc.). This discipline is distinct from computational biology and not the focus of this review. The principal focus of mathematical epidemiology is on the understanding and computation of the reproductive number ($\mathcal{R}$) in models of various kinds of homogenous or heterogeneous populations (Brauer, 2009).

Over several years, an effort has intensified to develop mathematically-epidemiological models to enable timely response to pandemics of emerging and re-emerging diseases. Epidemiologic models are mainly assembled by statistical, mathematical and machine-leaning based models (Siettos and Russo, 2013). Developing mathematically-epidemiological models of infectious diseases is useful in the study of complex phenomena such as the population dynamics of infectious diseases and has been exploited for IAV to better understand its transmission dynamic patterns, allow a rapid assessment, and forecast capability of combating epidemics (De Jong, 1995; Coburn et al., 2009; Siettos and Russo, 2013). A simple compartment model includes either Susceptible-Infectious (SI) or Susceptible-Infectious-Recovered (SIR) models. The models are conceptualized and constructed to simulate situations of IAV transmission.

Epidemiological transmission process of infectious diseases can be mathematically described by a simple SIR model. Briefly, an infectious individual (I) was introduced into a closed -susceptible population (S); then a susceptible individual becomes infected with transmission rate β. Next, infectious individual (I) is recovered (R) with recovery rate γ. At the end of epidemics, the equilibrium point is evaluated for S, I, and R (Coburn et al., 2009; Brauer et al., 2010; Brauer and Castillo-Chávez, 2013). In

addition, the SIR model can be extended by adding other compartments such as; latent (L), symptomatic (S), a symptomatic (A), or Death (D) compartments. Also, intervention compartments may be added into the SIR model such as vaccine (V), treatment (T) or quarantine (Q) compartments, depending on an objective of researchers (Medley and Nokes; Scherer and McLean, 2002; Gandon et al., 2003; Park et al., 2004; Coburn et al., 2009; Lu et al., 2009; Brauer et al., 2010; Brauer and Castillo-Chávez, 2013). The example of SIR model with the system of ordinary differential equations (ODEs) was expressed as:

$$\frac{dS}{dt} = -\text{R}\gamma SI$$

$$\frac{dI}{dt} = \text{R}\gamma SI - \gamma I$$

$$\frac{dR}{dt} = \gamma I$$

where S, I, R and Ŗ are the number of susceptible, infectious and recovered individuals, and the reproductive number, respectively. Model descriptions and derivatives are described elsewhere (Allen et al., 2008; Keeling and Rohani, 2008).

Why is transmission modeling important?

A study of IAV transmission modeling is consequential to better understand the speed at which IAV is transmitted, to deal with epidemic, emerging and re-emerging, to rapidly control, and perhaps to eradicate IAV infection in swine population, including implementation of a vaccination protocol (Mills et al., 2004). A fundamental measure of transmissibility of infectious diseases is the reproductive number (Ŗ) (Mills et al., 2004), which is the most important epidemiological parameter for assessing the disease

transmissibility (Woolhouse et al., 1997; Mills et al., 2004; Chowell et al., 2013). The term “reproductive” may be in place of “reproduction," and the term “number” may be in place of “rate or ratio”(Allen et al., 2008). $\mathcal{R}$ has been other derivatives such as the basic reproductive number ($\mathcal{R}_0$), the effective reproductive number ($\mathcal{R}_E$) and the control reproductive number ($\mathcal{R}_C$). Differences are based on the use and the definitions. $\mathcal{R}_0$ is quantitatively defined as the number of secondary cases produced by a primary infectious case in the absence of any constraints on the spread of infection during the course of epidemic in a completely susceptible population (Woolhouse et al., 1997; Mills et al., 2004; Chowell et al., 2006a; Brauer, 2009; Brauer et al., 2010; Brauer and Castillo-Chávez, 2013). $\mathcal{R}_E$ is defined as the number of secondary cases produced by a primary infectious case in a partially immune population. $\mathcal{R}_C$ is defined as the number of secondary infectious cases with a control measure, both vaccinated and unvaccinated, generated by a primary infectious case both in completely susceptible (unvaccinated) and partially immune (vaccinated) population (Allen et al., 2008). In addition, another derivative of $\mathcal{R}$ has been described by a Generalized reproductive number (G_0), defined as $\mathcal{R}$ that explicitly accounting for spatial distributions of interested populations and disease transmission (Gatto et al., 2012).

$\mathcal{R}$ is relied upon the threshold principle and is a function of time spent in the infection state. $\mathcal{R}$ magnitude determines the intensity of measure required to halt the transmission (Mills et al., 2004). An important threshold is defined by the condition with $\mathcal{R}$ =1 being the threshold (Woolhouse et al., 1997).When $\mathcal{R}$ is greater than one, an epidemic potentially occurs. However, when $\mathcal{R}$ is less than one, the epidemic will be unlikely to occur but some secondary cases may be reported (Woolhouse et al., 1997;

Chowell et al., 2006a; Hall et al., 2008; Brauer et al., 2010; Reeth et al., 2012; Brauer and Castillo-Chávez, 2013; Chowell et al., 2013). Therefore, in evaluating any intervention measure, R has to be less than one to be considered successful in the intervention implementations. Estimating R can be obtained using either epidemiologic data or compartmental models.

Firstly, epidemiologic data are used to estimate R (or other parameters) by standard approaches (Diekmann et al., 1990; Dietz, 1993; Diekmann and Heesterbeek, 2000; Van den Driessche and Watmough, 2002; Brauer et al., 2010; Hens et al., 2012; Brauer and Castillo-Chávez, 2013). Standard approaches are given (**Table 1-2)** such as; R is estimated from (i) the intrinsic growth rate or the early phase reports (Chowell et al., 2007b; Boëlle et al., 2009), (ii) from the peak of an outbreak reports (Chowell et al., 2007b), (iii) from the epidemic compartment models (i.e. SIR, SEIR) (Chowell et al., 2007b; Romagosa et al., 2011; Allerson et al., 2013b), (iv) from Bayesian epidemic models (i.e. SIR, SEIR) (Cauchemez et al., 2004; Chowell et al., 2007a; Bouma et al., 2009) and (vi) lastly from the real time epidemic report, which is important to estimate the probability of happening both epidemic and pandemic transmissions (Halloran et al., 2010).

Secondly, from the compartment model, R can be estimated by solving the next-generation matrix approach (Diekmann and Heesterbeek, 2000; Van den Driessche and Watmough, 2002; Heffernan et al., 2005; Lanzas et al., 2008; Brauer et al., 2010; Brauer and Castillo-Chávez, 2013). This approach can be used for models with age or spatial structures (Heffernan et al., 2005). Nevertheless, the limitation of this method is that it was assumed that transmission probabilities between states are constant and time arrival

within each compartment is exponentially distributed (Heffernan et al., 2005). Ɍ arising from the next-generation matrix is defined by the dominate eigenvalue or spectral radius (Diekmann et al., 1990; Heffernan et al., 2005).

Although Ɍ is the fundamental measure of an infectious disease transmissibility, there is not only one way to estimate Ɍ, leading to be inconstant of estimations (Yang et al., 2007b; Brauer and Castillo-Chávez, 2013). Besides, the degree of Ɍ depends on both the infectious agent and the host population. Once taking into account for other potential factors, including latitude-longitude, size-density of populations, age-sex distributions or other factors, Ɍ may become significantly unbiased-estimated either toward from the null or away from the null (Mills et al., 2004). Ɍ may inconsistently be estimated in specific circumstances. Firstly, the estimate obtained from epidemiological data was inconsistent among several methods (Chowell et al., 2007a). Secondly, when a disease is transmissible and dynamic, Ɍ will relatively be very non-informative (Chowell et al., 2007b; Yang et al., 2007b). Thirdly, Ɍ hinges enormously on a transmission model specification. Different model specifications may provide different estimates (Chowell et al., 2007b; Yang et al., 2007b). Fourthly, a constant Ɍ assumption may not be appropriate. For instance, during early infection period, pathogens may be more viable and virulent leading to higher Ɍ. During late infection period, viruses may be less viable and virulent, leading to lower Ɍ (Yang et al., 2007a). Fifthly, infectious doses affected the estimated Ɍ as reported from H5N1 experimental setting (Bouma et al., 2009). Lastly, if an animal-to-animal transmission has not been taken in place during a course of disease dissemination, Ɍ estimates may be nonzero, but they are meaningless (Yang et al., 2007b).

Alternatively, Ɍ can obtain from simulation method as Bayesian approach with time-dependence transmission. Another matter of Ɍ is that a constant Ɍ assumption may not be appropriate for IAV transmissibility. For example, during early infection period, viruses may be more viable and virulent leading to higher Ɍ. During late infection period, viruses may be less viable and virulent leading to lower Ɍ.

Seasonal influenza transmission model

Disease seasonality is defined as the cycle or changes of thing(s) depending upon the seasons or may be defined as "systematic periodic fluctuations within the course within a year that can be characterized by the magnitude, timing and duration of a seasonal increase" (Naumova and MacNeill, 2007).

In humans, seasonality of infectious diseases has been documented with different pathogens, for instance, measles, diphtheria, chickenpox, cholera, rotavirus, malaria, gonorrhea, and pneumococcal infections (Dowell, 2001; Dowell et al., 2003; Grassly and Fraser, 2006). Seasonal influenza infections in humans during the wintertime followed by fade out have been demonstrated in temperate regions throughout the word, including the USA (Kim et al., 1996; Säynäjäkangas et al., 2001; Donaldson and Keatinge, 2002; Müller-Pebody et al., 2002; Dowell et al., 2003; Crighton et al., 2004; Dushoff et al., 2004; Chowell et al., 2008). This periodic pattern which occurs at the predictable time each year has been detected through the use of publicly available epidemiological or diagnostic laboratory submission data (Crighton et al., 2004; Chowell et al., 2008; Chowell et al., 2010; Brauer and Castillo-Chávez, 2013).

A relationship between seasonal influenza in human and swine populations may be connected. However, it remains difficult to estimate the full extent of bidirectional (human-to-swine and swine-to-human) seasonal transmission, owing to large gaps for IAV surveillance in swine populations geographically and in past decades (Nelson and Vincent, 2015).

In swine, IAV can circulate among swine throughout the year. However, information regarding the periodicity of infections is scarce and conflicting. A study concluded that IAV infections occurred at a certain age with a predictable pattern (Rose et al., 2013). Whereas two other studies concluded that periodic IAV infection was weak and estimates were not robust since it depended on mode assumptions or there was no periodicity between incidence and seasons (Kyriakis et al., 2013; Poljak et al., 2014). Another study was reported that influenza A virus is present in growing pigs throughout the year and groups of pigs are more likely to have positive test results by RT-PCR during the spring and summer than in the fall and winter (χ^2=9.66, df=3, p-value=0.022) (Corzo et al., 2013a).

Pathogenesis of seasonal influenza in both human and swine populations has remained unclear, poorly understood and still heavily debated (Dushoff et al., 2004; Chowell et al., 2008). The previous report suggested that seasonal infection could have appeared during a time within the year when other factors are present such as cold weather, indoor heating, bad air quality inside barns (i.e. air ventilation or bulk aerosol transport), coinfection, crowding, air transportation, and El Nino (Straub, 1994; Brown, 2000; Lofgren et al., 2007b). However, probable pathogenesis of seasonal influenza may be suggested and graphed as shown in **Figure 1-3**. Briefly, during wintertime, the

respiratory mucosal immunity is expected to be lower and lack of exposure to the sun (among human population) that leads to lower vitamin D levels that are involved in intracellular pathogen killing. In conjunction, viral survivability and viability is favored in the cold environment (Dushoff et al., 2004; Viboud et al., 2004; Cannell et al., 2006). In a temperate region (i.e. State of Minnesota, USA), a ventilation system in a swine barn is worked at lower efficiency during winter than summer seasons. Lower air ventilation rate has been documented enormously changing R (viral transmissibility) (Lofgren et al., 2007b). This may be one of the several causes that influenza infection has been higher observed during winter-fall seasons.

Seasonal transmission modeling was generally proposed under the assumption that such transmission is assumed to be sinusoidal and expressed as: $\beta(t)=\beta_0(1+\alpha cos(2\pi t))$, where $\beta(t)$ denoting the transmission parameter, β_0 denoting the mean transmission parameter, α denoting the amplitude of seasonal variation, typically referred to as the strength of seasonal forcing ($0 \leq \alpha \leq 1$), *t* denoting time *t* which could be measured for time scale (i.e month, year) (Schwartz and Smith, 1983; Dushoff et al., 2004; Grassly and Fraser, 2006; Brauer et al., 2010). R_0 for a seasonal transmission model is defined by: $R_0 = D \int_0^1 \beta(t)dt$, where D denoting the average duration of infection (Grassly and Fraser, 2006). However, the reproductive number can be estimated from compartment models (i.e. SIR or SEIR, etc.) such that $R_0 = \frac{\beta_0}{\gamma}$ (Keeling and Rohani, 2008).

A primary effort for modeling seasonal transmission was performed. A data characteristic is for mortality cases caused by IAV infection in USA human populations. Dataset was available and extracted from Centers for Disease Control and Prevention

(CDC) website from 2004-2013. Before performing a statistical analysis, data was aggregated weekly. **Figure 1-4** represents time-series analysis with wavelet-based bootstrapping (above) and with Fourier spectra (below). By wavelet-based bootstrapping analysis, seasonal influenza A mortality is trending to be increasing over years (by ignoring year of 2009). This may rise to public health concern in evaluating new intervention methods to reduce seasonal mortality in a near future. By Fourier spectral analysis, seasonal influenza A periodic signals has two waves with the highest wave on the second week of February every year.

Bayesian approach

Why is Bayesian approach appropriate for transmission modeling of IAV?

Infectious disease modeling involves in several states or compartments (i.e. Susceptible-S, Exposed-E, Infectious-I, Asymptomatic-A, Symptomatic-Sy, Recovered-R, Immune-I, Vaccinated-V, Quarantine-Q, etc.). Basically, each epidemic generation depends only on the state of the epidemic system in the previous time-point, so-called conditional independence, for example, I at time $t+1$ depending only on I on time t. In addition, I time $t+1$ is also conditional on S at time t for SIR model. The conditionally independent principle in epidemic systems is well-suited for Markov chain (discrete-time Markov chain) and Bayesian approach. In addition, the classical formulation of the SIR transmission model is a continuous time, continuous state and deterministic model. The deterministic models have advantages over the stochastic models in that the deterministic models are more agreeable to theoretical analysis and require less computational

efficiency (Halloran et al., 2010). However, in order to incorporate the likelihood framework, a stochastic model needs to be performed.

Likelihood inference

For the likelihood inference to estimate the IAV transmission, the probability that the epidemic data were produced was given by the model and the model's parameters. The transmission rate (β) was parametrized as $\beta = Ɍ^* \gamma$. The system of ordinary differential equations (ODEs) of SIR model was constructed as previously expressed. The number of infectious individuals (I_t) over the susceptible individuals (S_t) at time *t*, is represented by the probability (p) of RCS pigs. The model assumed that (1) all susceptible individuals have the same probability (p) of exhibiting RCS, (2) individuals exhibited RCS independently, and (3) the distribution of infectious pigs was assumed as binomially distributed. The likelihood ($L(p \mid \cdot)$ of the probability of exhibiting RCS in any individual pig with a particular β value was expressed by;

$$L(p|i_1, i_2, \dots, i_t) = \prod_t \binom{S_t}{i_t} p_t^{x_t} (1 - p_p)^{S_t - i_t},$$

where i_t are the number of exhibiting RCS pigs at time *t*; p_t are the observed prevalence of the number of not exhibiting RCS (S_t) at time *t*. Maximum likelihood estimation method (MLE) was used to fit the dynamic SIR model to the prevalence data and to find β that maximize the likelihood. As β in ODEs of the SIR model was expressed in terms of Ɍ and γ, the parameters (Ɍ and γ) eventually maximize the likelihood (Ferrari, 2009; Bellan, 2014). The Nelder-Mead algorithm called through *"optim"* function was used to navigate the likelihood to MLE and the 95% confidence intervals of the parameters (Ɍ and γ) were estimated using profile likelihood methods.

Bayesian inference

As monitoring reports for an infectious disease, the number of infected cases can only be obtained from some intervals (i.e. weekly or monthly). A useful stochastic epidemic model is the chain binomial model. The chain binomial models are dynamic model developed from the simple binomial model. The chain binomial model fundamentally relies on a discrete-time Markov chain model in Bayesian framework (Gani and Jerwood, 1971). Examples of the chain binomial models are such as the Reed-Frost and Greenwood model. The Reed-Frost model is widely used while the Greenwood model is rarely applied (Halloran et al., 2010). The Reed-Frost stochastically-epidemic model was developed by the biostatistician, Lowell Reed, and the epidemiologist, Wade Hampton Frost, during 1930 at John Hopkins University (Halloran et al., 2010).

Usefulness of incorporating the Bayesian framework into the chain binomial model (Reed-Frost) is due to the fact that (i) when dealing with infectious disease outbreak circumstances, the actual infectious process and disease transmission are unobserved or partially observed; (ii) also, data for an infectious disease outbreak are complicated, inherently dependent, usually incomplete and may be missing (O'Neill and Roberts, 1999; O'Neill, 2002). The likelihood function needs to be evaluated, but it may become very difficult to evaluate as aforementioned by two rationales. Such challenges can be alleviated by incorporating the Bayesian framework. What's more? When epidemiological data are progressively accumulating, Bayesian approach is well-appropriate for infectious disease models due to the flexibility to incorporate prior information and be practically suitable to a conditional probability and complexities

(Shoemaker et al., 1999; Sorensen and Gianola, 2002; Carlin and Louis, 2008). The flexibility of incorporating prior information is that it is sequentially updated as more complete/ partially-complete outbreak data becoming available (Chowell et al., 2007b; Bettencourt and Ribeiro, 2008; Chowell et al., 2013).

Due to difficulty to evaluate the likelihood function as mentioned earlier, analyzing infectious disease data and modeling with frequentist approach (classical statistic method) may become less efficient (O'Neill and Roberts, 1999). Besides, when dealing with sequentially update in several epidemic compartments, using such approach is computationally intractable, especially in cases of partially-complete outbreak data (O'Neill and Roberts, 1999; Sorensen and Gianola, 2002). Dealing with partially-complete outbreak and unobserved-infectious process data, data augmentation (imputation) in Bayesian framework is a technique to generate unobserved-infectious process of disease transmission, or to impute incomplete or missing data, generating from Markov Chain Monte Carlo (MCMC) sampling by either Gibbs sampler or the Metropolis-Hastings algorithm (Metropolis et al., 1953; Hastings, 1970; Geman and Geman, 1984; Gelfand and Smith, 1990; Cauchemez et al., 2004; Carlin and Louis, 2008). For that, data augmentation makes an evaluation of the likelihood function much easier (Jewell et al., 2009).

In measuring disease outcome in a transmission model, diagnostic test may be imperfect, leading to misclassification of such outcome. To account for that misclassification, Bayesian approach can be implemented by incorporating uncertainty of test Se and Sp to adjust for a misclassification bias (Bernatsky et al., 2005; Kostoulas et al., 2009; Correia-Gomes et al., 2014). In measuring an error from imperfect diagnostic

test, RCS observation has been routinely used for on-farm diagnosis and monitoring of swine respiratory diseases. However, RCS observation is imperfect, and failure to adjust for the imperfection may bias measurements of diseases outcome. Thus, it is crucial to account for imperfect measurement to estimate IAV transmission within pig populations (Kostoulas et al., 2009).

In estimating transmission rate (β) from S to I, the formula y_{obs_t} is the observed number of new RCS cases based on imperfect tests at time *t* and assumes $y_{obs_t} \sim Bin(p_t, S_t)$, where p_t is the probability of susceptible pigs at time *t-1*, becoming positive to RCS observation at time *t* and S_t is the number of susceptibles. The probability p_t due to the imperfect RCS observation was expressed as $p_t = \psi_t Se + (1 - \psi_t)(1 - Sp)$, where $\psi_t$denoted the expected probability of expected RCS positivity due to IAV transmission within pig populations, where Se and Sp are the sensitivity and specificity of RCS observation, respectively (Kostoulas et al., 2009). Subsequently, it was assumed $y_{true_t} \sim Bin(\psi_j,\ S_t)$, where y_{true_t} is the true number of new cases after adjusting for an imperfect test at time *t*. The ψ_t was modeled using $cloglog(\psi_t) = \log(\beta) + \log(\frac{I_{t-1}\Delta t}{N_{t-1}})$, with binomial regression with complementary log-log link function, where β is the transmission rate; I_{t-1} is the true number of infectious pigs at the end of time step (t-1); N_{t-1} is the total number of pigs; and Δt is time step in day ($\Delta t = 1$) (Correia-Gomes et al., 2014). By imperfect RCS observation, the expected number of I can also be represented as a sum of two binomial distributions expressed by $I = I_{TP} + I_{FN}$, where I_{TP} ~Bin(Se, I_{obs}) and I_{FN}~Bin(1-Sp,N- I_{obs}). I_{TP} is the number of positive IAV pigs as correctly classified and I_{FN} is the number of true IAV positive pigs as misclassified.

From the previous expression, transmission rate *(β)* was estimated by exponentiating $\log(\beta)$.

In estimating recovery rate (γ) from I to R, the number of new recovered pigs at time *t* was assumed as $R_t \sim Bin(pi_t,\ I_t)$, where pi_t is the probability of infectious pigs at time *t-1* becoming recovered at time t, and I_t is the number of infectious pigs at time *t*. The probability pi_t was modeled using $cloglog(pi_t) = \log(\gamma)$ with binomial regression with complementary log-log link function, where γ is the recovered rate, assuming exponential distribution. Ɍ is calculated by Ɍ =β/γ.

Bayesian epidemic model can be classified as two main categories; Markov and non-Markov epidemic models. The difference between Markov and non-Markov epidemic models is that the assumption of an infection period of an individual follows as exponentially distributed for a Markov model while follows as non- exponentially distributed with some specified probability distributions (i.e. Weibull distribution, etc.). The similarity of both models is that during this time, infectious contact occurs with each susceptible according to a Poisson process of rate *β/N* (Heffernan et al., 2005; Neal and Roberts, 2005). Markov SIR epidemic models will be the focus of this review.

In general setting of Markov SIR epidemic model, a population of N individuals is in the epidemic system. At time t, there are: S_t susceptibles, I_t infectives and R_t recovered individuals. In closes population, $S_t + I_t + R_t = N$ for all t. There are two parameters: transmission rate *β* (rate at which susceptibles become infectives), and recovery rate γ (rate at which infectives become recovered individuals). Initially, $(S_0 + I_t + R_0) = (N\text{-}1,1,0)$. Each infectious individual remains for a length of time $T_i \sim \exp(\gamma)$. During this time, infectious contact occurs with each susceptible according to a Poisson

process of rate β/N. Thus, the overall infectious rate is $\beta S_t I_t/N$. Let infectious times be $i_1 \leq i_2 \leq i_3 \ldots \leq i_n$, where i_1 is time that initial infective begins their infectious period and n is random variable for total numbers that were infected. Besides, let recovered times be $r_1 \leq r_2 \leq r_3 \ldots \leq r_n$, where r_1 is time that initial recovered individual begins their recovered period. Let define $\mathbf{i} = (i_2, i_3, \ldots, i_n)$ and $r = (r_1, i_2, \ldots, i_n)$ be vectors. By Bayes' theorem, Markov SIR epidemic model can be expressed as:

$$\pi(\beta, \gamma, i_1 | \mathbf{i}, \mathbf{r}) \propto L(\mathbf{i}, \mathbf{r} | \beta, \gamma, i_1)\, p(\beta, \gamma, i_1),$$

where $\pi(\cdot)$, $L(\cdot)$ and $p(\cdot)$ denoting posterior, likelihood and prior distributions, respectively. In performing MCMC, the joint likelihood for the model can be written as: $L(\mathbf{i}, \mathbf{r} | \beta, \gamma, i_1) = \left(\prod_{j=2}^{n} (\beta/N) S_{i_j} I_{i_j}\right) \left(\prod_{j=1}^{n} \gamma I_{r_j}\right) exp\left(-\int_{i_1}^{r_n} [(\beta/N) S_t I_t + \gamma I_t] dt\right)$. The prior distributions of β and γ follow Gamma distribution (O'Neill and Roberts, 1999; O'Neill, 2002). Furthermore, Bayesian epidemic models are constructed by three hierarchical levels such as the observed data level, unobserved transmission process level and the level of prior probability distribution (Cauchemez et al., 2004).

Bayesian approach has been used to study infectious disease transmission for several studies (Cauchemez et al., 2004; Lekone and Finkenstädt, 2006; Chowell et al., 2007b; Clancy and O'Neill, 2008; Yang et al., 2008; Yang et al., 2010; Chowell et al., 2013), used to estimate R_0 (Chowell et al., 2007b; Clancy and O'Neill, 2008; Bouma et al., 2009; Chowell et al., 2013), and used to study monitoring and surveillance system of epidemic and pandemic emerging, or reemerging diseases (Abbas et al., 2004; Farewell and Spiegelhalter, 2006; Fienberg and Shmueli, 2006; Sebastiani et al., 2006; Höhle, 2007). Examples of using Bayesian approach in studying influenza transmission have

been reported such as H1N1 in humans, H1N1 in chickens and H7N7 in humans (Carlin and Louis, 2008; Bouma et al., 2009; Chowell et al., 2013).

Computer software packages performing Bayesian modeling

Bayesian epidemic modeling can successfully be performed by several computer software packages. The first package has been developed during1980s, namely BUGS (standing for Bayesian inference Using Gibbs Sampling). It can be grouped by three generations of BUGS development: ClassicBUGS (1990-1996), WinBUGS (1996-2007) and OpenBUGS (2007- present). ClassicBUGS runs on DOS platform. WinBUGS runs on Win32x platform and OpenBUGS runs on both Win32x and Win64x platforms (Lunn et al., 2000; Spiegelhalter et al., 2003; Thomas et al., 2006). The most-recent development of BUGS language written in C++ is JAGS (standing for Just Another Gibbs Sampler), currently version 4.1.0. This package runs on all major platforms (Windows, Mac, Linux)(Plummer, 2003). Another software package written in C++ but language syntax is totally different from BUGS family is Stan (named for Stanislaw Ulam who was the first thought and develop Monte Carlo methods). Stan runs on all major platforms (Windows, Mac, Linux), which has several interfaces such as RStan (run in R), PyStan (run in Python), CmdStan (run in shell, command-line terminal), Matlabstan (run in MATLAB), "Stan.il" (run in Julia) and StataStan (run in Stata)(Stan Development Team, 2015). Another interfaces run in in Python is PyMC (Patil et al., 2010). Also, SAS can run Bayesian analysis with MCMC procedure (Chen, 2009). In addition, the R Packages calling BUGS family language (i.e. BUGS or JAGS) from R have been developed in several packages such as BRugs, R2OpenBUGS, rjags, R2jags

and runjags (Thomas, 2004; Sturtz et al., 2005; Sturtz et al., 2010; Plummer, 2015b; Su and Yajima, 2015; Denwood, In press).

Tables and Figures

Table 1-1: Cough-sneeze score based on respiratory clinical signs to swine influenza infections.

Score	Definition
0	No coughing or sneezing
1	Observed coughing/sneezing once while in the room
2	Observed coughing/sneezing 2-3 different times while in the room
3	Observed continual coughing/sneezing while in the room

Note: Detailed elsewhere (Detmer et al., 2013)

Table 1-2: A summary reported of the reproductive number (Ɍ) estimated by different approaches for IAV infection in human and animal populations.

Estimation method	**$Ɍ_0$**	**Lower 95% CI**	**Upper 95% CI**	**Host**	**Subtype**	**Remarks**	**References**
Exponential growth rate							
	3.0	2.7	3.3	humans	H1N1		(Chowell et al., 2007b)
	2.2	2.4	2.8	humans	H1N1		(Boëlle et al., 2009)
	2.6	2.4	2.8	humans	H1N1		(Boëlle et al., 2009)
	3.1	2.9	3.5	humans	H1N1		(Boëlle et al., 2009)
	3.2	2.72	3.82	pigs	H1N1		(Rose et al., 2013)
	6.9	4.12	10.50	pigs	H1N1		(Rose et al., 2013)
	2.5	1.91	2.94	pigs	H1N1, H1N2		(Rose et al., 2013)
	4.1	2.01	6.89	pigs	H1N1, H1N2		(Rose et al., 2013)

	5.9	4.23	7.96	pigs	H1N2		(Rose et al., 2013)
SIR							
	2.98	2.77	3.02	pigs	-	[1]RCS	(Homwong et al., In prepare)
	10.4	6.6	15.8	pigs	H1N1		(Allerson et al., 2013b)
	10.7	6.6	16.5	pigs	H1N1		(Romagosa et al., 2011)
	2.4	2.2	2.6	humans	H1N1		(Chowell et al., 2007b)
SEIR							
	1.49	1.45	1.53	humans	H1N1	first wave	(Chowell et al., 2006a; Chowell et al., 2007b)
	3.75	3.57	3.39	humans	H1N1	second wave	(Chowell et al., 2006a, b)
	2.2	1.6	2.1	humans	H1N1		(Chowell et al., 2007b)
	2.7	2.3	3.4	humans	H2N1	median	(Mills et al.,

							2004)
[1]Bayesian approach							
	3.04	2.66	3.46	pigs	-	[2]RCS	(Homwong et al., In prepare)
	4.19	1.98	26.29	pigs	-	[3]RCS	(Homwong et al., In prepare)
	2.1	1.1	3.0	humans	H1N1		(Chowell et al., 2007b)
	1.6	0.9	2.5	chicken	H5N1		(Bouma et al., 2009)
				human	H3N2		(Cauchemez et al., 2004)
	0.11	0.003	0.42	human	2013 A/H7N9	Zhejiang, China	(Chowell et al., 2013)
	0.17	0.01	0.49	human	2013 A H7N9	Shanghi, China	(Chowell et al., 2013)
Real-time estimation							

	3.2	2.1	4.0	humans	H1N1		(Boëlle et al., 2009)
	1.6	1.5	1.7	humans	H1N1		(Chowell et al., 2007a)
	3.1	2.8	1.7	humans	H1N1		(Chowell et al., 2007a)
	1.2	0.8	1.7	humans	H1N1	median	(Chen and Liao, 2010)
	1.4	0.9	2.2	humans	H3N2	median	(Chen and Liao, 2010)
	1.3	1.2	1.4	humans	-		(Chowell et al., 2008)
	2.0	1.1	3.0	humans	H1N1		(Mills et al., 2004)
	1.3	1.3	1.4	humans	H1N1		(Tuite et al., 2010)
	1.4	1.4	1.5	humans	H1N1		(Pourbohloul et al., 2009)

[1]Bayesian approach 95% confidence limits is called 95% credible interval

[2]RCS stands for respiratory clinical signs as measured of disease progress, assuming perfect

[3]RCS stands for respiratory clinical signs as measured of disease progress, assuming imperfect tests

Figure 1-1: Hypothetical representation for classifying the diagnostics results between positive and negative results

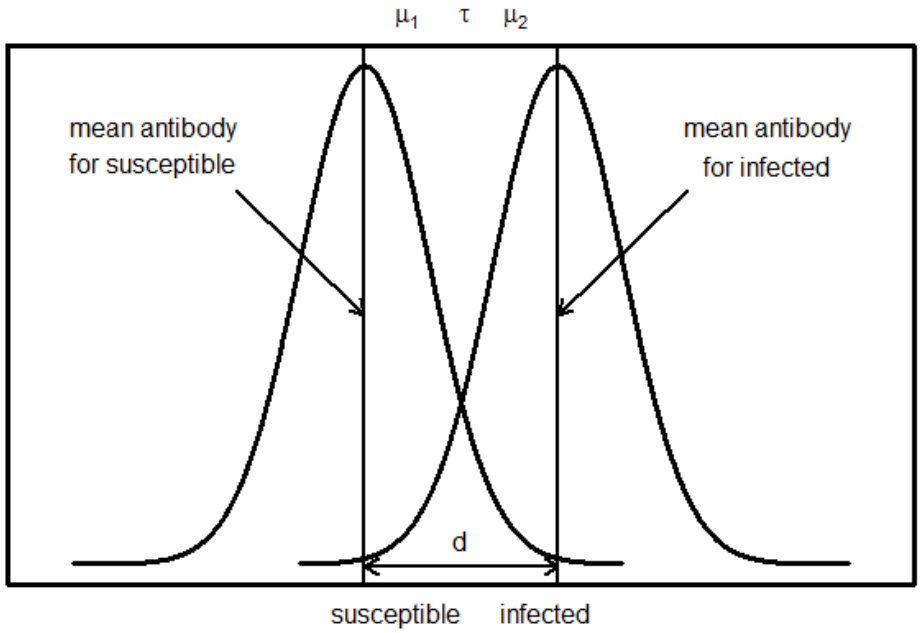
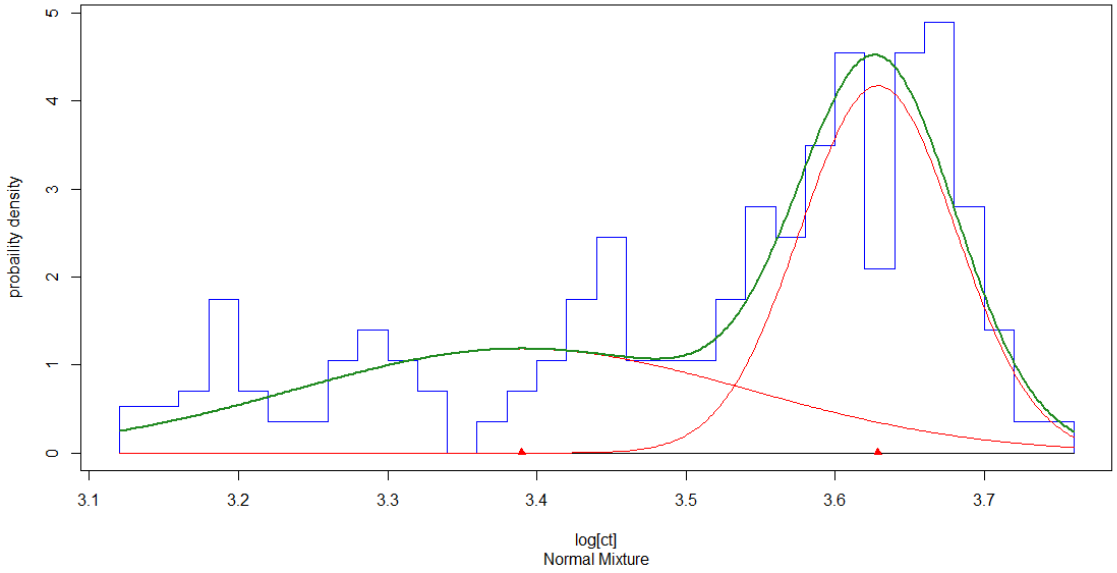


Figure 1-2: Theoretical representation for estimating the optimal RRT-PCR Ct cutoff value cutoff diagnosed for IAV infection using Bayesian two-component mixture model



Theoretically, the lines in red represented the positive and negative sample distributions. The line in green represented the probabilistic distribution function of submitted samples (cumulative distribution). Given no information regarding to diseases status of submitted samples, the optimal cutoff value was estimated to be exp(3.57)= 35.52, thereby minimizing misclassification rate. The estimated probability of positive samples was 0.453 with an average Ct value of 29.66 while that of positive samples was 0.547 with an average Ct value of 37.71. On the log-scale, the standard deviation of positive sample distribution was 0.153 and of negative sample distribution was 0.052.

Figure 1-3: Demonstration of probable pathogenesis of seasonal influenza

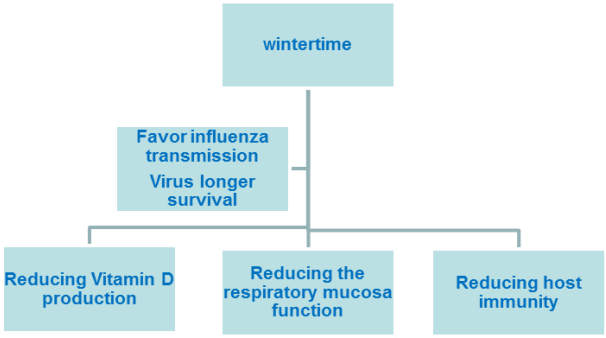
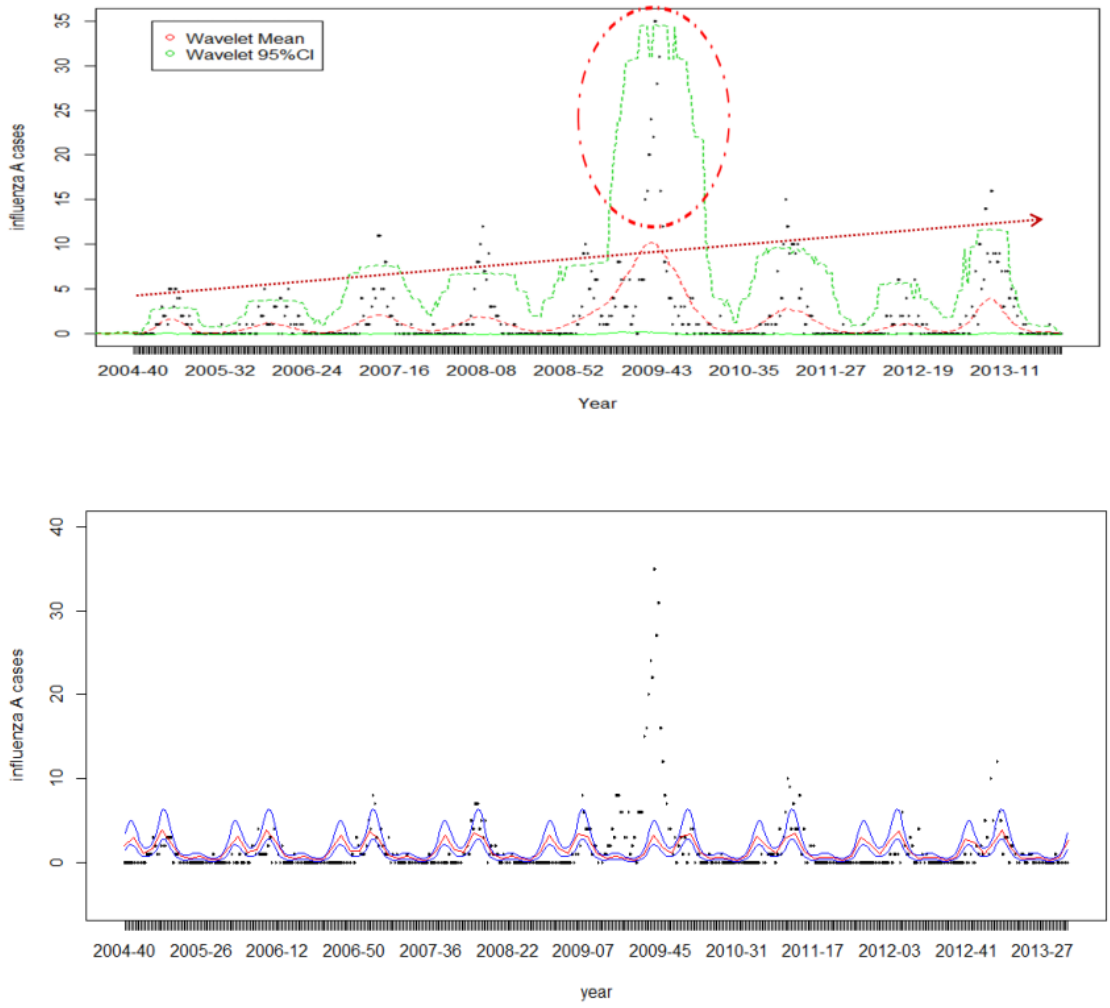


Figure 1-4: Time series analysis with wavelet-based bootstrapping (above) and with Fourier spectra (below) for seasonal influenza A analyzed by human influenza A mortality data



Data were extracted from Centers for Disease control and Prevention (CDC) from 2004-2013. The analysis was as aggregated weekly of influenza A mortality cases in US human population. By wavelet-based bootstrapping analysis, seasonal influenza A mortality cases in humans are trending to be increasing over years. By Fourier spectral analysis, seasonal influenza A periodic signal has two waves with the highest wave being on the second week of every February.

Chapter 2 : A Bayesian approach for inductive reasoning to clinical veterinary medicine: The math of experience

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Summary

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, veterinarians 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.

Keywords: 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 (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 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 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 (i.e. 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) (Hald, 1998; Dale, 1999). 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)"(Bayes and Price, 1763; Stigler, 1986; Fienberg, 2006; Aldrich, 2008).

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 change as well as the lists of differential diagnoses will be reduced. A utility of BA for veterinary diagnostic test has been well-addressed elsewhere (Greiner and Gardner, 2000b; Gardner, 2002; Branscum et al., 2005; Toft et al., 2005; Bonde et al., 2010; Paul et al., 2013). Therefore, the objectives of the current article were to examine the state of BA used for inductive reasoning in veterinary medicine 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 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 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 (Bayes and Price, 1763; Stigler, 1986; Fienberg, 2006; Aldrich, 2008). 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; Gardner, 2002; Dowd and Meyer, 2003; Basáñez et al., 2004; Fienberg, 2006). A classic example of applying BA in order to make inductive reasoning from an effect to a cause is during 1855-1865 in London where John Snow had used BA as his inductive reasoning to scientifically convince audiences that a source of cholera transmission was a private water supplier company (Koch and Denike, 2006).

Components of a Bayesian approach

A Bayesian approach comprises three mathematical terms: i) *evidence*[1], "p(x)", (a.k.a. the marginal likelihood, or the probability of evidence), ii) the *prior experience*[2], "p(θ)" and iii) strength of evidence[3], "p(x|θ)" (a.k.a. likelihood of evidence given a hypothesis).The *posterior experience*, "p(θ|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

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

Simply, the posterior experience is proportional to the strength of the new evidence times the prior experience (Higgins et al., 2012b; Higgins et al., 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 infer using a posterior clinical experience (posterior probability) as shown in **Figure 2-1**.

[1] "x" is data that has been observed.
[2] "θ" is a prior clinical experience about a disease.
[3] "x|θ" is data that have been observed based on a prior clinical experience.
[4] "θ|x" is a potential disease after having seen the data.

However, the coughing could be caused by multiple pathogens including metastrongylus, *mycoplasma hyopneumoniae*, porcine respiratory coronavirus, classical swine fever virus, porcine circovirus type 2 virus, porcine reproductive and respiratory syndrome (PRRSv) virus, or IAV, etc. (Zimmerman et al., 2012) (**Figure 2-2)**.

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

$$p(flu|coughing) = \frac{p(coughing|flu)*p(flu)}{p(coughing|flu)*p(flu) + p(coughing|\sim flu)*p(\sim flu)}.$$

The denominator is called the "probability of evidence" of coughing event (marginal likelihood). The Bayesian terms, notations and definitions were detailed in **Table 2-1**.

Figure 2-3 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 (Olsen et al., 2000; Choi et al., 2002b). 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, $p(coughing|flu)$. If the probability of IAV infected pigs having coughing as a clinical sign, $p(coughing|flu)$, is, for example , 0.3, the posterior clinical experience, $p(flu|coughing)$, is 0.17. The calculation is illustrated in **Figure 2-3**. As

BA measures a degree of prior clinical experience (hypothesis), from such posterior experience, it is implied from his/her clinical experience 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 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 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 are 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 (i.e. the prevalence of IAV was 29% with 13% SD(Olsen et al., 2000; Choi et al., 2002b), by prior clinical experience of H1N1-IAV prevalence in US swine herd) (**Figure 2-4)**. 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 compared to the prior clinical experience, represented by narrower credible interval (confidence interval used in BA) as shown in **Figure 2-5**. By choosing narrower credible intervals, inference and decision-making are better served, compared to chance (Poole, 2001). More 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 those 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 in **Figure 2-3**, 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|θ)", 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(θ|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|θ)" has been found or theoretically when sample sizes is large (as $n \to \infty$) 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 those 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).

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 *Ehrlichia 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 *Ehrlichia canis* infection requires BA. In statistical terms, what it a probability that the serological positive test result would really be caused by *Ehrlichia canis* infection? This is the mathematical way of incorporating the serological evidence accompanied with prior clinical experience concerning the previous prevalence of *Ehrlichia canis*. One then updates the estimate of how likely is the serological positive test caused by *Ehrlichia 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 *Ehrlichia 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 *Ehrlichia 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 is (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 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 contented) 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.

Conclusions

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 those veterinarians, 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 the 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.

Acknowledgments

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Table and Figures

Table 2-1: 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	$p(flu)$	the probability that pigs have IAV infection (prevalence)
The strength of evidence	$p(coughing\|flu)$	the probability that IAV infected pigs are coughing as a clinical sign
-	$p(coughing\|\sim flu)$	the probability that pigs negative to IAV infection have coughing as a clinical sign
-	$p(\sim flu)$	the probability that pigs have no IAV infection
Posterior clinical experience	$p(flu\|coughing)$	the probability that coughing is caused by IAV infection

Figure 2-1: The representation of inductive inference from posterior clinical experience

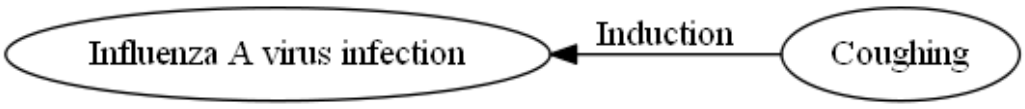


Table 2-2: The processes of deduction (from diseases to observed clinical signs) and induction (from observed clinical signs to diseases) used in veterinary inference with an example of partially-selected swine diseases

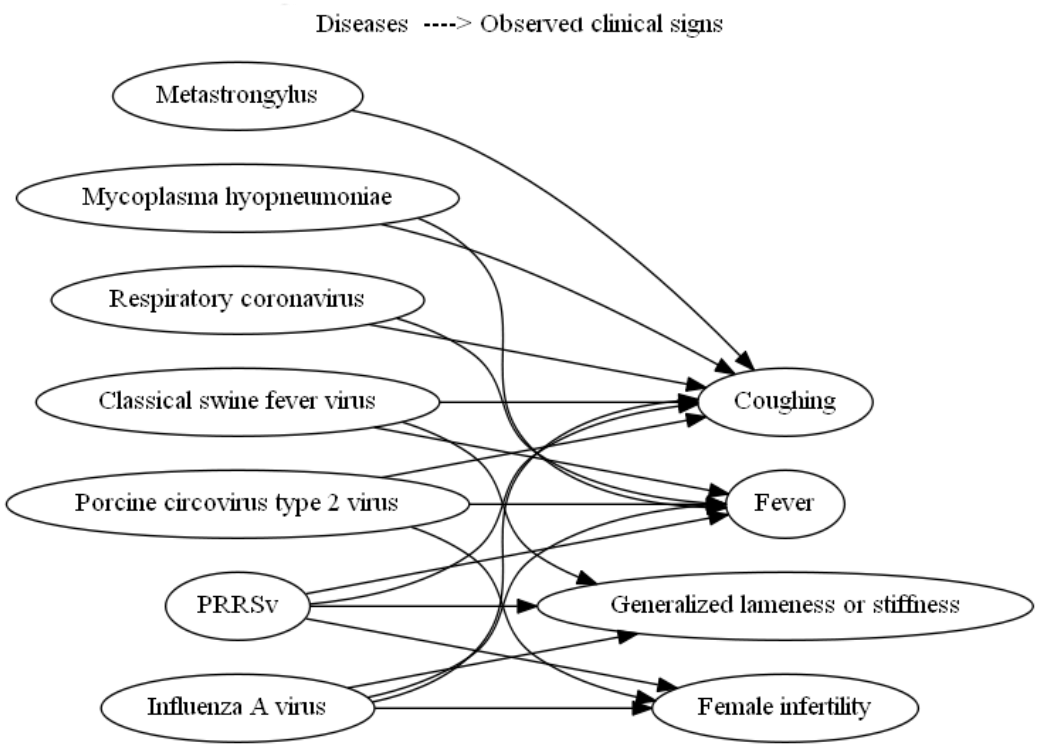
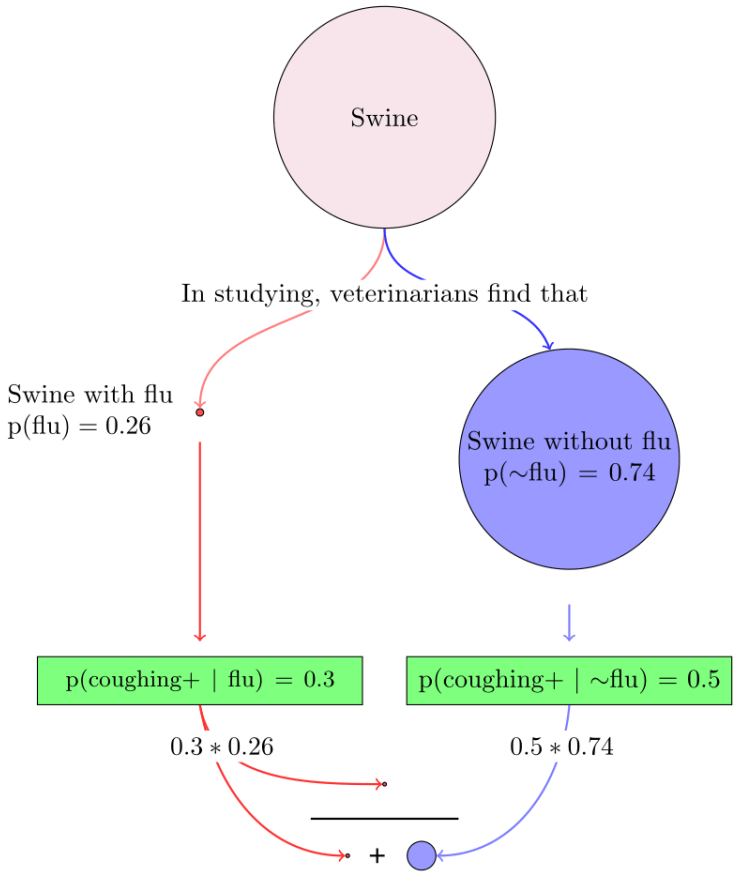


Figure 2-2: The calculation using Bayes' theorem for inductive inference processes from coughing to influenza A virus infection



$$p(\text{flu} \mid \text{coughing+}) = \frac{p(\text{coughing+} \mid \text{flu}) * p(\text{flu})}{p(\text{coughing+} \mid \text{flu}) * p(\text{flu}) + p(\text{coughing+} \mid \sim\text{flu}) * p(\sim\text{flu})}$$

$$0.17 = \frac{0.3 * 0.26}{(0.3 * 0.26) + (0.5 * 0.74)}$$

Figure 2-3: 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%)

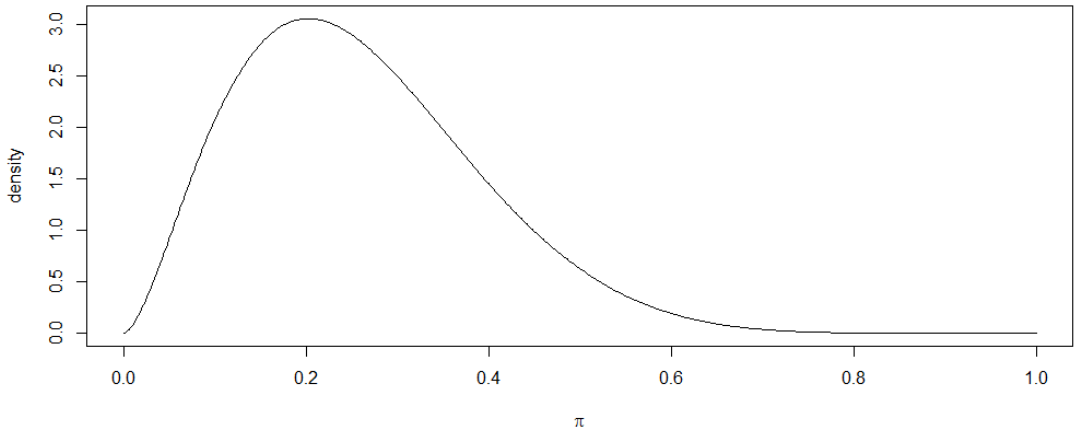
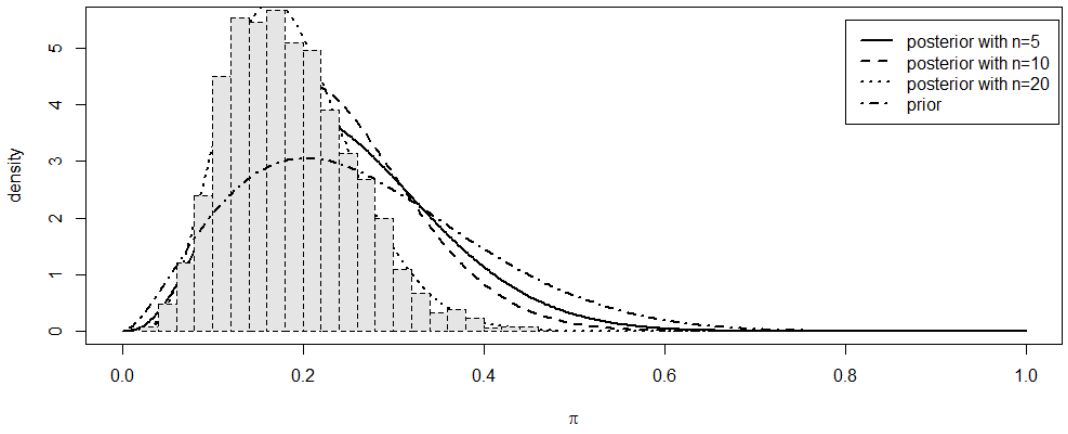


Figure 2-4: The representation of increasing strength of evidence as probabilistic graph using Beta-binomial model with 1, 2 and 3 positive samples out of 5, 10, 20 samples respectively (a horizontal axis is the prevalence)



Chapter 3 : Bayesian estimation to test accuracy for influenza A infection via respiratory clinical signs in the absence of a gold standard

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Summary

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 (1) IAV vaccination reduced only clinical manifestations, but not infection (basic model), or where (2) vaccination reduced both. By the basic model, the Se and Sp of RCS from posterior distributions were 0.38 (95% Credible 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). 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.

Keywords: 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 (Romagosa et al., 2011; Torremorell et al., 2012; Allerson et al., 2013b). 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) (Vincent et al., 2008; Deblanc et al., 2012; Fablet et al., 2012; Rose et al., 2013). 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 $3.23-10.31/head (Donovan, 2008; Dykhuis Haden et al., 2012).

Important control measures for IAV in pigs include surveillance, monitoring, prevalence estimation, and risk factor studies (Greiner and Gardner, 2000b). 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, 2000a), which do not account for sampling methodology (Joseph et al., 1995), and the variability within and between herds (Enøe et al., 2000; Greiner and Gardner, 2000a; Davies, 2006). 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; Toft et al., 2007; Praud et al., 2012; Paul et al., 2013). 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. However, one might consider PCR's as reference tests with some fixed values.

In a context where a gold standard or a reference test is absent, as deemed in this case, we thus focus on using full Bayesian model as our main analytic tool to estimate parameters of Se, Sp and true the prevalence. Therefore, the objectives of this study were: in scenario 1 (i) to estimate Se and Sp of RCS, and NS and OF RRT-PCRs; (ii) to

estimate the true prevalence using both RCS and NS, and in scenario 2 (iii) to understand how vaccination affects estimates of the test accuracy and true prevalence.

Materials and Methods

Datasets

This study utilized published data from two studies: (1) a field study on active surveillance of swine influenza infection in growing pig populations in the Midwestern United States (US) (Corzo et al., 2013a), and (2) an experimental challenge study of IAV in swine (Romagosa et al., 2012) **(Table3-1)**. 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 minutes 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 (Vincent et al., 2008; Corzo et al., 2013a; Rose et al., 2013). 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 manuscript, 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: (1) Se and Sp of RCS, (2) Se and Sp of OF and NS RRT-PCR, (3) the prevalence and (4) 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., 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 we 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% (Olsen et al., 2000; Choi et al., 2002b).
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*[5] 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 3-2)**.

Sensitivity analysis of the prior distribution

Since the duration of infection affects the Se (Greiner and Gardner, 2000a), we categorized the priors into three groups based upon our initial assumptions: (I) if samples were taken within one week of infection, (II) if samples were taken within two weeks of infection and (III) if samples were taken without any information on the course of infection. The prior distributions of (I) 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 of (II) 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 in (III).

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

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, we tested two approaches: RCS relying on visual observation of clinical outcomes, which is a subjective measure, while NS RRT-PCR, and RNA-based technique, which 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, we 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 (Enøe et al., 2000; Gardner et al., 2000; Branscum et al., 2005). 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 (Johnson et al., 2001; Branscum et al., 2005; Bouwknegt et al., 2008). The 4 combination 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, 2015a) and constructed following previously described methods (Branscum et al., 2005; Toft et al., 2005; Geurden et al., 2008; Nérette et al., 2008). The detailed model structure is included **(Table 3-4, Appendix A)** as well as a conceptual model with Directed acyclic graph **(Figure 3-1)**. The JAGS model codes were written in R v3.2.0 (R Core Team, 2015) The "rjugs" and "R2jags" packages were used as an add-on for calling JAGS from R to perform Gibbs sampling (Plummer, 2015b; Su and Yajima, 2015). The analysis of MCMC chains and graphics was performed by using the "CODA," "ggmcmc" and "ggplots2"packages (Plummer et al., 2006; Hadley, 2009; Marin, 2013). 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 Traceplots, Gelman-Rubin R-hat (Potential Scale Reduction Factor) and diagnostic Geweke z-score plots (Geweke, 1991; Gelman and Rubin, 1992). 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 (Spiegelhalter et al., 2002; Carlin and Louis, 2008).

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 3-2)**. In summary, after selecting the final model (based on DIC), the models were run six times in total.

Results

Diagnostic test results 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 with two populations. 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 3-3 and 3-4**). 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 3-5)**. The Se and Sp of 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 in (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 3-2)**. 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 3-5)**.

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 3-6).** 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 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 3-6)**.

With sensitivity analysis, the priors of the test accuracy (varied by time-of-sampling) were reviewed from Goodell and colleagues (2013), and used non-informative priors **(Table 3-2).** 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. However, 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 of 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 our 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. (2013a), 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 and colleagues (2013) indicated that the prevalence estimated by targeted sampling of pigs displaying RCS may be slightly overestimated compared to simple random sampling (Allerson et al., 2013a). 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, our four model assumptions were constructed. 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, we 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, our 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 (Olsen et al., 2000; Choi et al., 2002b; Poljak et al., 2008a). 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., 2002b). This study contained a large sample size, consisting of 111,418 samples submitted to the University of Minnesota Veterinary Diagnostic Laboratory. However, our 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., 2008a). 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., 2008b). 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 our estimates, we 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, which seems comparable to weeks 2 and 3. A similar characteristic was also found in scenario 2. If RCS is used for monitoring IAV infection in swine herds, it will create more false-negative results in an endemic herd. As Allerson and colleague et al (2013) reported, positive growing pigs may not exhibit RCS (Allerson et al., 2013a). 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.

Conclusions

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 USA 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.

Chapter appendix

Appendix A

Bayesian model, namely $y_{ijk} \sim multinomial(n_i, (p_{i11}, p_{i12}, p_{i21}, p_{i22}))$, 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: $k_\eta = \frac{2*\gamma_\eta}{\eta_1(1-\eta_3)+\eta_3(1-\eta_1)}$, $k_\theta = \frac{2*\gamma_\theta}{\theta_1(1-\theta_3)+\theta_3(1-\theta_1)}$ (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 3-1)** 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 3-1)**.

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 3-4**, 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.

Tables and Figures

Table 3-1: 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

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

Table 3-2: Description of the prior distribution for Se and Sp of RCS, OF and NS RRT-PCR, and prevalence in field and experimental populations

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-	η_n	0.50	0.03-0.98	0.08	Beta (1,1)	Non-informative
PCR	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)	(Olsen et al., 2000; Choi et al., 2002b)
Experimental prevalence	π_e	0.92 0.17	0.80-0.99 0.14-0.12	0.01 0.01	Beta (24.75,2.04) Beta (100.9,496.4)	(Romagosa et al., 2012)

[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

Table 3-3: 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

[1]DIC= Deviance+ pD

Table 3-4 : Final Bayesian model selected by Deviance information criteria to estimate Se and Sp of RCS and OF as well as NS RRT-PCR and prevalence

Population *i*	Probability[1]	Structure of the model[2]
1	Conditionally independent[3]	
	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}	$\pi_1(1-\eta_1)(1-\eta_2)+(1-\pi_1)\theta_1\theta_2$
2	Conditionally dependent	
	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}	$\pi_2[(1-\eta_1)(1-\eta_3)+\gamma_\eta]+(1-\pi_2)[\theta_1\theta_3+\gamma_\theta]$

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.

[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

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

		Sensitivity analysis (Time-of-sampling)								
		Within 1 week of infection			**Within 2 weeks of infection**			**Unknown course of infection**		
Parameters[1]		**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-	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
PCR	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-	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
PCR	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 $\pi_e \sim$Beta(24.75, 2.04)

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

		Sensitivity analysis (Time-of-sampling)								
		Within 1 week of infection			**Within 2 week of infection**			**Unknown course of infection**		
Parameters[1]		**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-	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
PCR	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-	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
PCR	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)

Figure 3-1: 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.

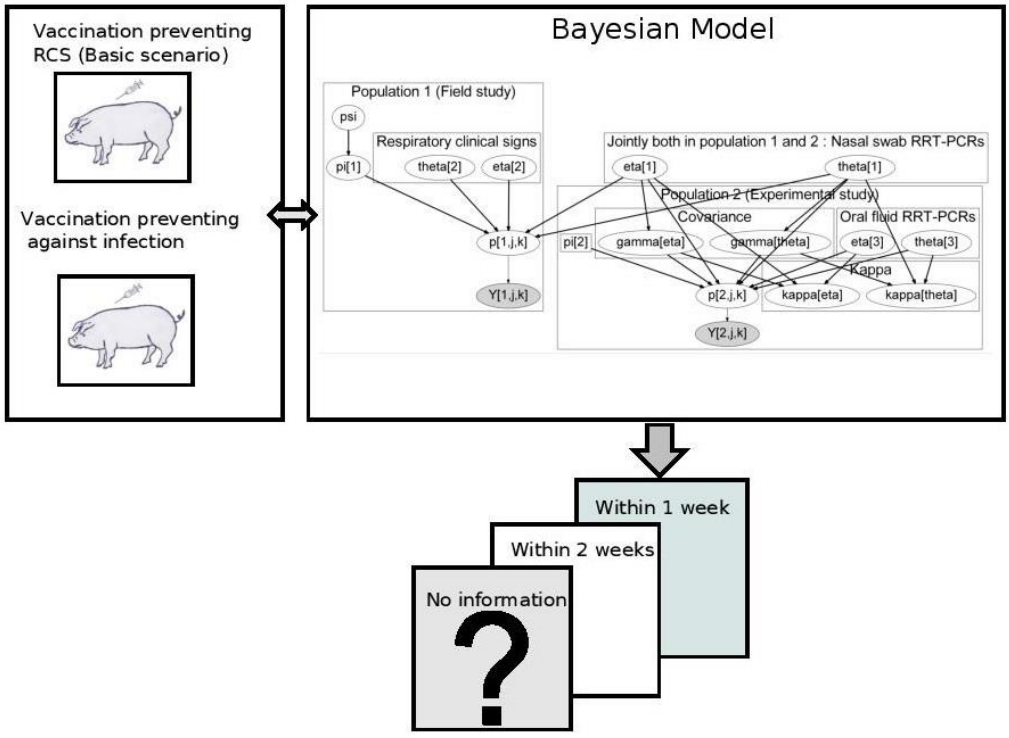
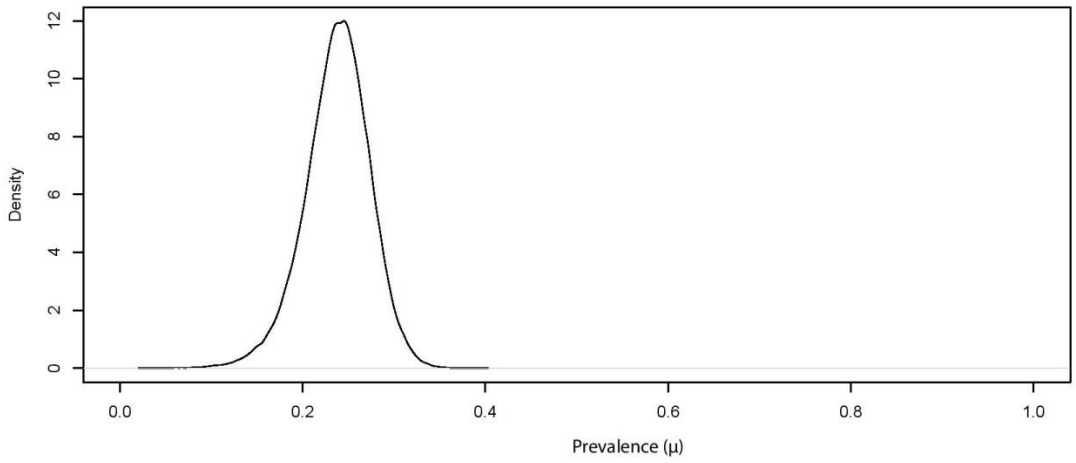


Figure 3-2: A probability distribution represents herd prevalence of IAV infection in the Midwestern US growing pig populations

The x-axis represents herd-level prevalence. The probability distribution was generated from three MCMC chains with 250,000 with 5000 discarded each chain.



The BUGS codes

The BUGS codes for the final Bayesian model run in JAGS 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. The prior distributions introduced as a reflection of uncertainty about knowing time-of-sampling before data is observed (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). The posterior distributions reflect the uncertainty about test accuracy and prevalence (model parameters) after data has been observed.

```
#2  population model
model{

  x1[1:4] ~ dmulti(pr1[1:4], n1)
  x2[1:4] ~ dmulti(pr2[1:4], n2)

  #Posterior predictive model
  pred.x1[1:4] ~ dmulti(pr1[1:4], n1)
  pred.x2[1:4] ~ dmulti(pr2[1:4], n2)

 #Population 1, two independent tests RRT-PCR vs respiratory clinical sign
  pr1[1] <- pi1*(SeNS*SeRes) + (1-pi1)*((1-SpNS)*(1-SpRes))
  pr1[2] <- pi1*(SeNS*(1-SeRes)) + (1-pi1)*((1-SpNS)*SpRes)
  pr1[3] <- pi1*((1-SeNS)*SeRes) + (1-pi1)*(SpNS*(1-SpRes))
  pr1[4] <- pi1*((1-SeNS)*(1-SeRes)) + (1-pi1)*(SpNS*SpRes)

  #Population 2, two dependent tests RRT-PCR with nasal oral vs fluid samples
  pr2[1] <- pi2*(SeNS*SeOF+covDp) + (1-pi2)*((1-SpNS)*(1-SpOF)+covDn)
  pr2[2] <- pi2*(SeNS*(1-SeOF)-covDp) + (1-pi2)*((1-SpNS)*SpOF-covDn)
  pr2[3] <- pi2*((1-SeNS)*SeOF-covDp) + (1-pi2)*(SpNS*(1-SpOF)-covDn)
  pr2[4] <- pi2*((1-SeNS)*(1-SeOF)+covDp) + (1-pi2)*(SpNS*SpOF+covDn)


  ################################################
  #upper and lower limits of conditional covariance positive
  lpos <- max(-(1-SeNS)*(1-SeOF), -SeNS*SeOF)
  upos <- min(SeNS*(1-SeOF), SeOF*(1-SeNS))

  #upper and lower limits of conditional covariance negative
  lneg <- max(-(1-SpNS)*(1-SpOF), -SpNS*SpOF)
  uneg <- min(SpNS*(1-SpOF), SpOF*(1-SpNS))

  ####################################################
  #conditional covariance positive
  covDp ~ dunif(lpos, upos)
  #conditional covariance negative
  covDn ~ dunif(lneg, uneg)

 #Conditional Correlation
  rhoDp <- covDp / sqrt(SeNS*(1-SeNS)*SeOF*(1-SeOF))
  rhoDn <- covDn / sqrt(SpNS*(1-SpNS)*SpOF*(1-SpOF))

  #test covariances and kappa statistic (Gardner et al., 2000)
  kSe <- 2*covDp/(SeNS*(1-SeOF)+SeOF*(1-SeNS))
  kSp <- 2*covDn/(SpNS*(1-SpOF)+SpOF*(1-SpNS))

  #version 1 from Geurden et. al, 2008
    for (i in 1:4){
        d1.v1[i] <-x1[i]*log(max(x1[i],1)/(pr1[i]*n1))
        d2.v1[i] <-x2[i]*log(max(x2[i],1)/(pr2[i]*n2))
        ####
        pred.d1.v1[i] <-pred.x1[i]*log(max(pred.x1[i],1)/(pr1[i]*n1))
        pred.d2.v1[i] <-pred.x2[i]*log(max(pred.x2[i],1)/(pr2[i]*n2))

}
   G0.v1 <- 2*(sum(d1.v1[])+sum(d2.v1[]))
   pred.G0.v1 <- 2*(sum(pred.d1.v1[])+sum(pred.d2.v1[]))
```

```
   #Bayesian p-values
    baye.p.v1 <-step(G0.v1-pred.G0.v1)


#####################################################################
#Population 1
#Prior
  pi1~ dbeta(2.7,7.68) ##mode=0.28, with SD=0.13
  #(Olsen et al., 2000; Choi et al., 2002)

  SeRes ~ dbeta(1, 1) #non-informative prior
  SpRes ~ dbeta(1, 1) #non-informative prior

#############################################################
 #within 1 week infection
  #the prior of sensitivity and specificity(Goodell et al., 2013)
  SeNS ~ dbeta(71.23,12.28)      ###0.85(0.77,0.92)
  SpNS ~ dbeta(24.75,2.03)       ###.97(0.80-0.99)

 #within 2 week infection
  #SeNS ~ dbeta(0.38,0.34)       ### (Goodell et al., 2013)
  #SpNS ~ dbeta(1,1)         ### non-informative prior

 #Unknown course of infection
  #SeNS ~ dbeta(1,1)         ### non-informative prior
  #SpNS ~ dbeta(1,1)         ### non-informative prior


#####################################################################
#Population 2
#Prior
#scenario 1
  pi2~dbeta(24.75,2.04)  #median=0.92(0.80,0.99) SD=0.01(Romagossa et al., 2011)
#scenario 2
  #pi2~dbeta(100.92, 496.41)  #the prior prevalence was 0.17 with 95% sure that
                             #the prevalence was in between 0.14 and 0.20

 #within 1 week infection
  SeOF ~ dbeta(77.85,15.75)    ###0.83(0.75-0.90)(Goodell et al., 2013)
  SpOF ~ dbeta(39.97, 4.344)     ###0.95(0.80-0.97)

#within 2 week infection
  #SeOF ~ dbeta(1.22,0.60)  ### (Goodell et al., 2013)
  #SpOF ~ dbeta(1, 1)          ### non-informative prior

#Unknown course of infection
  #SeOF ~ dbeta(1,1)         ### non-informative prior
  #SpOF ~ dbeta(1,1)             ### non-informative prior

#############################################################

}
```

Chapter 4 : Bayesian estimation of the reproductive number for influenza A virus infection accounted for imperfect diagnostic test of respiratory clinical sign observation in growing pig populations

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[in prep, to be submitted to J Vet Epi]

Summary

Influenza A virus (IAV) infection is the cause of an economically important respiratory disease in growing pigs. On-farm assessment of health on an individual level for IAV infection may need the use of respiratory clinical sign (RCS) and behavioral observations, and such observational data are prone to misclassification. The elimination of misclassification bias introduced by imperfect diagnostic test of RCS was modeled to estimate transmission rate (β) and the effective reproductive number (Ŗ) of IAV transmission within a herd. Data were from an observational study by investigating RCS presentation of growing pigs during IAV outbreak. Growing pigs of 100 each from two cohorts (cohort 3 and 5) were randomly chosen and tagged. Pigs were observed daily in total of 70 days by a one trained observer to investigate RCS on an individual level during IAV outbreak. Bayesian Susceptible-Infectious-Recovery (SIR) models were constructed to estimate β and Ŗ accounted for imperfect diagnostic test of RCS observation. β was estimated to be 1.40 day^{-1} (95%Cridible Interval (CrI): 0.42-5.52) and Ŗ was estimated to be 4.19 (95%CrI: 1.98-26.29). 41.45% of the true infected pigs do not exhibited RCS to IAV infection. Hence, the current study emphasizes the importance of accounting for imperfect diagnostic test of RCS observation to measure IAV transmission, avoid underestimating, falsely interpreting and providing new metrics of Ŗ estimation to assist veterinarians and practitioners to evaluate alternatives for measuring IAV transmission in growing pig populations.

Key words: Bayesian estimation; the reproductive number; influenza A virus; imperfect diagnostic test; respiratory clinical signs; growing pigs

Introduction

Influenza A virus (IAV) is commonly found in global human and pig populations (Fouchier et al., 2005; Russell et al., 2008; Nelson et al., 2012). Belonging to the family *Orthomyxoviridae*, IAV is an enveloped virus containing 8 segments of negative single-stranded RNA virus (Pleschka, 2013). Swine IAV infects the upper and lower respiratory tract associated the porcine respiratory disease complex (PRDC), and cause of an economically important respiratory disease in growing pigs (Vincent et al., 2008; Qi et al., 2011; Deblanc et al., 2012; Detmer et al., 2012; Fablet et al., 2012; Rose et al., 2013).

Respiratory clinical signs (RCS) caused by IAV infection are typically characterized by dyspnea, nasal discharge, sneezing, coughing, and other signs such as a rapid onset with high fever, anorexia, lethargy, ocular discharge, and sometimes, conjunctivitis (Olsen et al., 2000; Van Reeth, 2007; Reeth et al., 2012). While morbidity associated with IAV infection is high and can reaching up to 100%, mortality associated with an IAV infection is generally low and recovery occurs within 7-10 days. Due to high fever and anorexia, pigs may lose of bodyweight due to reduce the feed consumption and average daily weight gain. Thus additional feed and longer duration to reach optimal bodyweight for market are required, resulting increased costs in production (Rose et al., 2013; Er et al., 2014). With consistent clinical signs associated with respiratory infections, studies have suggested that low levels of exposure to IAV might prelude clinical signs in pigs (Van Reeth et al., 1999; Van Reeth, 2000; Van Reeth, 2007). However, subclinical infections associated with IAV infections are very common, and pigs may not exhibit clinical signs or RCS when infected with IAV (Allerson et al., 2013a). Usually, swine IAV clinical signs are frequently seen in only 20 to 30 % of pigs

in a herd (Brown, 2000). In a recent IAV field study, 34.6% pigs exhibited clinical signs but only 22.9% were positive for IAV infection while, 20.9% of the pigs were positive IAV but lack clinical signs (Corzo et al., 2013a). Furthermore, a cough-sneeze score is used as a group-based measurement for disease severity caused by IAV infection, which is based on RCS (Detmer et al., 2013).

RCS observation is a diagnostic tool used to gathered information on animal respiratory diseases and can establish pathways of probable causes of the disease from RCS exhibition through inductive reasoning (Homwong et al., 2015a). For instance, if some pigs infected with IAV, IAV transmission can occur between pigs within a barn due to social behavior or interaction. RCS observation offers a unique opportunity to measure IAV spreading conditional on RCS and may be significantly important in detecting and understanding the dynamics and pattern of IAV transmission. Similarly, RCS observation can be used as a quantitative measure for assessing changes in clinical disease (Baadsgaard and Jørgensen, 2003).

Two main challenges are observed in using RCS as a measurement of disease transmission. First, RCS observation are considered an imperfect diagnostic test, with very low accuracy of the diagnostic ability because IAV infected pigs may lack RCS exhibition; on other hand, such exhibition may not be associated with IAV infections (Allerson et al., 2013c; Homwong et al., 2015b). Second, when dealing with disease outbreak circumstances with exhibiting clinical signs, the actual infectious process and transmission are unobserved or only partially observed. Furthermore, RCS data are complicated by the fact that such data are inherently dependent, usually incomplete and may be missing (O'Neill and Roberts, 1999; O'Neill, 2002). The likelihood function may

become very difficult to evaluate. Consequently, analyzing RCS data with conventional statistical approaches is less efficient (O'Neill and Roberts, 1999).

On the contrary, the two challenges associated with RCS analysis can be overcome by using Bayesian approach, mainly by two advantages. First, the imperfect diagnostic test of RCS observation can be incorporated for the test uncertainty and the test misclassification (Bernatsky et al., 2005; Kostoulas et al., 2009; Correia-Gomes et al., 2014). Second, as mentioned previously, the actual infectious process and transmission are unobserved or only partially observed. The use of datum augmentation (imputation) technique generating from Markov Chain Monte Carlo (MCMC) algorithm is to generate unobserved infectious process and disease transmission, or to impute incomplete or missing data, making an evaluation of the likelihood function much easier (Jewell et al., 2009).

As the IAV ecology has become more complex due to viral reassortment and mutation, the important to quantitatively measure IAV transmission in pig populations is important for veterinarians and researchers. Ɍ has been estimated to understand swine IAV transmission dynamics obtained by several epidemic models such as susceptible-infectious-recovery (SIR) (Romagosa et al., 2011; Allerson et al., 2013b) and an exponential growth rate model (Rose et al., 2013). The SIR model is one of the basic compartment models that take interested stages of infectious diseases process while the exponential growth rate model takes only during the early stages of infectious diseases process. The SIR model is an attractive tool and widely used in better comprehends of disease transmission dynamics.

The IAV transmission has been measured using quantitative RRT-PCR (Romagosa et al., 2011; Allerson et al., 2013b; Rose et al., 2013). Although RCS observations have been routinely used for on-farm diagnosis and monitoring of swine respiratory diseases, the use of RCS observation as a quantitative measurement of IAV transmission in a growing pig population has yet been reported. In addition, it may be alternative measurement of IAV transmission. Due to imperfect test of RCS observation, failure to adjust for misclassification disease outcome of RCS observation may introduce bias measures of diseases (Kostoulas et al., 2009). Therefore, the objective of this study was to estimate the IAV transmission rate and the effective reproductive number (R_t) accounting for imperfect diagnostic test of RCS observation through Bayesian estimation in growing pig populations.

Materials and methods

A cohort study herd

A wean-to-finish farm with a previous history of IAV outbreak during the first month was selected according to the veterinarian's knowledge and previous experience with swine IAV infection. Piglets arrived at the farm from two different batches obtained from the same breeding herd. Such herd was routinely checked for *Mycoplasma hyopneumoniae* and porcine respiratory and reproductive syndrome viruses by ELISA or PCR, and was considered as a negative herd. At 45 days of age, 200 weaned-pigs were randomly selected and ear tagged from two cohorts (cohort 3 and 5, 100 pigs each) to investigate the RCS representation to IAV infection in pigs. The wane-to-finish facility is located in the province of Saskatchewan, Canada. From each cohort, 30 pigs were

randomly selected to achieve the power of statistical test at 0.8, and blood samples were taken at day 1 and 70. The serum samples (n=120) were analyzed for IAV antibodies by ELISA, and S/N ratios ≤ 0.67 were considered positive (IDEXX Laboratories Inc., Westbrook, ME, USA)(Ciacci-Zanella et al., 2010).

The 200 ear-tagged pigs were individually investigated for the representation of RCS for 20 minutes a day, in a total of 70 days. Pigs were required to stand up for at least a minute. If a pig exhibited coughing, sneezing, nasal discharge, respiratory distress, or inactivity, "presence" of RCS was documented. If no RCS were notable, "absent" was noted (Vincent et al., 2008; Corzo et al., 2013a; Detmer et al., 2013; Rose et al., 2013; Homwong et al., 2015b). Cohorts 3 and 5 were recorded for prevalence and incidence of RCS exhibition, respectively. The cohort 3 pigs were followed-up as a group of 100 pigs, then counting the number of representing RCSs while the cohorts 5 pigs were followed-up individually as the number of new cases within a day. To reduce the measurement error, RCS representation was not ranged according to its severity (Gore, 1981) and thus the cough-sneeze score (group-based investigation) was not documented. The on-side investigation was performed by trained farm personnel.

Disease transmission modeling

The SIR model

The IAV transmission process among growing pigs was modeled in accordance with SIR model. The model assumes that in the total population (N), an infectious pig was introduced into a closed, partially-susceptible population with the transmission rate (β) and the recovery rate (γ). Piglets were derived from an historic IAV positive sow

herd. Thus, the piglets were assumed to have maternally-derived IAV immunity. A function of time spent in the infection state and is defined as the number of secondary cases produced by a primary infection in a partially immune population was defied as the effective reproductive number, Ɍ (Woolhouse et al., 1997; Mills et al., 2004; Chowell et al., 2006b; Brauer, 2009; Brauer and Castillo-Chávez, 2013). Since the probability of IAV positive pigs is significantly associated with pig density (Maes et al., 2000; Poljak et al., 2008a), and as population size increases, contact rata increases; thus density-dependent transmission was assumed, which leads to increase of IAV transmission (Anderson and May, 1979; Reynolds et al., 2014).

Likelihood inference

Estimation of the IAV transmission in pigs using prevalence data of RCS was performed using the dynamic SIR model using the likelihood framework. β was parameterized as $\beta = Ɍ^* \gamma$. The system of ordinary differential equations (ODEs) in SIR model was expressed as;

$$\frac{dS(t)}{dt} = -Ɍ\gamma S(t)I(t)$$

$$\frac{dI(t)}{dt} = Ɍ\gamma S(t)I(t) - \gamma I(t)$$

$$\frac{dR(t)}{dt} = \gamma I(t)$$

where S, I and R are the number of susceptible, infectious and recovered individuals, respectively.

The probability (p) of RCS was defined as the number of infectious individuals (I_t) over the susceptible individuals (S_t) at time *t*. The model assumed that (1) all

susceptible individuals have the same probability (p) of exhibiting RCS, (2) individuals exhibited RCS independently, and (3) the distribution of infectious pigs was assumed as binomially distributed. The likelihood ($L(p \mid \cdot)$ of the probability of exhibiting RCS in any individual pig with a particular β value was expressed by;

$$L(p|i_1, i_2, \dots, i_t) = \prod_t \binom{S_t}{i_t} p_t^{x_t} (1 - p_p)^{S_t - i_t},$$

where i_t are the number of exhibiting RCS pigs at time *t*; p_t are the observed prevalence of the number of pigs that were not exhibiting RCS (S_t) at time *t*, and $t \in (1,2, \dots ,70)$. Maximum likelihood estimation method (MLE) was used to fit the dynamic SIR model to the prevalence data to find β that maximize the likelihood. As β in ODEs of the SIR model was expressed in terms of Ʀ and γ, the both parameters eventually maximize the likelihood (Ferrari, 2009; Bellan, 2014). The Nelder-Mead algorithm called through *"optim"* function was used to navigate the likelihood to MLE and the 95% confidence intervals of the parameters (Ʀ and γ) were estimated using profile likelihood methods. The analysis was performed on prevalence data of the cohorts 3, 5 pigs and their combination with the R packages "deSolve" and "fields", and implemented on R version 3.2 (Soetaert et al., 2010; Nychka et al., 2015; R Core Team, 2015).

Bayesian inference

An estimation of the transmission parameters of IAV positive pigs using incidence data of cohort 5 pigs was performed using the stochastic SIR model implemented in Bayesian framework.

Estimation of transmission rate (β) from S to I

The formula y_{obs_t} is the observed number of new RCS case based on imperfect tests at time *t* and assumes $y_{obs_t} \sim Bin(p_t,\ S_t)$, where p_t is the probability of a susceptible pigs at time *t-1*, becoming positive to RCS observation at time *t* and S_t is the number of susceptibles accounting for imperfect diagnostic test to estimate IAV trasmission. The probability p_t due to the imperfect RCS observation was expressed as $p_t = \psi_t Se + (1 - \psi_t)(1 - Sp)$, where $\psi_t$denoted the expected probability of expected positive RCS results due to IAV transmission occurrences within the growing pig population. The Se and Sp are the sensitivity and specificity of the RCS observation, respectively (Kostoulas et al., 2009).

Subsequently, it was assumed that $y_{true_t} \sim Bin(\psi_j,\ S_t)$, where y_{true_t} is the true number of new cases after adjusting for imperfect test at time *t*. The ψ_t was modeled using $cloglog(\psi_t) = \log(\beta) + \log(\frac{I_{t-1}\Delta t}{N_{t-1}})$, using binomial regression with complementary log-log link function, where β is the transmission rate; I_{t-1} is the true number of infectious pigs at the end of time step (*t-1*); N_{t-1} is the total number of pigs; and Δt is time step in day ($\Delta t = 1$) (Correia-Gomes et al., 2014). By imperfect RCS observation, the expected number of I can also be represented as the sum of two binomial distribution expressed by $I = I_{TP} + I_{FN}$, where I_{TP} ~Bin(Se, I_{obs}) and I_{FN}~Bin(1-Sp, N-I_{obs}). I_{TP} is the number of positive IAV pigs as correctly classified and I_{FN} is the number of true IAV positive pigs being misclassified. Hence, β was estimated by exponentiating of $\log(\beta)$.

Estimation of recovery rate (γ) from I to R

The number of new recovered pigs at time *t* was assumed as $R_t \sim Bin(pi_t,\ I_t)$, where pi_t is the probability of infectious pigs at time *t-1* becoming recovered at time t, and I_t is the number of infectious pigs at time *t*. The probability pi_t was modeled using $cloglog(pi_t) = \log(\gamma)$ using binomial regression with complementary log-log link function, where γ is the recovered rate, assumed as exponentially distributed.

Estimation of the effective reproductive number (Ʀ)

The effective reproductive numbers (Ʀ) was calculated by Ʀ=β/γ.

Prior information

In Bayesian framework, the estimated parameters were considered random variables, and the prior distributions are expressed as the uncertainty about their qualifications of the data. Since information is lacking regarding the parameter to be estimated, log (β), which is a log-transformed scale of β, non-informative prior was applied and modeled using log (β)~Norm(0, 1000). The prior (γ) was obtained from the previous estimation by likelihood inference. However, the Se and Sp of RCS observation for IAV infection in growing pigs were modeled using Beta distributions, obtained from previous report, Se=0.38 [95%CrI: 0.28-0.48] and Sp=0.66 [95%CrI: 0.61-0.71], respectively (Homwong et al., 2015b).

Scenarios

The two stochastic SIR models with Bayesian framework were run, assuming (1) a perfect or (2) imperfect diagnostic test. The point estimates of Ʀ from two scenarios

were used to simulate the epidemic curves to evaluate hidden pig proportion (infected but not shows RCS).

Bayesian computation

The two stochastic SIR models were computed using Markov Chain Monte Carlo (MCMC) implemented in JAGS 3.4.0 (Plummer, 2015a). The JAGS model codes were run in R v3.2.1 and "rjugs" and "R2jags" packages (Plummer, 2015b; R Core Team, 2015; Su and Yajima, 2015). The 100,000 iterations with three chains for posterior samples were run, and the first 2,000 iterations were discarded. The results were sampled every 5 iterations. The analysis of MCMC chains was performed and graphed using the "CODA", "ggmcmc" and "ggplots2"packages (Plummer et al., 2006; Hadley, 2009; Marin, 2013). The convergence and mixing of the chains were assessed by visual inspection using Traceplots, and by quantitative inspection using Gelman-Rubin R-hat, diagnostic Geweke z-score plots and Monte Carlo errors (<5%) (Geweke, 1991; Gelman and Rubin, 1992). MCMC sample mean and median were presented as a point estimate while the 2.5 and 97.5 percentiles were presented as 95% credible intervals (CrI). Positive predictive check of Bayesian p-value was used to assess the predictive probability and to measure goodness of fit the model (close to 0.5) (Carlin and Louis, 2008; Geurden et al., 2008; Lunn et al., 2012). The final model was selected based upon DIC, where a lower difference of 5 is substantially a better model (Spiegelhalter et al., 2002; Carlin and Louis, 2008). In the final model, sensitivity analysis of the prior was not performed because non-informative prior has employed.

Results

Sixty samples were taken from two cohort pigs at sampling day 1 and 70. For cohort 3, the ELISA results yield 2 (6.7%) and 27 (90%) to seropositive IAV samples at day 1 and 70. For the cohort 5, the ELISA results yielded 0 and 28 (93.3%) positive IAV samples at day 1 and 70, respectively. Time-series plot for the epidemic curve of the cohort 3 and 5 pigs represents RCS. The epidemic curve illustrates a progression of representing RCS in an outbreak over time (day). The peak of RCS was approximately at day 20 **(Figure 4-1**). The epidemic curve analyses were performed to estimate IAV transmission parameters, Ʀ and β (1/infectious period). For the cohort 3, Ʀ was estimated to be 2.91 (95%CI: 2.73-3.10) and β was estimated to be 0.23 (95%CI: 0.21-0.24) day^{-1} **(Table 4-1).** For the cohort 5, Ʀ was estimated to be 2.73 (95%CI: 2.55-3.93), and β was estimated to be 0.22 day^{-1} (95%CI: 0.20-0.23). In the combined analysis of the cohort 3 and 5 pigs, Ʀ was estimated to be 2.98 (95%CI: 2.77-3.02) and β was estimated to be 0.19 (95%CI: 0.18-0.21). Besides, fitted epidemic curves using previous estimated parameters were simulated and superimposed on observed epidemic curves for the cohorts 3 and 5 **(Figure 4-2)**.

With Bayesian framework, the stochastic SIR models were run with two scenarios (perfect and imperfect diagnostic tests). With the perfect diagnostic test scenario, β was estimated to be 0.64 day^{-1} (95%CrI: 0.56-0.73) and Ʀ was estimated to be was 3.04 (95%CrI: 2.66-3.46) while the imperfect diagnostic test scenario, β was estimated to be 1.40 day^{-1} (95%CrI: 0.42-5.52) and Ʀ was estimated to be 4.19 (95%CrI: 1.98-26.29) **(Table 4-2)**. The posterior distribution of β was represented in **Figure 4-3** and that of Ʀ was graphed in **Figure 4-4**. The predictive probabilities of Ʀ that was greater than or

equal to 2 or 3 were estimated to be 0.97 or 0.71, respectively. Recalled the current modeling framework with the imperfect diagnostic test scenario includes the Se and Sp of RCS observation being the prior information. The posterior distributions of estimated Se and Sp were 0.53 (95%CrI: 0.13-0.98) and 0.99 (95%CrI: 0.98-0.99) **(Table 4-2)**. The point estimates from two scenarios were used to simulate the epidemic curves. By comparing two scenarios, the proportion of infected pigs but not exhibits RCS was 41.5% **(Figure 4-5)**.

The goodness of model fit was acceptable with a Bayesian p-value of 0.71 and 0.67 for perfect and imperfect scenarios, implying no significant difference between posterior predictive distributions. The convergence and mixing of the MCMC chains were well-mixed by investigating the Gelman-Rubin statistic R-hat and diagnostic Geweke z-score plots **(Supplemental material)**. Monte Carlo errors for parameters were less than <5% for the estimated parameters of two scenarios. The difference in DIC for the models with accounting for imperfect test of RCS observation (latter scenario) was 7.85 (240.62-232.77) lower than the perfect test scenario (the fist scenario), implying that imperfect diagnostic test scenario was better model.

Discussion

The term “clinimetrics” has been proposed by Feinstein in order to clearly define characteristics of clinical signs (Feinstein, 1987). Using more precise clinical definition can reduce the uncertainty of clinical diagnostic test (Somoza and Mossman, 1992). However, the relationship between pathological changes and the clinical status is very complex (Baadsgaard and Jørgensen, 2003). Considerations of imperfect test do not have

interaction on the underlying epidemiological dynamics (Keeling and Rohani, 2008). In addition, observed clinical signs are purely a function of the test accuracy (Se and Sp of the imperfect test). Thus, imperfect RCS observation is able to be applied to measure quantitative IAV transmission, which were considered and accounted for the current study.

IAV often has been described as an occasional outbreak with a time-limited impact on herd health. On-farm assessment for IAV infection requires the use of respiratory clinical and behavioral observations (Baadsgaard and Jørgensen, 2003). It is by the fact that as soon as one day post-infected, pigs are able to exhibit clinical signs such as coughing (Choi et al., 2004). However, as a population level, the test accuracy of RCS observation was rather low with Se of 38% and Sp of 66%, which are prone to misclassification (Homwong et al., 2015b). Correct classification of IAV infection using RCS in pigs is difficult since RCS is a subjective measure. RCS observation lacks of Se and pigs may not display clinical signs in the first days of infection. Misclassification can create much more uncertainty in estimations of parameters, measurements of association, and between-individual observers (Baadsgaard and Jørgensen, 2003; Dohoo et al., 2010).

The problem of estimating transmission rate via RCS observation without accounting for the imperfect results of a diagnostic test is that transmission rates do not compare across populations because it presents the joint effect of observed bias. Additionally, the prevalence calculated from RCS observation may underestimate β and R_0, thus resulting in being potentially biased, and misleading for control, prevention and perhaps for IAV elimination from swine herds (Dohoo et al., 2010; Otten et al., 2013). In our current study, Bayesian models employed RCS observation that was considered

perfect or imperfect diagnostic tests and the Rs were estimated to be 3.04 (95%CrI: 2.66-3.46 and 4.19 (95%CrI: 1.98-26.29), respectively. The failure to account for imperfection of a test leads to underestimate. In addition, the CrI obtained from the model that was assumed that RCS observation was imperfect was wider than the CrI obtained from that RCS observation was perfect RCS, indicating that misclassification error can create much more uncertainty in estimating parameters.

Epidemic data for RCS observation was modeled by using likelihood and Bayesian frameworks. The likelihood framework assumes data are random, and the interpretation is based on repeated sampling. Bayesian framework assumes data are fixed after they have been observed, and the interpretation is based on its priors and data (Greenland, 2006). Infectious diseases in field setting were quite improbable to reproduce having similar effects as an experimental challenge study. Therefore, the likelihood frameworks with repeated sampling for infectious disease outbreak data would fail to analyze an outbreak event (Clancy and O'Neill, 2008). Event though, our estimates from both approaches are comparable, i.e.2.98 (95%CI: 2.77-3.02) and 3.04 (95%CrI: 2.66-3.46) for the likelihood and Bayesian frameworks, respectively. Unsurprisingly, it is because in Bayesian model, non-informative priors were applied. By incorporating prior knowledge of imperfect diagnostic capability of RCS observation and extending models that accounted for such diagnostic capability, Bayesian approach offers a great power, flexibility and facilitates adjusting the misclassification errors of disease outcomes as compared to the likelihood framework (Clancy and O'Neill, 2008).

To the best of our knowledge, the current study is the first report for estimating R for IAV transmission measured by RCS observation in growing pigs through Bayesian

probabilistic model, which was incorporating prior knowledge of RCS imperfect diagnostic test. While non-informative priors establish a baseline scenario, it is reasonable to use informative priors based on epidemiological beliefs (O'Neill, 2002). The current study investigated hided pig proportion (infected but not show RCS) by comparing two scenarios without and with considering the imperfection of RCS observation. The proportion of hidden pigs was 41.45% over the expected infected population after accounting for imperfect RCS observation. Ŗ is used for measuring and understanding IAV transmissibility to both prevent and intervene IAV infections. Specific intervention such as vaccination may apply. The critical coverage of vaccination required to control IAV in a randomly mixing growing pig population can be determined by Ŗ (Nishiura and Chowell, 2009). In the current study, Ŗ was estimated to be 4.19 (95%CrI: 1.98-26.29), and the probabilities that Ŗ is greater than 1, 2 and 3 was 1.00, 0.97 and 0.71, respectively, indicating that IAV is rapidly spreading in a growing pig population with partial immunity. If veterinarians wish to control IAV transmissions in this population by vaccination and vaccine effectiveness (VE) against infection was 98.62% (95%CI: 92.96-99.73%) (Homwong et al., 2015b), at least 74.6% $[VE * (1 - \frac{1}{Ŗ})]$ ranging from 48.5% to 94.3% of this population needs to be vaccinated. A large confidence interval was due to uncertainty in Ŗ as aforementioned.

Many pigs in the study were vaccinated with IAV H1N1 vaccine or already have antibodies due to natural infection with H1N1, and the pigs should be protected from severe clinical signs due to IAV H1N1 infection. IAV subtypes cannot be differentiated by RCS observation as a study reported that pigs infected with H3N2 or H1N1 have same RCS representation, and the same field reports suggest that H3N2 infections are more

severe than H1N1 infections (Rose et al., 2013). At least, these estimates may potentially be a measurement of IAV within-herd transmission when accounting as imperfect diagnostics test characteristic for RCS. By not considering the imperfect diagnostic test, the estimates are underestimated, and should not be used.

R_t in the current study (R_t=4.2) was similar to previous research (R_t=4.1) (Rose et al., 2013). The assumption behind those estimates is that populations have partial immunity, and both are from field settings. Unsurprisingly, the current estimate was in between previous reports of the effective reproductive number (i.e. R_t=7.1 and R_t=0.99) (Romagosa et al., 2011; Allerson et al., 2013b). It is important to note that an assumption behind both estimates is of heterogeneous vaccine challenging populations. Therefore, different level of immunity may affect estimates. In addition, R_t may differ because of age at infection or may differ due to waning immunity (waning immunity is a function of infection time and levels of maternal immunity). Firstly with levels of immunity, our study population has historically IAV infection in sows and the piglets received passive immunity while the previous studies used naïve piglets. Piglets receive high titer of IAV maternal immunity, and parity 4 sows have significant shorter for infectiousness duration, leading lower the R estimate (Rose et al., 2013). Secondly, age of infection affects infectious period with significantly shorter in older pigs and longer in younger in younger pigs, leading to difference R_t with 6.9 and 3.2, respectively. The same study concluded IAV-subtypes had no direct relationship with R_t estimates (Rose et al., 2013). Thirdly with waning immunity, the older pigs, especially pigs greater than 80 days were found that higher R estimates due to waning immunity, arising arising lower level of maternal immunity (Rose et al., 2013). Another reported that at approximately 80 days of age, 80%

of growing pigs do not have maternal immunity against IAV when entering the finishing facilities(Loeffen et al., 2003). By considering the R estimate, IAV maintains in the wean-to-finish pigs, and most of the pigs were positive with 90.0% and 93.3% for the cohorts 3 and 5, respectively.

The observation period was 70 days, and the observer was not re-trained during a period of the study. Thus, RCS observation may create more misclassification and increase uncertainty associated to the estimate, indicated by the wilder credible interval (CrI). The estimate for the recovery rate is not affected by misclassification because our models did attempt to estimate this parameter since it was calculated from the likelihood inference method. Due to flexibility of Bayesian modeling, more complicated/flexible models can be formulated to estimate misclassification rate. Since our models were designed to combine misclassification effects as a whole over the period, the correct classification of IAV infection status in pigs is difficult because of the subclinical signs. In cases of an acute IAV infection, a change on the behavior may reduce the likelihood of exhibiting the RCS and may increase the Se of RCS (reduce imperfect testability).

Limitations of this study are that data RCS might not be present during times of conducting observations. In addition, the severity of the clinical signs varied, ranging from only coughing to a combination of coughing, labored breathing, and decrease in feed consumption. IAV was detected in the farm by ELISA and the viruses were not subtyped because different subtypes of IAV might cause different RCS. Using RCS in some circumstances, a case definition is difficult to include and exclude every true case of the diseases (Smith, 2006).

Conclusions

Bayesian framework for RCS observation, with adjustments for imperfect diagnostic tests, proved quantifying transmission rate and the reproductive number. This study emphasized the way of alleviating the problem with the reliability of RCS observation and the magnitude of the reproductive number in modern growing pig production. The lack of Se caused an underestimation of β and Ɍ. After accounting for its imperfect test ability, RCS observation is potentially a quantitative measure of IAV transmissibility, and Ɍ was estimated to be 4.19. The more precise the case definition of RCS is, the better the decision of veterinarians's diagnosis will be.

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Conflict of interest

The authors declare they have no conflict of interest.

Chapter appendix

The representation of the estimation framework for observably infectious number as a function of true infectious number after accounting for misclassification

	Truly positive	**Truly negative**	**Observed probability**	**Observed Number**	**Observably infectious number as a function of I_{true}**
Observably positive	Se	1-Sp	$\Psi Se+(1-\psi)(1-Sp)$	I_{obs}	$Se*I_{true}+(1-Sp)*(N-I_{true})$
Observably negative	1-Se	Sp	$\Psi(1-Se)+(1-\psi)Sp$	$N-I_{obs}$	$(1-Se)*I_{true}+Sp*(N-I_{true})$
Expected probability	ψ	$1-\psi$	1	-	-
Expected number	I_{true}	$N-I_{true}$	-	N	N
Expected number as a function of I_{obs}	$Se*I_{obs}+(1-Se)*(N-I_{obs})$	$(1-Sp)*I_{obs}+Sp*(N-I_{obs})$	-	N	N

The ψ is the expected probability of expected positive RCS results due to IAV transmissibility in a growing pig population.

The $\Psi Se+(1-\psi)(1-Sp)$ is the observed probability of observed negative RCS results based on the imperfect test for RCS observation.

The Se is sensitivity of the imperfect test for RCS observation.

The Sp is specificity of the imperfect test for RCS observation.

I_{obs} is the observed number of positive individuals by RCS observation.

I_{true} is the true number of positive individuals by after adjusting for imperfect RCS observation.

N is the total number of population size.

The expected number of I_{true} is represented as the sum of two binomial distributions expressed as $I_{true} = I_{TP}+ I_{FN}$, where for stochastic models, $I_{TP} \sim Bin(Se, I_{obs})$ and $I_{FN} \sim Bin(1-Sp,N- I_{obs})$ and for deterministic models, $I_{true} = Se*I_{obs}+(1-Se)*(N-I_{obs})$.

Similarly, the recorded number of I_{obs} is also represented as the sum of two binomial distributions expressed as $I_{obs} = I_{TP}+ (N-I_{TP})_{FP}$, where for stochastic models, $I_{TP} \sim Bin(Se, I_{true})$ and $(N-I_{TP})_{FP} \sim Bin(1-Sp,N- I_{true})$ and for deterministic models, $I_{obs} = Se*I_{true} + (1-Sp)*(N-I_{true})$. In the close population SIR model without having a measurement error, as N=S+I+R, the quantity of $(N-I_{TP})_{FP}$ is referred to as N-I=S+R.

Tables and Figures

Table 4-1: Summary for the reproductive number and recovery rate (1/infectious period) for influenza A virus transmission measured via RCS observation modeled by ordinary differential equation through maximum likelihood estimation (MLE) using prevalence data

Population	Parameter				
	The reproductive number (Ṟ)		Recovery rate (γ), 1/day		Infectious period (1/γ), day
	Estimate	95%CI	Estimate	95%CI	Estimate
Cohort 3	2.91	2.73-3.10	0.23	0.21-0.24	4.35
Cohort 5	2.73	2.55-3.93	0.22	0.20-0.23	4.64
Combined population	2.98	2.77-3.02	0.19	0.18-0.21	5.14

Figure 4-1 : Summary for the transmission parameters and the reproductive number for IAV transmission measured via RCS observation obtained from the cohort 5

Parameters	Median	Mean	SD	95% CrI	Rhat	Dbar	pD	DIC
RCS observation assumed as perfect diagnostic test						239.6	0.98	240.6
β	0.64	0.64	0.04	0.56-0.73	1.0			
Ʀ	3.04	3.04	0.21	2.66-3.46	1.0			
RCS observation assumed as imperfect diagnostic test						225.6	7.10	232.8
β	0.88	1.40	1.36	0.42-5.52	1.0			
Ʀ	4.19	6.68	6.48	1.98-26.29	1.0			
Se	0.53	0.54	0.25	0.13-0.98	1.0			
Sp	0.99	0.99	0.03	0.98-0.99	1.0			

The two Bayesian SIR models were modeled with two assumptions of perfect and imperfect RCS observation using incidence data for the cohort 5 growing pig population.

β denotes transmission rate from susceptible *S* to infectious *I*.

Ʀ denotes the reproductive number

Se denotes sensitivity

Sp denotes sensitivity

Figure 4-2: Time-series plot for the epidemic curve for pigs represents RCS for the cohort 3 and 5

The epidemic curve shows progression of representing RCS in an outbreak over time (day). During ongoing outbreak RCS observation, the epidemic curve is updated as new RCS reporting data become obtainable.

Legend:

The horizontal axis (x-axis) is the day when a growing pig exhibits RCS. The day 1 of the study is approximate to 45 days of pig age

The vertical axis (y-axis) is the number of pig exhibits RCS each day.

The dash line reflects the cohort 3 of the growing pig population.

The solid line reflects the cohort 5 of the growing pig population.

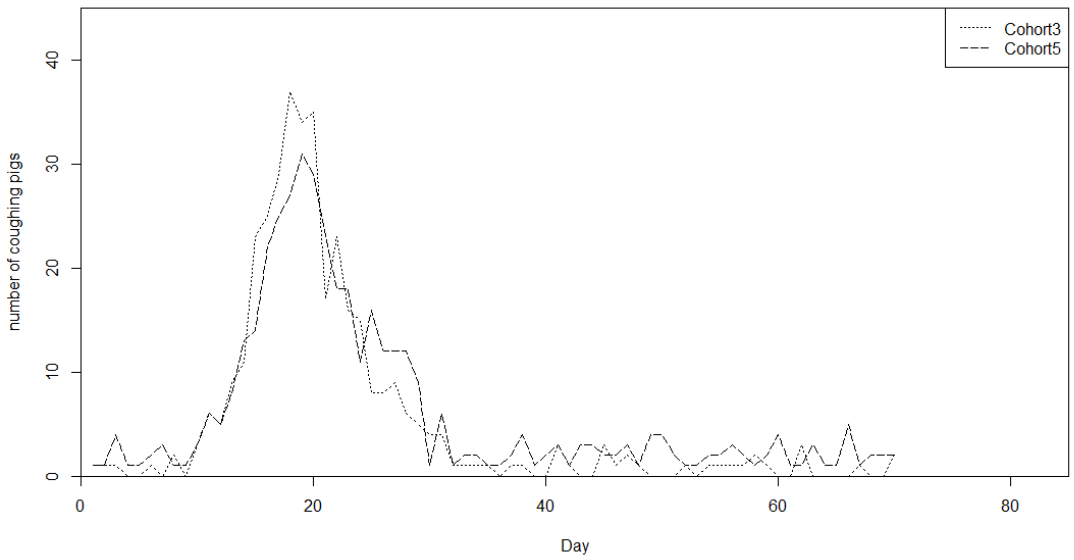
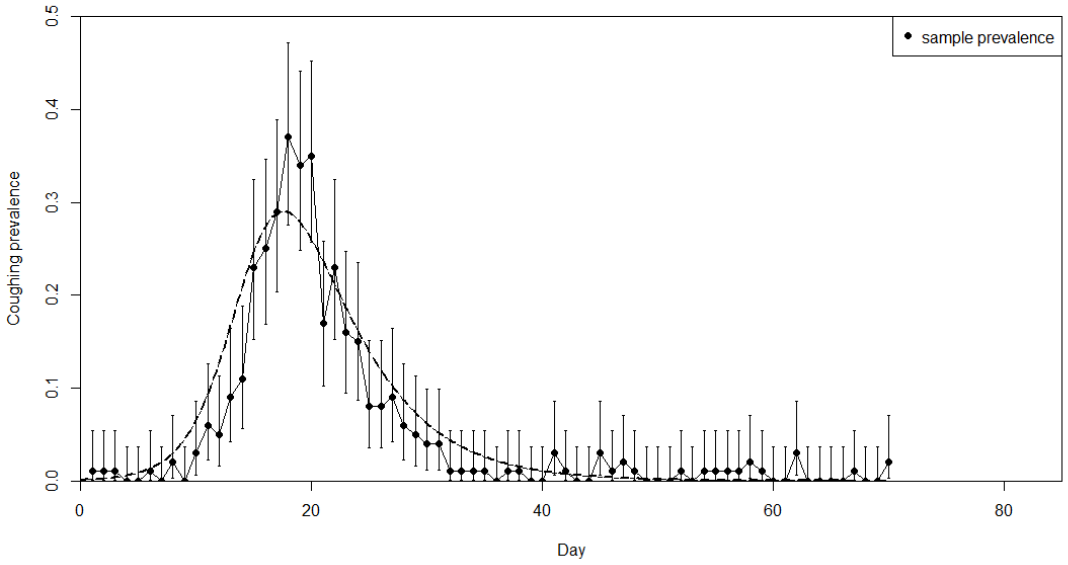


Figure 4-3: The fitted epidemic curve superimposed on the observed epidemic curve with the vertical line being 95%CI around the observed epidemic curve for the cohort 3 (above) and for the cohort 5 (below)



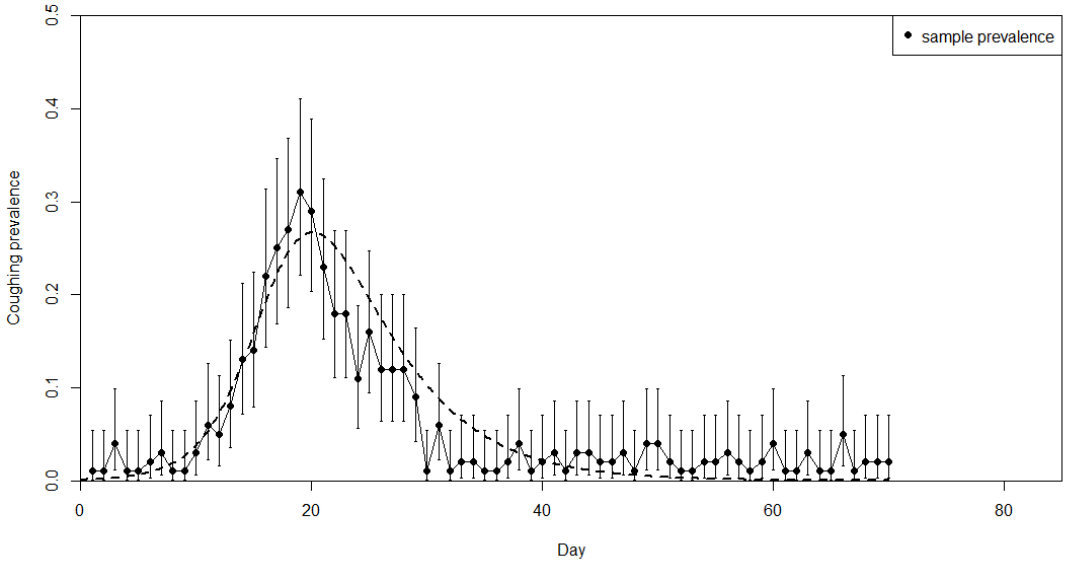


Figure 4-4: The posterior distribution of the transmission rate parameter (β) indicating the rate at which IAV spreads from susceptible to infectious pigs

Legend:

Top-left) Boxplots of the posterior samples used to generate the transmission rate plot where the thick line in the plot reflects the median assuming the perfect test for RCS observation.

Bottom-left) Plots of the posterior distribution for the transmission rate parameter assuming the perfect test for RCS observation.

Top-right) Boxplots of the posterior samples used to generate the transmission rate plot where the thick line in the plot reflects the median assuming the imperfect test for RCS observation.

Bottom-right) Plots of the posterior distribution for the transmission rate parameter assuming the imperfect test for RCS observation.

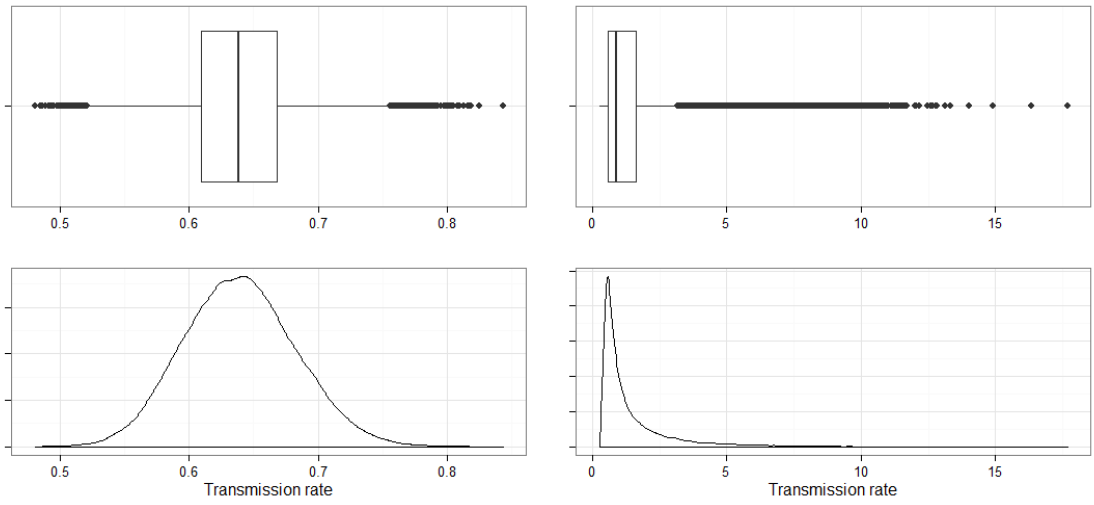


Figure 4-5: The posterior distribution for the effective reproductive number (Ṟ), indicating the number of new infected pigs from one infectious pig in partially immune population

Legend:

Top-left) Boxplots of the posterior samples used to generate the reproductive number plot where the thick line in the plot reflects the median assuming the perfect test for RCS observation.

Bottom-left) Plots of the posterior distribution for the reproductive number assuming the perfect test for RCS observation.

Top-right) Boxplots of the posterior samples used to generate the reproductive number plot where the thick line in the plot reflects the median assuming the imperfect test for RCS observation.

Bottom-right) Plots of the posterior distribution for the reproductive number assuming the imperfect test for RCS observation.

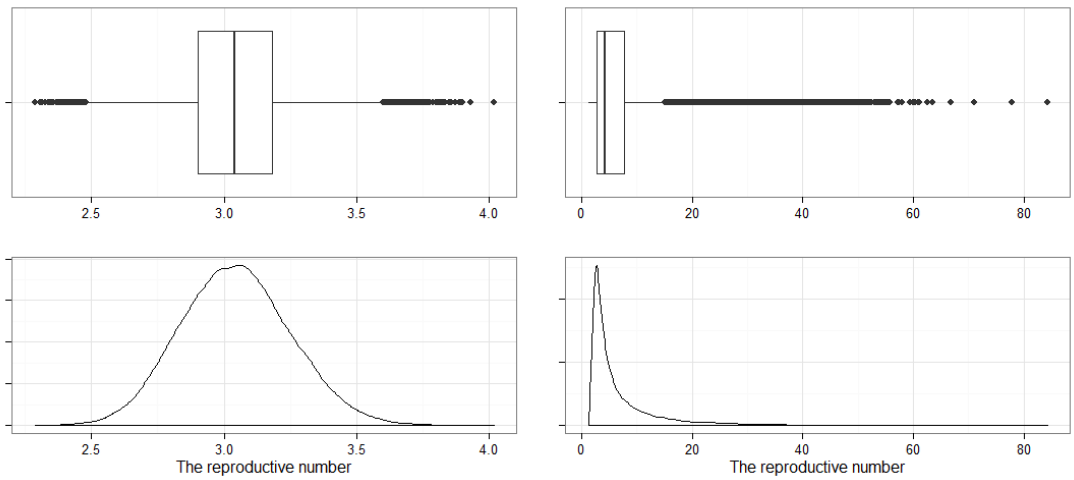


Figure 4-6: The SIR simulations for the epidemic curve by which RCS observation assuming as the perfect test or such observation accounting for the imperfect test for IAV transmission in the growing pig population

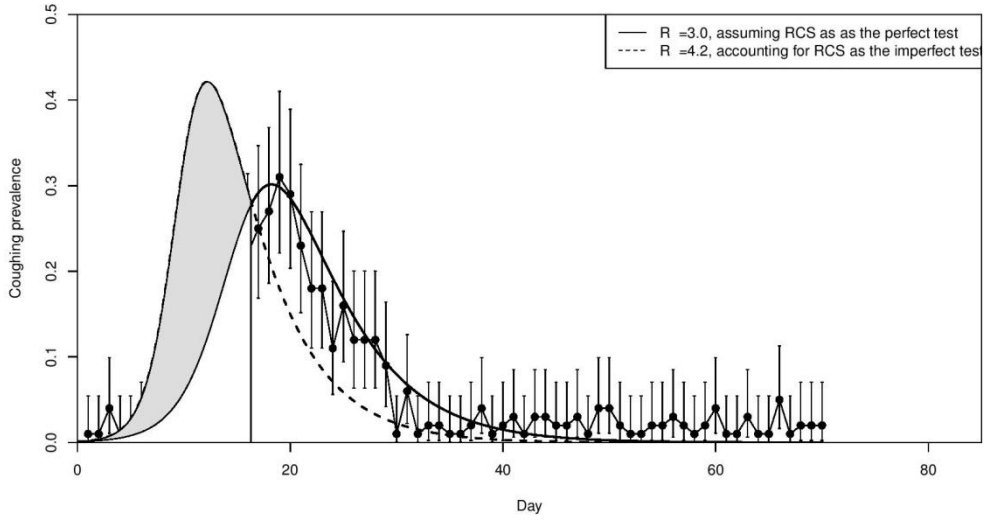
Legend:

The dash line reflects the epidemic curve for IAV transmission measured by RCS observation by accounting for such measurement has imperfect test ability.

Solid points represent the sample prevalence corresponding to their 95% exact confident intervals.

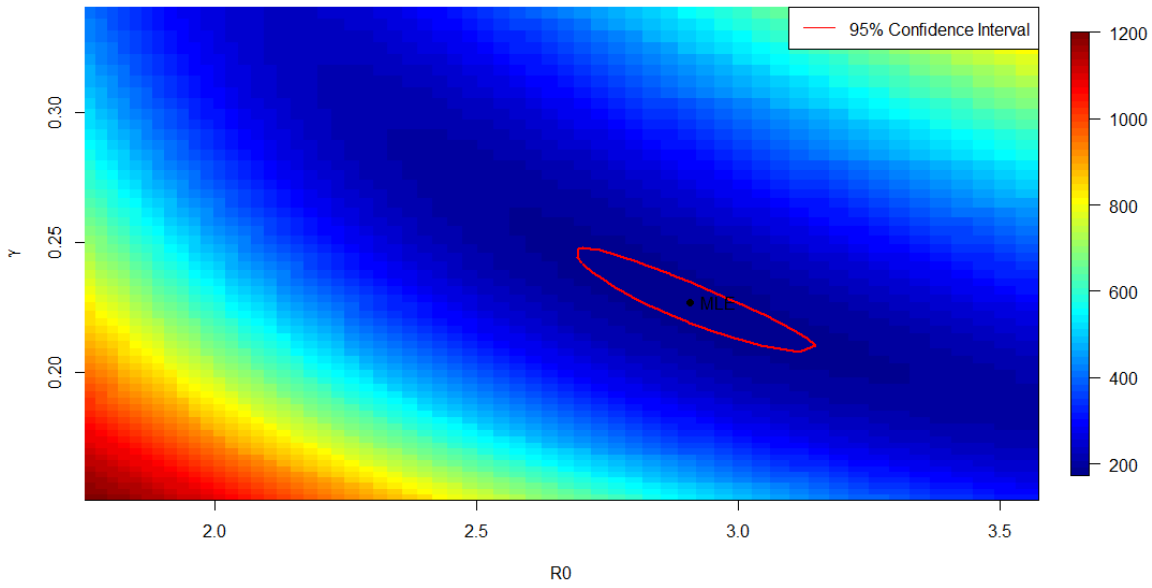
The solid line reflects the epidemic curve for IAV transmission measured by RCS observation by assuming that such measurement has perfect test ability.

The gray area represents the proportion of hidden pigs that have not been able to observe RCS after IAV outbreaks in the population.

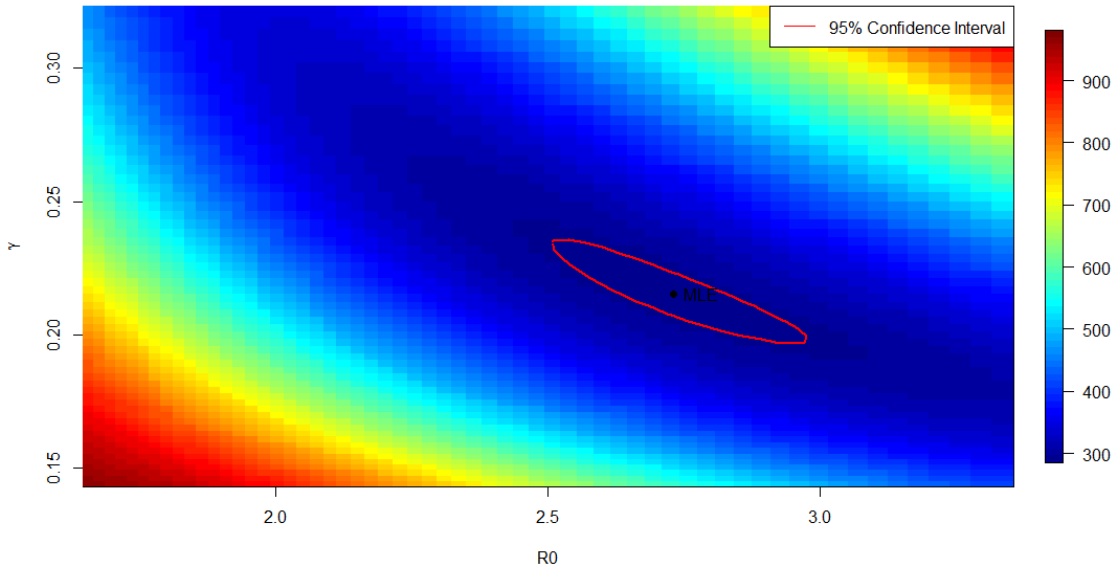


Supplemental materials

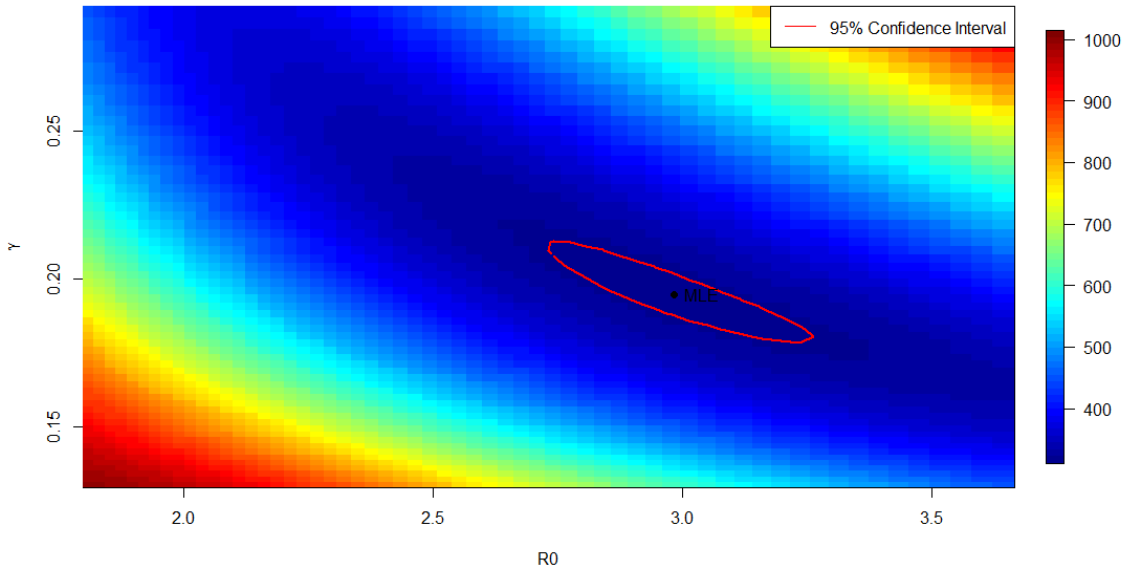
Supplemental material 1 represents an analysis of the negative log-likelihood of the SIR model **for the cohort 3**. The gradient color scales represent the values of negative log-likelihood using the likelihood profiles while the black point is the point estimate of the best parameter combination (MLE) to the likelihood. The red ellipse represents the contour for the 95% profile likelihood confidence intervals corresponding to bivariate MLE for γ and Ɍ estimate. The y axis represents the estimated recovery rate (γ) and the x axis represents Ɍ estimate.



Supplemental material 2 represents an analysis of the negative log-likelihood of the SIR model **for the cohort 5**. The gradient color scales represent the values of negative log-likelihood using the likelihood profiles while the black point is the point estimate of the best parameter combination (MLE) to the likelihood. The red ellipse represents the contour for 95% profile likelihood confidence intervals corresponding to bivariate MLE for γ and Ʀ estimate. The y axis represents the estimated recovery rate (γ) and the x axis represents Ʀ estimate.



Supplemental material 3 represents an analysis of the negative log-likelihood of the SIR model **for the combined cohorts 3 and 5**. The gradient color scales represent the values of negative log-likelihood using the likelihood profiles while the black point is the point estimate of the best parameter combination (MLE) to the likelihood. The red ellipse represents the contour for 95% profile likelihood confidence intervals corresponding to bivariate MLE for γ and Ʀ estimate. The y axis represents the estimated recovery rate (γ) and the x axis represents Ʀ estimate.



Supplemental material 4. The posterior distribution for the recovery rate (γ), indicating the rate at which IAV spreads from infectious to recovered pigs.

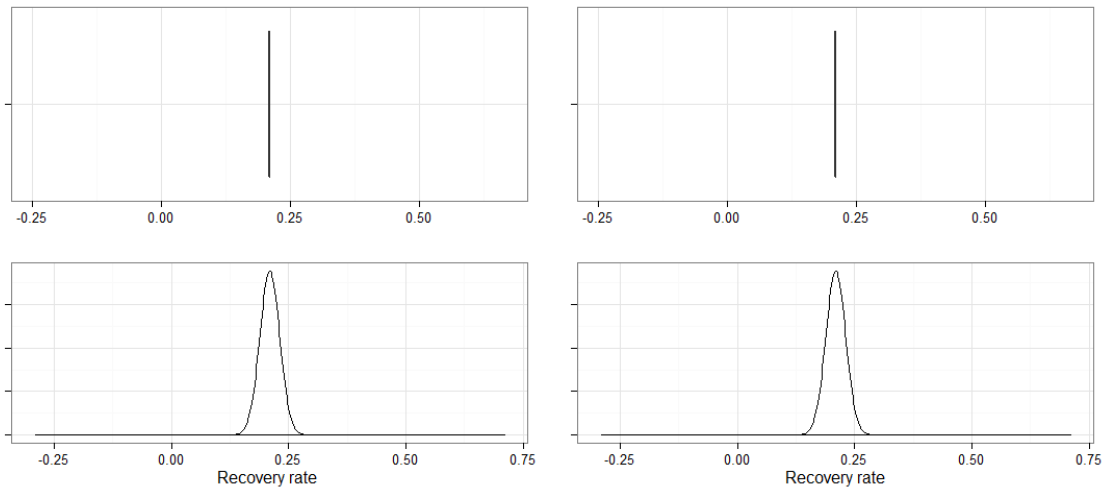
Legend:

Top-left) Boxplots of the posterior samples used to generate the recovery rate plot where the thick line in the plot reflects the median assuming the perfect test for RCS observation.

Bottom-left) Plots of the posterior distribution for the recovery rate assuming the perfect test for RCS observation.

Top-right) Boxplots of the posterior samples used to generate the recovery rate plot where the thick line in the plot reflects the median assuming the imperfect test for RCS observation.

Bottom-right) Plots of the posterior distribution for the recovery rate assuming the imperfect test for RCS observation.



Supplemental material 5. The posterior distribution for the Se and Sp of RCS observation.

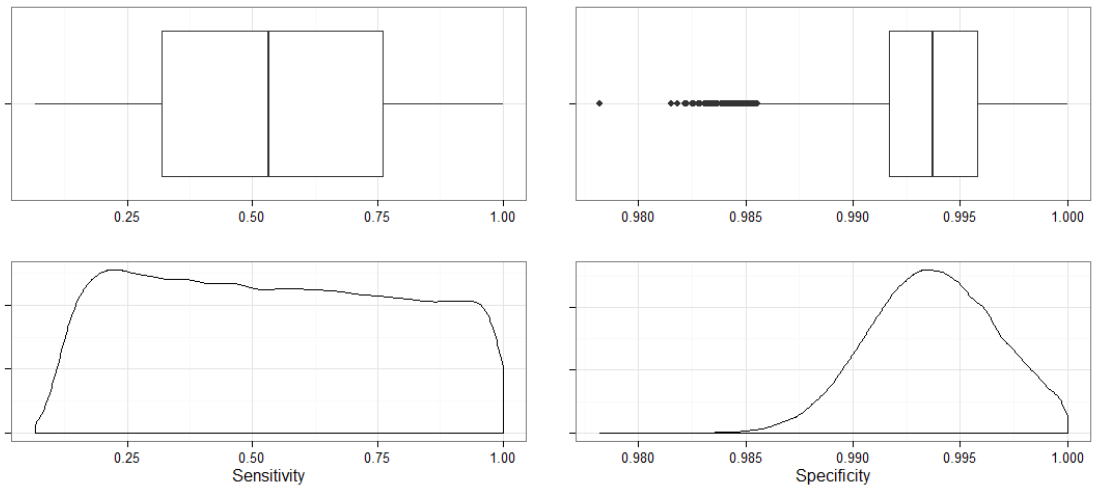
Legend:

Top-left) Boxplots of the posterior samples used to generate the Se and Sp plot where the thick line in the plot reflects the median assuming the perfect test for RCS observation.

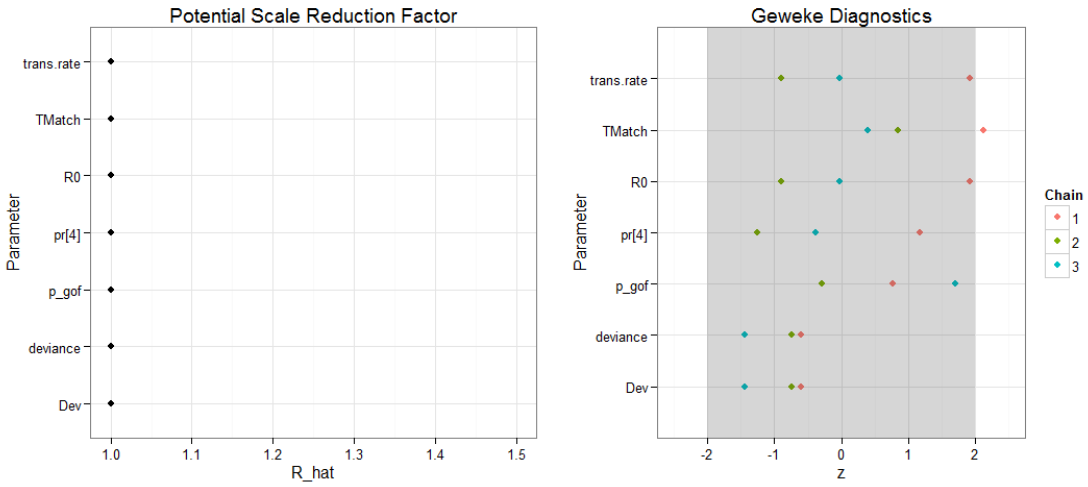
Bottom-left) Plots of the posterior distribution for the reproductive number assuming the perfect test for RCS observation.

Top-right) Boxplots of the posterior samples used to generate the reproductive number plot where the thick line in the plot reflects the median assuming the imperfect test for RCS observation.

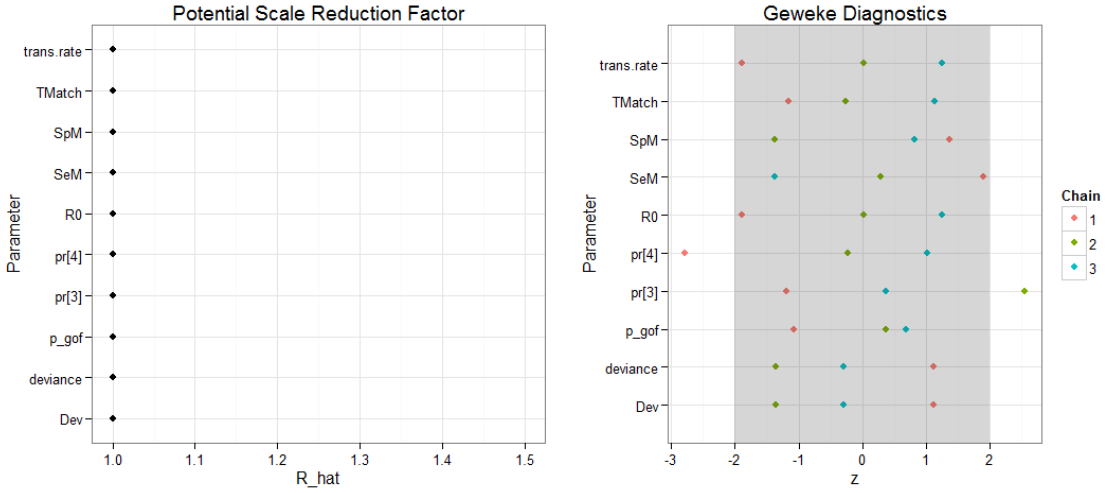
Bottom-right) Plots of the posterior distribution for the reproductive number assuming the imperfect test for RCS observation.



Supplemental material 6. The assessment of the convergence and mixing of the MCMC chains quantitative inspections using Gelman-Rubin R hat (left), diagnostic Geweke z-score (right) plots for which the model was assumed as perfect diagnostic test of RCS observation.



Supplemental material 7. The assessment of the convergence and mixing of the MCMC chains quantitative inspections using Gelman-Rubin R hat (left), diagnostic Geweke z-score (right) plots for which the model was assumed as imperfect diagnostic test of RCS observation.



Supplemental material 8. Bayesian model of within-herd IAV transmission

Transmission rate (β) from S to I

$y_{obs_t} \sim Bin(p_t,\ S_t)$,
$p_t = \psi_t Se + (1 - \psi_t)(1 - Sp)$,
$y_{true_t} \sim Bin(\psi_j,\ S_t)$,
$cloglog(\psi_t) = \log(\beta) + \log(\frac{I_{t-1}\Delta t}{N_{t-1}})$,

$I = I_{TP} + I_{FN}$

$I_{TP} \sim Bin(Se, I_{obs})$

$I_{FN} \sim Bin(1-Sp, N- I_{obs})$

Recovery rate (γ) from I to R

$R_t \sim Bin(pi_t,\ I_t)$,

$cloglog(pi_t) = \log(\gamma)$,

Prior information

$\log(\beta) \sim Norm(0, 1000)$

$Se \sim Beta(33.50, 55.27)$

$Sp \sim Beta(226.59, 116.27)$

γ =0.21, obtained from likelihood inference

$u_t \sim Norm(0, \sigma_u^2)$

$Se \sim beta(a_{se}, b_{se})$

$Sp \sim beta(a_{sp}, b_{sp})$

Chapter 5 : Stochastic modeling effects of waning maternal immunity on influenza A virus transmission in wean-to-finish pig populations

Summary

Influenza A virus (IAV) is considered one of the top three respiratory diseases in terms of frequency of appearance in North American swine herd. It has been reported that 80% of growing pigs do not have maternal immunity against IAV at an entry of finishing facilities. Therefore, the objectives of this study were to 1) estimate the rate of waning maternal immunity of IAV-H1N1 and 2) perform a simulation of epidemic curves of different proportions of maternally derived antibody (MDA). 15 weaned pigs from 15 immune sows were bled every week in total 14 weeks. Sera were tested for IAV-H1N1 using IDEXX ELISA. The SP ratio data were modeled using binomial regression with clog-log link to estimate the rate of waning MDA (ψ). A stochastic-MSIR model using Gillespie's direct method was constructed simulating 500 weaned pigs. Parameters in the stochastic models were ψ and transmission rate (β) and recovery rate (γ), 0.646 and 0.23 day^{1}. Scenarios were combinations of initially-infected pigs (I=1, 5, and 10) and proportions of pigs having maternal immunity at weaning (pM=0.5, 0.7 and 0.9) (9 scenarios). Statistical modelling and simulations were performed in the R statistical package. Estimated waning MDA rate (ψ) for IAV H1N1 was 0.016 day^{-1} (95%CI: 0.013, 0.019). The estimated time to lack of MDA maternal immunity was 64.09 days (95%CI 60.77- 77.40). Based on the stochastic-MSIR simulations with medium (pM=0.7) or low MDA (pM=0.5) with sufficiently-initially infected pigs, an epidemic may or may not occur at any point in time during a wean-to-finish period with more than one epidemic peak. In conclusion, in a herd health management perspective, heterogeneous MAD in weaned pigs plays a crucial role in enhancing IAV transmission. Waning MAD has interfered with vaccination to create more subclinical infections.

Veterinarians should focus on the herd immunity management to control IAV transmission in wean-to-finish pig populations.

Keywords: weaning maternal immunity; modeling; stochastic; influenza A virus; wean-to-finish pig populations

Introduction

Influenza A virus (IAV) is considered one of the top three respiratory diseases affecting the United States of America (USA) swine industry (Holtkamp et al., 2007). IAV is widespread in the USA pig populations with the individual prevalence of 5% in growing-finishing sites and 44% in breeding herds (Allerson et al., 2013c; Corzo et al., 2013a). Among the IAV positive results, prevalence of H1N1, H1N1pdm09, H1N2 and H3N2 were estimated to be 18.0%, 14,5%, 16.0% and 7.6%, respectively (Corzo et al., 2013a). IAV infection causes low mortality but more morbidity can reach up to 100%, resulted in high fever and anorexia. Then, growing pigs may lose of bodyweight due to reduce the feed consumption and average daily weight gain. Thus, additional feed and longer duration to reach optimal bodyweight for market are required, resulting increased costs of production (Rose et al., 2013; Er et al., 2014). Depending on the association with other respiratory pathogens, the estimated cost of IAV infection in market pigs ranges between $3.23 and $10.41/head (Donovan, 2008; Dykhuis Haden et al., 2012). Consequently, respiratory diseases associated with IAV infection in growing pigs cause substantial economic losses to the USA swine industry.

Maternal derived antibody (MDA) against IAV infection has been reported that it can protect piglets against clinical signs as well as reduce fever, pneumonia and viral excretion (Kitikoon et al., 2006) but cannot prevent infection (Loeffen et al., 2003; Choi et al., 2004; Kitikoon et al., 2006). Furthermore, it has been reported that 80% of growing pigs do not have maternal immunity against IAV infection at an entry of the finishing facilities (Loeffen et al., 2003). Similarly, a one study concluded that pigs at age greater than 80 days do not remain MDA (Rose et al., 2013). This may lead to growing pigs

showing clinical diseases with IAV infection on the last stage of production. A study with two cohorts of growing pigs reported that IAV seroprevalence at ages of 45 and 115 raised from 6.7% to 90% and 0.0% to 93.3%, each cohorts, respectively (Homwong et al., In prepare). This may suggest that IAV exposures increased at the last stages of production or the level of protective MDA is inconstant overtime. Furthermore, lasting MDA for market-bound pigs may alleviate and production loss from IAV infection in the last stage may be seen.

The loss of protective antibodies overtime is also known as waning immunity. Statistically, protective immunity is the probability of an individual pig *i* protected by immunity at age *t* (Hens et al., 2012). Waning immunity is only the counterpart of protective immunity. Therefore, the waning immunity is defined as the probability of an individual pig *i* not protected by immunity at age *t*. The information needed to characterize MDA against IAV in growing pigs has been only described that the waning rate from vaccination and the rate from infection may not be identical (Heffernan and Keeling, 2009). Being parallel of vaccination or of infection, the MDA waning rate from different IAV subtypes may not be identical as well. These studies not only describe the characteristics of waning MDA of individual weaned pigs under a field setting, but also allow one to quantify specific MDA waning rate against IAV H1N1 subtype. In addition, IAV transmission dynamics due to MDA wane in wean-to- finish facility is lack of study and has not been assessed in details. Therefore, to better understand those, the objectives of this study were 1) to estimate the rate of waning maternal immunity of IAV H1N1 and 2) to perform simulations of epidemic curves to better understand IAV transmission dynamics in the wean-to-finish facility.

Materials and methods

Animals

Fifth-teen sows were intramuscularly vaccinated with commercial trivalent vaccine (H1N1, H1N2, and H3N2: FluSure XP®, Zoetis, Florham Park, NJ, USA) at 6 and 2 week before parturition. On average age of 22 days, one weaned pig was randomly selected from one vaccinated sow. Fifth-teen weaned pigs born to those fifth-teen sows were bled every week for a total of 14 weeks. The 210 sera were tested using subtype-specific H1N1 commercial indirect enzyme linked assay (ELISA; HerdChek H1N1 Swine Influenza Virus Antibody Test Kit; IDEXX Laboratories, Westbrook, ME, USA) according to the manufacturer's instructions. The resulting sample to positive (SP) ratio of 0.4 was considered indicative of IAV H1N1subtype positivity (Pascu et al., 2012).

Statistical modeling and stochastic simulation

Waning immunity acts by moving individuals from a class where they are totally immune to the class where they are totally susceptible. Therefore, it was assumed that all individual piglets experienced exponential decay of MDA, which allows us to express the mean MDA level as log-transformed. The waning probability, $\pi(t_i)$, the counterpart of protective immune probability, $\kappa(t_i)$, for an individual *i* at a given time *t* postweaning in day was expressed as followed: $\pi(t_i) = 1 - \kappa(t_i) = 1 - e^{-\psi t_i}$, where ψ is the hazard of being susceptible so-called "the rate of waning MDA" or the rate at which describing the transition from being immune to being susceptible. To estimate the rate of waning MDA (ψ), the SP ratio data was modeled with binomial regression using log (t_i) as an offset

variable with complementary log-log (clog-log) link function. The standard error of ψ was obtained from the Hessian matrix of the log-likelihood function (Velthuis et al., 2003; Velthuis et al., 2007; Hens et al., 2012). The estimated time to lack of MDA was calculated and its standard error of a transformed regression parameter ($^{1}/_{\psi}$) was obtained using "δ" method implemented in the R "car" package (Fox and Weisberg, 2011).

To better understand the effect of waning MDA on IAV transmission dynamics in a wean-to-finish facility, the Maternally immune-Susceptible-Immune-Recovery (MSIR) model, which is describing the flow of individuals between mutually exclusive infection states, was constructed. A stochastic-MSIR epidemic model using Gillespie's direct method was constructed with 1000 iterations simulating the effect of waning MDA on IAV transmission dynamics (Keeling and Rohani, 2008; Drake and Rohani, 2014). The recursive iterations were modeled with 8 possible events: Transition events and corresponding rates for the simple stochastic MSIR model were shown in **Table 5-1**. Parameters in the stochastic models were the waning MDA rate (ψ) being estimated in the current work; transmission rate (β) and recovery rate (γ) were 0.646 and 0.23 day^{-1} obtained from a clinical disease study elsewhere (Homwong et al., In prepare). The closed system in the model was assumed, setting the flowing-in and flowing-out rate to the facility (μ) to very small number (i.e. 0.000001). Scenarios were comprised of 500 weaned pigs with combinations of initially infected pigs (I=1, 5, and 10) and proportions of pigs having MDA at weaning (pM=0.5, 0.7 and 0.9) in total of 9 scenarios **(Table 5-2)**. Statistical modelling and stochastic-MSIR simulations were performed in the R statistical package, v3.2 (R Core Team, 2015).

Results

The individual SP ratio profiles of MDA against IAV-H1N1 subtypes for 15 pigs from day 0 to day 91 postweaning were represented **(Figure 5-1)**. Two weaned pigs (#7 and #13) had low levels of MDA; however, the other weaned pigs had high levels of MDA at weaning. MDA SP profiles of 15 pigs were depicted as dots with their average as the line (in blue) according to SP ratio axis (left). The expected probability of being positive affected from MDA waning was lined (in green) according to the expected probability axis (left) **(Figure 5-2)**. Estimated MDA waning rate (ψ) for IAV H1N1 subtype was 0.013 day^{-1} (95%CI: 0.010, 0.016). On average, the estimated time to lack of MDA maternal immunity was 77.06 days (95%CI 60.28-93.85) after weaning.

Based on the stochastic-MSIR simulations, of initially one-infected pigs (I=1) or of the given MDA proportions of 0.9 in the pig population (pM=0.9) at weaning, five scenarios (C1, C4, C7, C8 and C9) were found that an epidemic cannot occur. In the pM=0.5 with I=5 or 10 scenarios (C2 and C3), an epidemic occurs **(Figure 5-3)**. The I=5 or I=10 with pM=0.7 scenarios (C5, C6), an epidemic may or may not occur. Interestingly, for those two scenarios, an epidemic can occur at any point in time during a wean-to-finish period with more than one epidemic peak **(Figure 5-4)**.

Discussion

IAV infection continues to be a major health problem for wean-to-finish pig populations. Vaccination in breeding sows remains an essential tool in controlling IAV

infection in those populations. Vaccination in breeding herds is usually recommended. The usefulness is not only providing herd immunity in breeding sows, but also increasing passive or maternal immunity through colostrum to the progeny. Passive immunity or MDA against IAV can protect only clinical signs but not protect infection. Nevertheless, the clinical signs may not be fully protected. At least, MDA protection during early infections may have accounted for the absence of clinical signs. MDA can also wane as older age, leading to the fact that clinical signs may be more severe during the late stage in grow-to-finish period. Vaccination in nursery period may be a choice to protect clinical signs at the late stage of feeding period. However, waning maternal immunity affects age appropriate vaccination in growing pigs. A report shown that the presence of MDA at vaccination negatively impacted vaccine efficacy as fever and clinical signs were prolonged, and expectedly IAV induced pneumonia was increased compared to pigs vaccinated in the absence of MDA (Kitikoon et al., 2006). Therefore if vaccination in growing pigs is needed, the waning rate should be taken into consideration.

IAV circulation can be identified in vaccinated breeding sow herds (Rose et al., 2013). Although, the absence of clinical signs related to infection can be observed, several IAV infections remain subclinical due to waning maternal immunity. Consequently, specification of MDA loss will provide useful information in terms of how fast MDA will decay per unit of time (i.e. waning rate), and when MDA will reach at a defined threshold where considered not having maternal immunity (i.e. waning duration). Our study demonstrated that the waning rate of MDA was estimated to be 0.013 day^{-1}(the rate at no having maternal immunity). One study reported that the half-lives of maternal antibodies for both H1 and H3 were 12 days (7/12=0.083 day^{-1}, the rate at having 50%

maternal immunity of the previous period) (Loeffen et al., 2003). MDA will remain until 64 days postweaning, between the late stage of nursery period and the beginning of growing period, leading to unprotected period until the end of finishing period. A research concluded the majority of IAV infection in finishing pigs took during finishing period, when pigs are at least 10 weeks old (Loeffen et al., 2003). In addition, waning immunity trends to increase the prevalence of susceptible individuals, decrease the force of infection, infer vaccination, create more subclinical infections and increase infectious individuals (van Boven et al., 2000; Glass and Grenfell, 2004; Homwong and Deen, 2014). The higher waning rates the more infectious pigs. The ways or pharmaceutical products that aid to reduce the waning rate may have maternal immunity covering the last period of market pigs, and may control the spread of IAV, reduce the number of infectious pigs and lessen area spread.

As in our stochastic MISR simulations, two factors involved in occurring epidemics (i.e. number of initial infectious pigs and level of maternal immunity) were considered to model. The outputs of 9 scenarios were grouped in three main categories: 1) scenarios that most likely not to be epidemic, 2) scenarios that most likely to be epidemic, and 3) scenarios that may or may not be epidemic if these do, more than one epidemic peak can be foreseen.

Firstly, several scenarios (C1, C4, C7, C8) were most likely not to be epidemic. The main features of this category are either low initial infectious or higher MDA pigs. Unsurprisingly, higher MDA was able to protect clinical outbreaks. One research revealed that increasing MDA levels through sow vaccination can protect piglets against clinical disease (Kitikoon et al., 2006). However, the levels of active immunity in sows or

of passive immunity in piglets are involved in multifactors such as vaccine effectiveness, colostrum intake and general farm management. Based on our reviews, there is no paper describing a number of initial infectious pigs from experimental or observational settings.

Secondly, scenarios (C2 and C3) were most likely to be epidemic. The main features of this category are higher infectious pig with low MDA. Epidemic can occur in the early phase postweaning. Not only those two factors, but it was suggested that movements of piglets, cross-fostering, mingling at weaning were associated to early infection and being epidemic (Rose et al., 2013). These factors are beyond scope of the current study and may need further study.

Thirdly, scenarios (C5, C6) may or may not be epidemic if these do, more than one epidemic peak could be foreseen. The main features are either medium maternal immunity or median-high infectious. The population in this category can be considered as a heterogeneous population as the population level has only 50% of the population. As individual level, weaned pigs may be segregated by different MDA levels of pig's subpopulations corresponding to different batches from different sows, thus spreading viruses from one to another, causing more than one epidemic during the wean-to-finish period as observed in the current stochastic simulation models. This phenomenon has been observed in a filed setting and authors called this phenomenon "poor internal biosecurity" (Rose et al., 2013). In addition, a study that observed more than one epidemic peak was described elsewhere (Diaz et al., 2013). The explanation may still not be clear why those happened but one explanation may be that viruses can jump from one higher to other lower immune piglets, as explained statistically by stochastic process as; we modeled in the current study.

Stochastic process is the natural way to describe the spread of disease and the probability of disease transmission is defined between two individuals, rather than stating certainly whether or not transmission will occur (Andersson and Britton, 2000). Similarly for IAV infection in wean-to-finish pig populations, Homwong and colleague implemented the stochastic process to estimate the reproduction number incorporating uncertain of diagnostics tests (Homwong et al., In prepare). With the current study, we represented stochastic models to describe viral dynamics stochastically for which viruses can jump from one to other wean-to-finish pigs. This simplification is necessary in order to be biologically realistic representation as close as possible. In addition an incorporation of explicit immune dynamics into a model for wean-to-finish pig populations would be more useful in terms of understanding immunity and viral transmission dynamics. However, a further study is needed to address this phenomenon.

We have chosen to describe medium-level infection and medium level of maternal immunity (C5 and C6) as "poor internal biosecurity" because of the heterogeneous maternal immunity. However, the important features of such combination are that it comes by population level with probability of having some MDA. The existence of piglet subpopulations with a decayed immunity depended on their age and the presence of remaining MDA with high spreading potential. Management of "poor internal biosecurity" requires the identification of subpopulations and appropriate management for these subpopulations within the same herd, which is the main focus being to limit mingling practice. Shorter of the infectious cycle between batches requires reinforcement of internal biosecurity and strict age-segregates rearing with an all-in/all-out practice at the compartment level. If vaccination is needed, vaccination should

optimally begin right after the time at disappearance of MDA, but this approach may be impracticable due to a high degree of variability between individuals; even more difficult if a herd has “poor internal biosecurity”. An epidemiological model comparison of various plausible vaccination ages is worthwhile because it can sometimes reveal vaccination ages for which disease incidence is substantially lower when compared with vaccination ages chosen on practical grounds. A further study is important to address such question.

Limitation of the study may rise in some aspects. We assumed that an individual with antibody SP ratio by ELISA below the threshold (0.4) has no protection, while an individual with that above the threshold has protection. It is suggested that the standard method for detecting IAV antibodies is the hemagglutination-inhibition (HI) tests (World Organisation for Animal Health (OIE), 2012). Another research concluded that serum HI antibody levels do not necessarily correlate with levels of heterologous protection (Heinen et al., 2001). Decaying maternal derived immunity may be measured by such test instead. However, the ELISA method was chosen in this study because it is fast and less expensive to perform. With comparing to test accuracy to HI test, the positive predictive value of the ELISA test to H1N1 SIV was 99% (Barbé et al., 2009). In addition, to estimate the waning rate, the outcomes of the current study were transformed to a probabilistic unit. Therefore, the estimated waning rate would not be significantly different from the estimate by a HI-transformed probabilistic unit. However, a further study is needed to address.

Conclusions

Modeling wane of MDA represented here was simplified of complicated transmission dynamics with loss of maternal immunity. In a herd health management perspective, heterogeneity of maternal immunity of weaned pigs plays a crucial role for IAV transmission dynamics. Waning MDA has interfered with vaccination to create more subclinical infections. Veterinarians should focus on the herd immune management to control IAV transmission in wean-to-finish pigs. Infectious outbreaks were mainly initiated by piglets born to sows delivering weak MDA and those from litters with numerous cross-fostered piglets.

Conflict of interest

The authors declare they have no conflict of interest. Mention of trade names or commercial products in this article is solely for the purpose of providing specification and does not imply recommendation.

Acknowledgments

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Tables and Figures

Table 5-1: Transition events and corresponding rates for a simple stochastic MSIR model with recursive iterations

Event	Description	Rate	Transition event	Recursive rate
1	Death of recovered pigs	μR	(M, S, I, R-1)	"default" $\mu N+\psi M+\mu M+\beta SI/N+\mu S+\mu I+\gamma I+\mu R$
2	Recovery of infected pigs	γI	(M-1, S+1, I, R)	$\mu N+\psi M+\mu M+\beta SI/N+\mu S+\mu I+\gamma I$
4	Death of infected	μI	(M, S, I-1, R)	$\mu N+\psi M+\mu M+\beta SI/N+\mu S+\mu I$
4	Death of a susceptible pigs	μS	(M, S-1, I, R)	$\mu N+\psi M+\mu M+\beta SI/N+\mu S$
5	Transmission event of infectious pigs	$\beta SI/N$	(M, S-1, I+1, R)	$\mu N+\psi M+\mu M+\beta SI/N$
6	Death of maternal immune pigs	μM	(M-1, S, I, R)	$\mu N+\psi M+\mu M$
7	Susceptible of maternal immune pigs	ψM	(M-1, S+1, I, R)	$\mu N+\psi M$
8	Birth of maternal immunity	μN	(M+1, S, I, R)	μN

In the MSIR model, in assuming constant population, μ is the flow-in and flow-out rate for wean-to-finish facility, ψ is the waning rate; β is transmission parameter, and γ is recovery rate. The M, S, I, R, and N represent the numbers of maternally-immune, susceptible, infectious, and recovered pigs in the epidemic compartment. The total rate equals $\mu N+\psi M+\mu M+\beta SI/N+\mu S+\mu I+\gamma I+\mu R$.

Event(s) will occur if the recursive rate/total rate of given events is greater or equal to U, where U is the random variable generated by uniform distribution ranged for [0, 1].

Table 5-2: Stochastic simulation scenarios were combinations between initially infected pigs and proportion of pigs having MDA at weaning in total of 9 scenarios (C1-C9)

Proportion of pigs having MDA at weaning (pM)	Initially Infected pig (I)		
	I=1	I=5	I=10
pM=0.5	C1	C2	C3
pM=0.7	C4	C5	C6
pM=0.9	C7	C8	C9

Figure 5-1: A representation of MDA by SP ratio profiles against IAV H1N1 subtype for 15 pigs by days postweaning (day 0 to day 91)

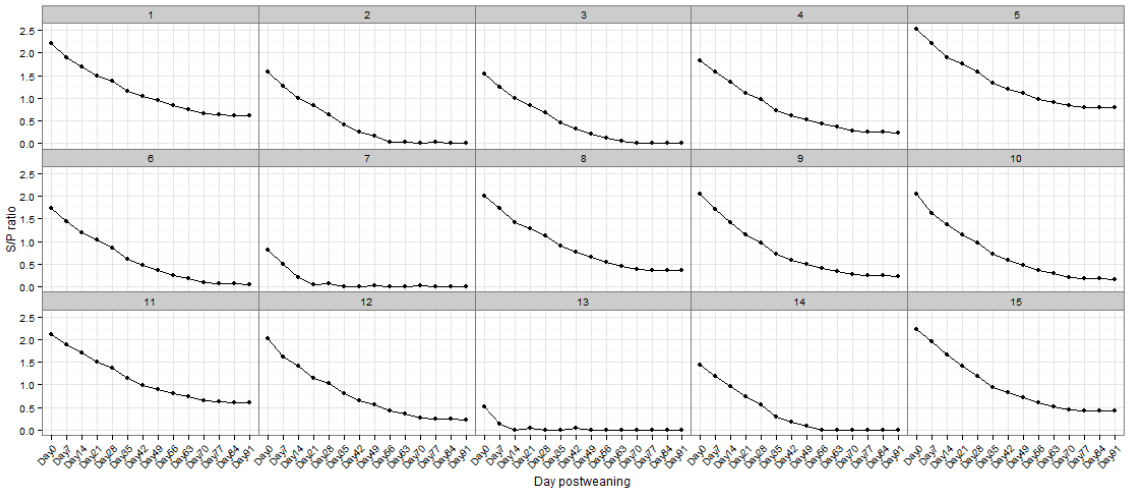


Figure 5-2: A presentation of SP profiles and probability of waning MDA of 15 growing pigs

The line in green belonging to the probability of being positive affected from MDA waning (left axis), the line in blue belonging to SP ratio (right axis), and the dots representing the SP ratio each pig at given day of blood collection.

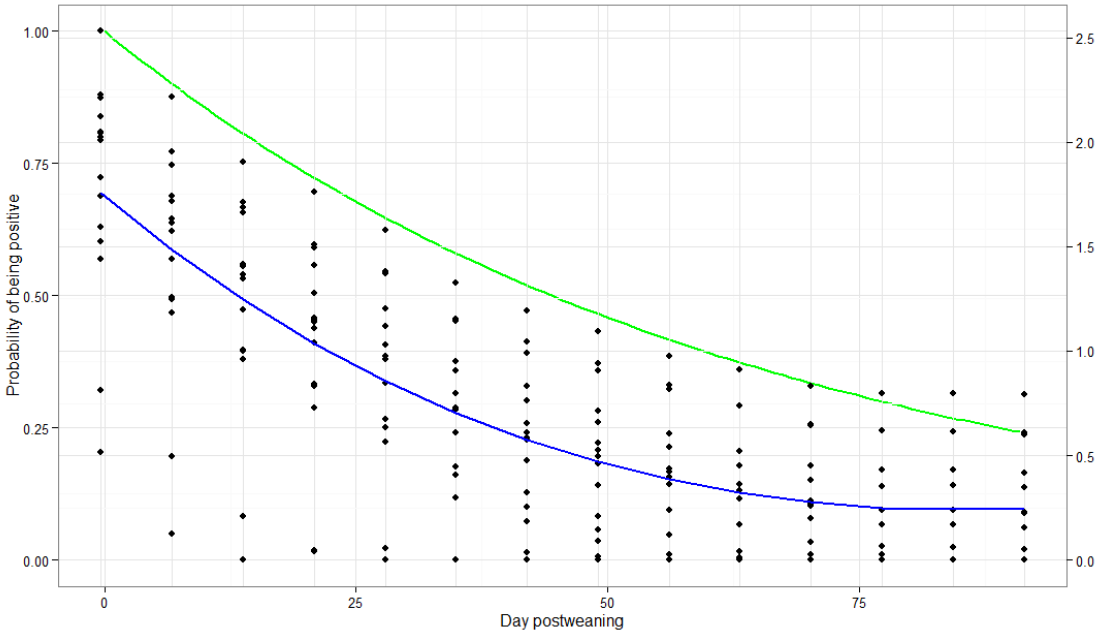


Figure 5-3: A representation of stochastic MSIR simulations for the pM=0.5 with I=5 or 10 scenarios (C2 and C3)

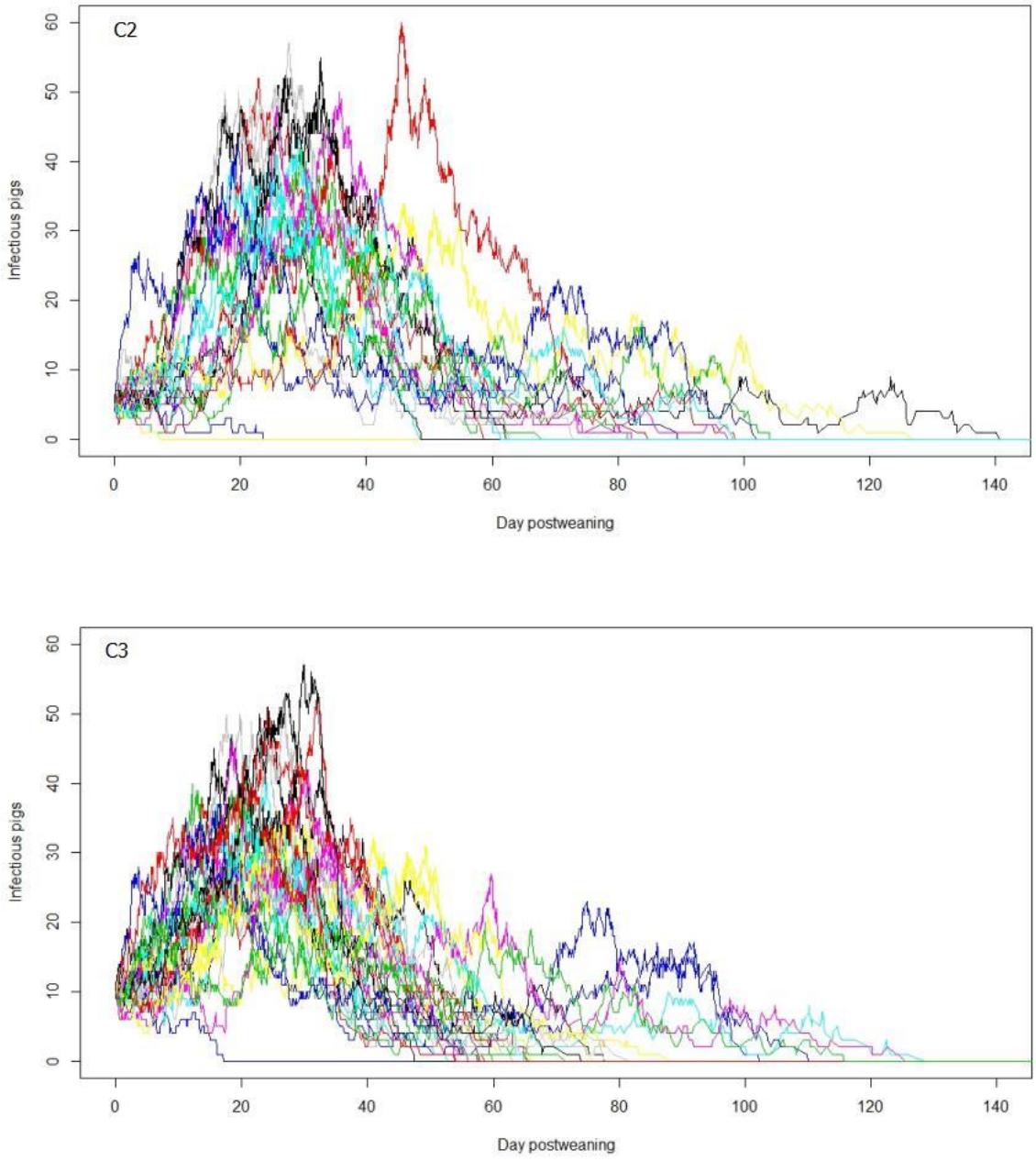
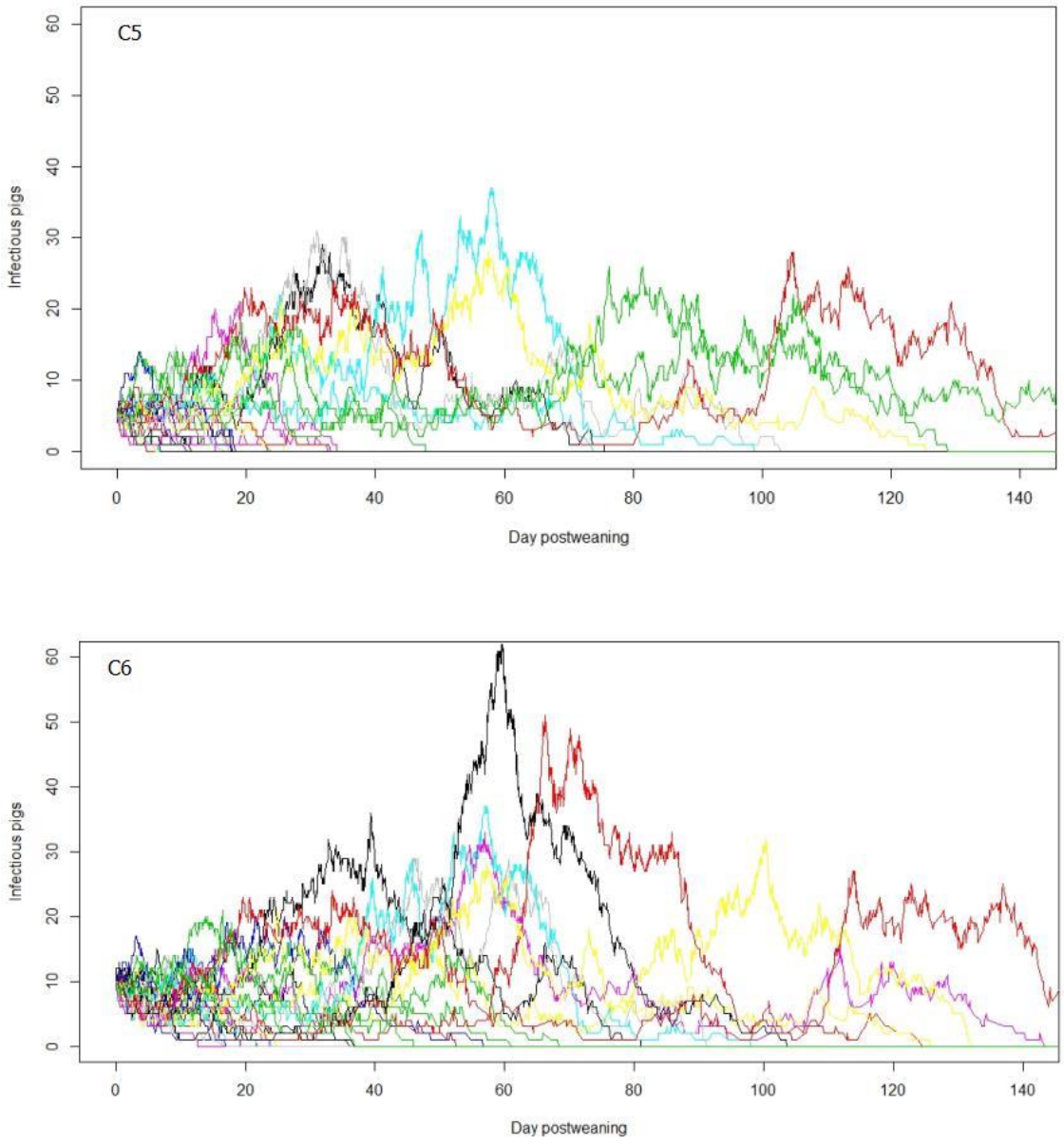


Figure 5-4: A representation of stochastic MSIR simulations for the I=5 or I=10 with pM=0.7 scenarios (C5, C6)



Chapter 6 : Periodic influenza A virus transmission in Midwestern United States growing pig populations: Harmonic regression and Fourier spectral analysis study

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[in prep, to be submitted to Prev Vet Med]

and

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Summary

Influenza A virus (IAV) is an important respiratory disease in pigs, especially in growing pigs. While periodic IAV transmission in human medicine has been well described, periodicity of IAV transmission in veterinary medicine has not been yet characterized in sufficient details. Therefore, the objective of this study was to examine periodic IAV transmission in Midwestern United States growing pig populations. Data from an active surveillance program for IAV conducted between 2009 and 2011 including a total of 16,170 nasal swab RT-PCR results were used. The log-binomial regression model with the Fourier spectra was applied to characterize the periodicity of IAV transmission pattern. IAV transmission pattern was elucidated as strong periodic transmissibility (p-value < 0.001) with medium amplitude of 0.44 and peak timing in mid-June. The average periodic prevalence was 0.20. The absolute IAV intensity was 0.18. The relative IAV intensity was 2.41, implying that prevalence intensity at the periodic peak was 2.41 times higher compared to that at the periodic nadir. The better understanding of periodic IAV transmission pattern and persistence should result in a better design of the optimal control strategies and periodic IAV vaccination in growing pig population.

Keywords: modeling; harmonic regression; Fourier spectrum; seasonality; periodicity; influenza A virus; swine; growing pigs

Introduction

Influenza A virus (IAV) is an enveloped segmented, negative sense single-stranded RNA virus belonging to the *Orthomyxoviridae* family (Vincent et al., 2008). Serologic and molecular surveys have concluded that the virus is widespread in pig population in the United States of America (USA) (Romagosa et al., 2011; Torremorell et al., 2012; Allerson et al., 2013b; Corzo et al., 2013a). IAV infection in pigs is considered one of the top three respiratory diseases in USA pig population (Holtkamp et al., 2007). Pigs infected with IAV may exhibit fever, sneezing, coughing, lethargy, conjunctivitis and oculonasal discharge (Reeth et al., 2012). Due to the clinical impact and mixed infections with other pathogens, the disease can cost producers between $3.23 and $10.31 per pig (Donovan, 2008; Dykhuis Haden et al., 2012). Infectious diseases of livestock contribute to significant economic losses and have to be carefully monitored via passive or active surveillance.

Surveillance systems established to track infections in humans allowed to observe remarkable periodicity for many pathogens, including measles, diphtheria, chickenpox, cholera, rotavirus, malaria, gonorrhea, and pneumococcal infections as well as influenza (Dowell, 2001; Dowell et al., 2003; Grassly and Fraser, 2006). Global periodic fluctuations in influenza infections in humans with the raise in the wintertime followed by fade out in summer months have been demonstrated in temperate regions throughout the word (Kim et al., 1996; Säynäjäkangas et al., 2001; Donaldson and Keatinge, 2002; Müller-Pebody et al., 2002; Dowell et al., 2003; Crighton et al., 2004; Dushoff et al., 2004; Chowell et al., 2008). The use of publicly available epidemiological laboratory-confirmed data enabled to describe seasonal transmission patterns in great details

(Crighton et al., 2004; Chowell et al., 2008; Chowell et al., 2010; Brauer and Castillo-Chávez, 2013).

In swine, information regarding the periodicity of infections is scarce and contradictory. One study concluded that influenza infections in pigs occur at a certain age with a predictable pattern (Rose et al., 2013); another study detected two peaks in March-May and in September-November in IAV positive samples (Beaudoin et al., 2012). A study by Poljak and colleague determined that periodic fluctuations in confirmed exposure were weak and estimates were not robust due to dependency on model assumptions (Poljak et al., 2014) and another found no association between incidence and season (Kyriakis et al., 2013).

Better understanding of seasonal transmission of infection in growing pig populations would provide valuable information, yet there have not been studies using an active surveillance data to investigate the periodicity of influenza infection in pigs. Therefore, the objective of this study was to evaluate periodic IAV trasmission pattern in Midwestern USA growing pig population with an active surveillance data using the log-binomial regression model analysis with Fourier spectra.

Materials and methods

Data

Nasal swab samples of 16,170 received from an active surveillance program between 2009 and 2011. Nasal swabs were collected on a monthly basis from 32 farms (30 samples per month per farms) in the Midwestern USA growing pig population. All samples were tested by real time reverse transcriptase polymerase chain reaction (RRT-

PCR) for IAV and also identified its subtypes to be H1N1, H1N2, H1N1P and H3N2, H3N2v and H1N2v (Corzo et al., 2013a). An analysis for IAV subtypes was considered farms that have two subtypes were considered two farms. On other hand, the farms that were identified for a subtype or missing subtype information were considered only as IAV. Because some farms had vaccination protocols in place and this may bias the results, a Pearson Chi-squared test was used to test whether the proportion of vaccinated farms was in place throughout the study or not. Thus, it was able to assume that vaccination was performed year around, equally influencing estimates throughout the study.

Statistical models

Monthly individual-level IAV prevalence was calculated by dividing the number of positive samples by the number of samples collected per herd in a given months. If at least one nasal swab sample tested positive, the herd was considered positive. The number of positive herds out of the total number of herds tested per month was used to calculate the herd-level prevalence. Time series of monthly individual-level and herd-level prevalence for IAV and its subtypes (i.e. IAV, H1N1, H1N2, H1N1P, H3N2, H3N2v, H1N2v) were plotted to provide a general overview. For a crude analysis of seasonality, the monthly values were categorized by seasons as winter (January-March), spring (April-June), summer (July-September) and fall (October-December).

Associations between IAV infection, its subtypes and seasons were assessed using Pearson's Chi-square test or Fisher's exact test (if $N < 5$). When Fisher's exact test was used, the p-value was calculated from a simulation with 10,000 iterations. To assess the

association as herd prevalence ratios between prevalence of monthly IAV and of its subtypes and seasons, log-binomial regression models were implemented expressing as: $E(log(\pi_i|st_i)) = \beta_0 + \beta_1(st_1) + \beta_2(st_2) + \beta_3(st_3)$, where the β_0 and β_i are intercept and regression coefficient of indicator variable for the seasons *i* (i=1, 2, 3 where the fall season is the predefined reference category and st_1 is a binary covariate, which is 1 for the winter and 0 otherwise, st_2 is that covariate, which is 1 for the spring and 0 otherwise, and st_3 is that covariate, which is 1 for the summer and 0 otherwise. Improvement-of-fit (IOF) was evaluated by comparing the current model to the intercept model.

Fourier spectral analysis has been used for capturing the periodicity and seasonality of infectious diseases in noisy time series data (Serfling, 1963; Cowpertwait and Metcalfe, 2009). The Fourier representation as a time series is a key approach for modeling and analyzing variance of a stationary time-series model over specific frequencies (Cowpertwait and Metcalfe, 2009; Barnett and Dobson, 2010). The stationary time-series framework was modeled using the binomial regression with log link function described elsewhere (Kedem and Fokianos, 2005). The extension of such framework with Fourier spectra (so-called seasonal or harmonic regression) was performed to fit the average seasonal pattern for the herd-level prevalence to non-epidemic months (Serfling, 1963; Choisy et al., 2006; Höhle, 2007; Shumway and Stoffer, 2011). The Fourier representation uses sinusoidal functions expressed by trigonometric functions (cosine and sine) (Barnett and Dobson, 2010). The model representation was expresses as followed:

$$\log(E[\pi_t|t]) = a_0 + b_0 t + \sum_{n=1}^{+k}[a_n \sin(2\pi\omega_n t) + b_n \cos(2\pi\omega_n t)],$$

where, $E[\pi_t|t]$ is a time series for the expectation of herd-level prevalence in the month $t^{th} \in (1,2,\ldots,12)$. The a_0 and b_0 are the average log-transformed prevalence, the secular trend parameter, respectively. For the last term, a sum of sinusoids represents the periodic (harmonic) mixture-components for seasonal patterns (mixture Fourier spectra). The periodic mixture-components have distinct frequencies of ω_n, for which in the current study ω_n=1/12, assuming that the periodicity makes one cycle per 12months. The seasonal pattern is characterized by the amplitude (A_n) and phase shift (ϕ_n). The subscript *n* is the number of mixture-components of periodic series. The regression coefficients for sine and cosine mixture-components, a_n and b_n, for n=1,…,+k are independent zero-mean random variables with variance $\sigma_{a_n}{}^2 and\ \sigma_{b_n}{}^2$ (ref). A_n and ϕ_n are independent random variables. A_n^2 is chi-squared distributed with 2 degrees of freedom and ϕ_n is uniformly distributed on [-π, π]. The number of periodic mixture-components of periodic series were estimated as decomposed into a sum of sinusoids (as n= 0, 1, 2, 3, 4 and 5) and tested using LR-χ^2. In addition, the peridogram was implemented to discover the number of periodic components. A final model was selected by Akaike Information Criterion (AIC)(Quinn, 1989). The generic representation of periodic disease transmission and its characteristics was depicted **(Figure 6-1)**.

Periodic IAV characteristics of time-to-peak and amplitude, various form of their representations, including phase, maximum seasonal peak, minimum seasonal nadir, absolute disease intensity and relative disease intensity were estimated from the result of the selected model elsewhere (Lofgren et al., 2007a; Naumova et al., 2007; Naumova and MacNeill, 2007; Lofgren et al., 2010; Wenger and Naumova, 2010). The measures of uncertainty for peak timing and amplitude such as the variance of amplitude, phase shift,

absolute disease intensity and relative disease intensity were estimated using "δ-method" as proposed by Naumova and MacNeill implemented in the R "car" package (Naumova and MacNeill, 2007; Fox and Weisberg, 2011). Differences were considered statistically significant when p-value <0.05. Graphs were produced using the "ggplot2" package (Hadley, 2009). Model's diagnostic was performed by examining Pearson's residual and Cook's distance and leverage. Statistical analysis and modeling was performed in the R statistical package, v3.2.0 (R Core Team, 2015).

Results

Of 16,170 nasal swab samples individually, 4.6% (746/16170) were positive for IAV. Scatter plots of time series data were used for data exploration monthly for both individual-level **(Figure 6-1)** and herd-level IAV prevalence as well as its subtypes (H1N1, H1N2, H1NP and H3N2, H1N2v and H3N2v) with 20 samples missing subtype information **(Figure 6-2)**. In regard with herd-level IAV prevalence, there was no association between vaccination and season (χ^2=0.58, p-value=0.90). An association was found to be statistically significant between IAV prevalence and seasons (winter, spring, summer and fall) (p-vale=0.045) but marginally statistically significant for H3N2 (p-value=0.056). The prevalence of IAV in spring and summer seasons were 1.62 (95%CI: 1.49-1.77) and 1.37 (95%CI: 1.25-1.51) times more likely compared to the prevalence in fall season. The H1N1 prevalence in winter, spring and summer, respectively, were 1.75 (95%CI: 1.35-2.56), 1.33 (95%CI: 1.02-1.74 and 2.82 (95%CI: 2.21-3.59) times more likely compare to the prevalence in fall season. The H1N1P prevalence in winter was found of 24% (PR=0.76) to be lower compared to the prevalence in fall season while not

statistically different from the other seasons. The H1N2 prevalence in winter and spring were 1.21(95%CI: 1.01-1.44) and 2.15(95%CI: 1.84-2.52) times more likely compared to the prevalence in fall season. The H3N2 prevalence in winter had a 82% (PR=0.18) reduction compared to the prevalence in fall season **(Table 6-1)**. The average monthly IAV prevalence was constant throughout the study (p=0.931). The periodic influenza transmission occurs in growing pig populations (Deviance=13.03, df=2, p-value=0.0015). The number of periodic mixture-components (*n*) in Fourier spectra was decomposed as n=1 and was statistically significant (p-value=0.0015) with lowest AIC of 118.11 **(Table 6-2)**.

For the final log-binomial regression model, an annual periodic cycle was detected for IAV transmission. The periodic peak was found in mid-June (month of 6.64). The amplitude was 0.440 with variance of 0.014. The maximal periodic IAV prevalence peaks and minimal periodic IAV prevalence nadir were 0.31 and 0.13. The absolute disease intensity (I) of IAV prevalence, which is the difference in the intensity between maximal SIV and minimal IAV prevalence, was 0.18. The 95%CI of I calculated by the R "car "package was ranged from 0.082 to 0.181 with variance estimate being 0.0025. The relative disease intensity (RI) of IAV, which is the ratio of the intensity between maximal IAV prevalence peak and minimal IAV prevalence nadir, was 2.41, implied that the disease prevalence intensity at maximum periodic peak was 2.41 time higher than that at minimal periodic nadir. The 95%CI of RI calculated by the R "car "package was ranged from 1.87 to 2.95 with variance estimate of RI being 0.359.The average seasonal prevalence (periodic threshold) across a year of IAV infected in growing pig population was 0.20 (1stQu-3rdQu:0.15-0.27) **(Table 6-3)**. The periodic IAV transmission was

intense and statically significant (p-value <0.001). The seasonal pattern was characterized by increasing in spring-summer then slightly decline with nadir in fall-winter **(Figure 6-4)**. In analyzing the peridogram, two frequencies were estimated to be at 0.1 and 0.4, suggesting that two patterns of transmission characteristics (sub-periodic and periodic) could be expected **(Figure 6-5)**.

Discussion

Disease seasonality is defined as "systematic periodic fluctuations within the course within a year that can be characterized by the magnitude, timing and duration of a seasonal increase" (Naumova and MacNeill, 2007). Disease seasonality has been well discribed in human medicine for several diseases, especailly influenza. However, in veterinary medicine, particularly swine medicine, it is poorly understood. To the best of our knowledge, this is the first report in swine medicine evaluating the IAV periodicity using an active surveillance database. Our study indicated that IAV prevalence in growing pig population rises and falls periodically on annual basis.

In humans, the duration of influenza periodic peak took 2 weeks in the USA. However, the cause of IAV seasonality remains unclear and is still heavily debated (Dushoff et al., 2004; Chowell et al., 2008). The previous suggestions reported causes of IAV seasonality may involve several factors such as cold-weather, bad air quality inside barns, and co-infection (Straub, 1994; Brown, 2000). Explanations of the winter peak for IAV transmission in humans have been suggesting that respiratory mucosal immune susceptibility, immune competency and longer survival in an environment can play a role in the periodic infection. Shorter sunlight exposure reduces the production of vitamin D,

thus reducing immunity (Dowell, 2001; Dushoff et al., 2004; Viboud et al., 2004; Cannell et al., 2006). Another factor such as age structure of susceptible population may influence periodic IAV transmission (Anderson et al., 1992; Sumi and Kamo, 2012). In regrading to those factors, it is difficult and unclear to identify the causes of periodicity of IAV transmission in growing pig populations.

Even though a seasonal IAV transmission has been well described in human medicine, variation on the peak time of the year when seasonal IAV transmission occurs has been described (Wenger and Naumova, 2010). Our study showed that the periodic IAV transmission peak time occurred between mid of June (summer) and nadir in December. Of approximate mid-August (1.23 month later from July), the IAV prevalence was reached to the average seasonal prevalence. However, as similar characteristics to human medicine, the variation in the peak time may also occur in swine populations.

The periodic peak may occur earlier or later depending upon several factors since the reason of peak variation is unclear and causes of periodicity are multifactorial factors (Lofgren et al., 2007b). Recent studies reported that the peak times of IAV positive submitted samples were found in April (March-May), October (September-November) (Beaudoin et al., 2012) and in June (Poljak et al., 2014). Variation of peak time in swine population can occur warranting the need for further studies.

IAV transmission was intensely periodic in growing pig populations and this periodic transmission may exist in breeding herds. However, the transmission peak in those herds may not be similar or identical to the peak in growing pig populations. The peak in breeding herds may be nearly similar to that in humans during winter-spring seasons since sows have longer longevity (about 3 years) than growing pigs (6 months).

Due to growing pigs being downstream production of breeding herds, seasonal characteristics (i.e. the amplitude, absolute intensity and peak time) in breeding herds may synchronize those characteristics in growing pig populations; therefore periodic transmission in breeding herds may occur earlier. A study reported that IAV infected sows are the source of infection to suckling piglets especially under actual management practices that have cross-fostering and commingling at weaning (Rose et al., 2013), and this may continue to growing pig populations.

Occasionally, periodic transmission pattern is difficult to evaluate. This may be explained by the previous simulation study demonstrated that if no between-herd variation in amplitude has been found but there is some between-herd variation in a phase shift, the periodic transmission was obvious and the expected periodic transmission dynamics and herd-level periodic transmission dynamics are the same (herd-level and average periodic transmission dynamic have equal amplitude). Whenever there are some variations in the phase shift, the expected periodic transmission dynamic has smaller amplitude than the herd transmission dynamics. In the extreme case, if the herd phase shifts for periodic transmission dynamics are spread evenly throughout the year, then the predictable periodic transmission dynamics will be flat (Barnett and Dobson, 2010). Our study indicated that the variance of the phase shift of IAV transmission in growing pig populations was low as 0.075 and thus periodic IAV transmission was obviously observed. The smaller the variation in herd phase shifts, the larger the expected periodic amplitude.

In the peridogram with the two frequencies at 0.1 and 0.4, for the periodic frequency of 0.1 (ω= 1/12=0.08), it is considered periodic since one periodic period is

completed within 12 months. Obviously, log-binomial regression with Fourier series provided the concordant result. On other hand, at the sub-periodic frequency of 0.4 (ω= 5/12=0.42), it is considered sub-periodic since it would be expected that the five periodic periods are completed within 12 months. The latter, our log-binomial model with Fourier spectra could not capture a high frequency. This might be because the sampling interval (monthly) was relatively wide compared to sub-periodic frequency, leading to the fact that the model is not able to capture periodic changes within 2-3 months. Both peridogram and the log-binomial model approaches are used complementarily. Therefore, the periodic IAV transmission is dominated by a 1-year period (and might have 2-3 month sub-periodically).

Fourier's theorem states that every periodic signal (i.e. seasonal IAV transmission) can be completely decomposed into a sum of sines and cosines of various amplitudes and frequencies; sines and cosines are, in fact, orthogonal (independent). Therefore, seasonal IAV transmission pattern with several peaks, nadirs, the amplitudes and frequencies – no matter what the shapes of a transmission within a year are – can be decomposed as periodic and sub-periodic transmission patterns.

The limitations in the study were that the sampling intervals were monthly (discrete time steps) but disease transmission is the continuous process. Therefore, the study may not well expand transmission process in between the sampling intervals, and information may lose about high-frequency periodic IAV transmission patterns as suggested by the peridogram. Our model did not account for distance between farms or farm location (latitude and longitude). If those are available, spatio-temporal analysis with Fourier spectral would be more fascinating. However, most of the growing pig

populations in the study are located in southern Minnesota and northern Iowa; estimates would not give much different given as spatial characteristics and environmental oscillation.

Conclusions

The periodicity of IAV transmission in growing pig populations was elucidated, with strong periodic, medium amplitude with low average periodic transmission rate. The periodic peak time occurred in mid of June. The relative IAV intensity in the periodic peak was 2.42 times higher comparted the relative IAV intensity in the periodic nadir. The log-binomial regression model with Fourier spectra is helpful to identify periodicity of IAV transmissions and using the active surveillance data provided quality data and increased the understanding of periodicity of IAV transmissions in the Midwestern USA growing pig populations. The better understanding of periodic IAV transmission and persistence is likely to result in a better understanding of the optima control strategies, for example, periodic IAV vaccination in growing pig populations. In addition, it is also important to explicitly consider the interaction of control strategies themselves with periodic transmission pattern of the optimal control.

Acknowledgments

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Conflict of interest

The authors declare they have no conflict of interest.

Table and Figures

Table 6-1: A representation of associations (prevalence ratio, PR) between seasons (winter, spring, summer, and fall) and herd-level IAV, and its subtypes (H1N1, H1N1P, H1N2, H3N2)

IAV	Season				N, (χ^2)	[d]p-value
	Winter	Spring	Summer	Fall		
IAV, +/-	19/105	36/99	34/101	21/107	522[a], (8.05)	0.045*
PR	0.94	1.62***	1.37***	(ref.)	-	<0.001***
(95%CI)	(0.84-1.04)	(1.49-1.77)	(1.25-1.51)			
H1N1, +/-	5/119	4/122	7/102	3/125	487[b], (2.86)	[c]0.414[ns]
PR	1.75	1.33	2.82	(ref.)	-	<0.001***
(95%CI)	(1.35-2.26)***	(1.02-1.74)*	(2.21-3.59)***			
H1N1P, +/-	5/119	7/119	6/126	7/121	510, (0.44)	0.932[ns]
PR	0.76	1.00	1.03	(ref.)	-	0.01*
(95%CI)	(0.62-0.93)**	(0.83-1.21)[ns]	(0.85-1.26)[ns]			
H1N2, +/-	8/116	15/111	7/125	7/121	510, (5.52)	0.137[ns]
PR	1.21	2.15	1.03	(ref.)	-	<0.001***
(95%CI)	(1.01-1.44)*	(1.84-2.52)***	(0.85-1.25)[ns]			
H3N2, +/-	1/123	6/120	1/131	6/122	510, (7.43)	[c]0.056[ns]
PR	0.18	1.01	0.00	(ref.)	-	<0.001***
(95%CI)	(0.12-0.26)**	(0.83-1.24)[ns]	(0.00-NA)[ns]			

Pearson's Chi-square test or Fisher's exact test and log-binomial regression model were used to estimate associations. One nasal swab was positive out of 30 nasal swabs considered positive for a group. Numbers in the table represented positive groups out of negative (+/-).

[a] Positive IAV samples of 20 were missing information of subtypes

[b]H1N1 records in 2009 July and September, H1N1 were exclude form the analysis because their Cook's distances were high (> 0.2)

[c]Due to cell counts being low (N < 5), p-values was calculated from Fisher's exact test for count data with simulated p-value based on 10,000 replicates

[d]The p-value of log-binomial model (prevalence ratio) was calculated for Improvement-of- fit χ^2 tests (IOF). IOFs were compared to of intercept model.

Statistically significant; *p-value < 0.05; **p-value < 0.01; ***p-value < 0.001

[ns] Not statistically significant

Table 6-2: Model selection for number of periodic mixture-components (n) in Fourier spectra for periodic IAV transmission fitted with binomial regression model with log link function

$a_0 + b_0 t + \sum_{n=0}^{k} [a_n \sin(w_n t) + b_n \cos(w_n t)]$	Residual deviance	LR χ^2 (df)	Pr (>χ^2)	AIC
n=0	47.44			127.34
n=1	34.41	13.03 (2)	0.0015	118.11
n=2	34.27	0.141 (2)	0.9319	122.02
n=3	31.41	2.857 (2)	0.2397	123.61
n=4	31.28	0.129 (2)	0.9374	127.56
n=5	31.18	0.099 (2)	0.9515	131.41

Table 6-3: Parameters, coefficients and standard error (SE) of the estimates for the final log-binomial regression model with one periodic mixture-component (n=1) in Fourier spectra for influenza transmission in growing pig populations

Description	**Notation**	**Expression**	**Estimate**	**Pr(>|z|)**[2]
Estimates from of log-binomial regression model[1]				
intercept	a_0	-	-1.615	<0.001***
Secular trend	b_0	-	0.002	0.931[ns]
Sine component	a_1	-	-0.144	0.232[ns]
Cosine component	b_1	-	-0.415	0.001***
SE of a_0	σ_{a_0}	-	0.090	-
SE of b_0	σ_{b_0}	-	0.025	-
SE of a_1	σ_{a_1}	-	0.121	-
SE of b_1	σ_{b_1}	-	0.124	-
Covariance of $a_1 and\ b_1$	$\sigma_{a_1 b_1}$	-	0.0003	-
Estimates of seasonal IAV characteristics				
Time to Peak	P	$12(1- \phi/\pi)/2$	6.64	-
Phase shift[3]	ϕ	$-\tan^{-1}(a_1/b_1)$	-0.335	-
Variance of ϕ	Var(ϕ)	$\frac{(a_1\sigma_{a_1})^2 + (b_1\sigma_{b_1})^2 - 2a_1b_1\sigma_{a_1b_1}}{(a_1^2 + b_1^2)^2}$	0.078	-
	Var(ϕ)	From the R "car" package	0.075	-

Amplitude	A	$\sqrt{{a_1}^2 + {b_1}^2}$	0.440	-
Variance of A	Var(A)	$\frac{(b_1\sigma_{a_1})^2 + (a_1\sigma_{b_1})^2 - 2a_1b_1\sigma_{a_1b_1}}{(a_1^2 + b_1^2)}$	0.014	-
	Var(A)	From the R "car" package	0.015	-
Maximal seasonal peak	Smax	$\exp(a_0 + A)$	0.31	-
Minimal seasonal nadir	Smin	$\exp(a_0 - A)$	0.13	-
Disease absolute intensity[4]	I	$\exp(a_0 + A) - \exp(a_0 - A)$	0.18	-
Variance of I		From the R "car" package	0.0025	
Relative intensity[5]	IR	$\exp(2A)$	2.41	-
Variance of IR	Var (IR)	From the R "car" package	0.359	-
Periodic threshold[6]	Sm	Sum(S(t))/30 $\cong$ $\exp(a_0)$	0.21$\cong$ 0.20	-

[1]One periodic component (n=1) in mixture Fourier spectra was expressed as followed:

$$a_0 + b_0 t + \sum_{n=1}^{1}[a_n \sin(2\pi\omega_n t) + b_n \cos(2\pi\omega_n t)].$$

[2]Statistically significant; *p-value < 0.05; **p-value < 0.01; ***p-value < 0.001

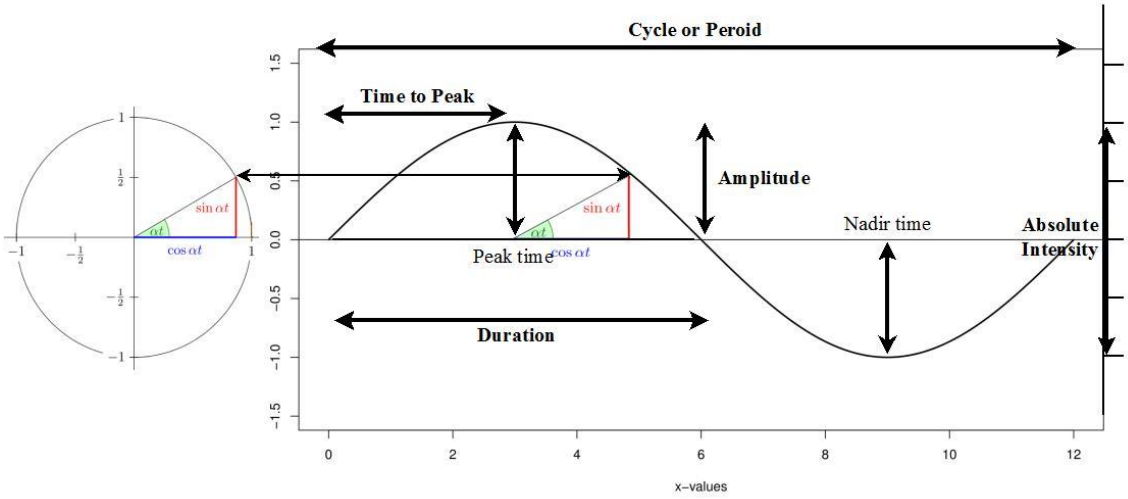
[3]Phase shift is distance of peak from beginning of series expressed in radian

[4]the difference between maximum and minimum IAV prevalence

[5]the ratio of the maximum and minimum IAV prevalence on the periodic curve

[6]the average IAV prevalence thought out the study

Figure 6-1: Generic representation of the characteristics of disease seasonality



Legend:

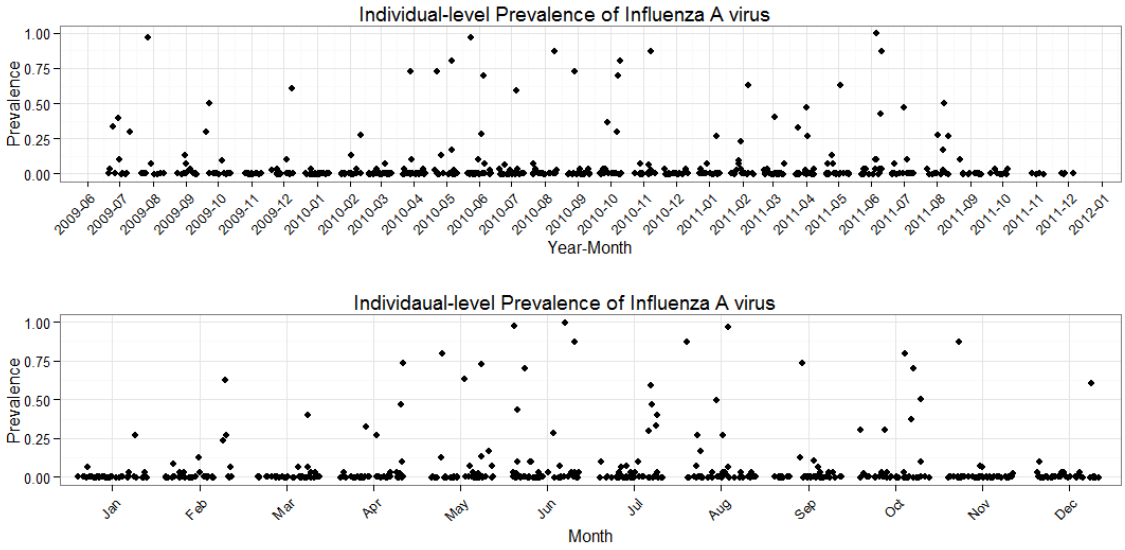
The figure represents a hypothetical disease seasonality with the one component of periodic series (one sinusoidal) characterized by the amplitude (A) and phase shift (ϕ), expressed as $A\cos(2\pi\omega t + \phi)$, where in the graph $\alpha = 2\pi\omega$. By a trigonometric identity as $cos(\alpha \pm \beta) = cos(\alpha)cos(\beta) \mp sine(\alpha)sin(\beta)$, hence

$A\cos(2\pi\omega t + \phi) = a_1\sin(2\pi\omega t) + b_1\cos(2\pi\omega t)$.

The parameter ω is a function of its frequency, in the current study ω =1/12, assuming that the periodicity makes one cycle per 12 months. One cycle per 12 months is equivalent to 2π radians per 12 months with rotational velocity of $2\pi\omega$ radians per 12 months. $2\pi\omega t$ is the angle in radians, meaning the distance around the circumference. The sine function, $sin(2\pi\omega t)$, is the projection of the radius onto the vertical axis. The cosine function, $cos(2\pi\omega t)$, is the projection of the radius onto the horizontal axis. The amplitude (A) is expressed by $\sqrt{{a_1}^2 + {b_1}^2}$, reflecting the height at the peak and nadir times while the phase shift (ϕ) is expressed by $-\tan^{-1}(a_1/b_1)$, reflecting the timing of the

peak relative to the origin. Time to peak is expressed by $12(1- \phi/\pi)/2$. The absolute disease intensity reflects the differences between the peak and nadir.

Figure 6-2: Representation of individual-level prevalence (per monthly-herd) of influenza infection in growing pig populations from 2009 to 2011



Dots represented proportions out of 30 nasal swabs (per monthly-herd) tested by RRT-PCR .The graph above was scaled by “year-month”; the graph below was scaled by “month” in any year.

Figure 6-3: Representation of herd-level IAV prevalence and subtypes (H1N1, H1N1P, H1N2, H3N2) monthly in growing pigs from 2009 to 2011

If at least one nasal swab (out of 30), considered that a herd was positive. Dots represented proportions of monthly positive herd(s) out of number of herds sampled in each month visited.

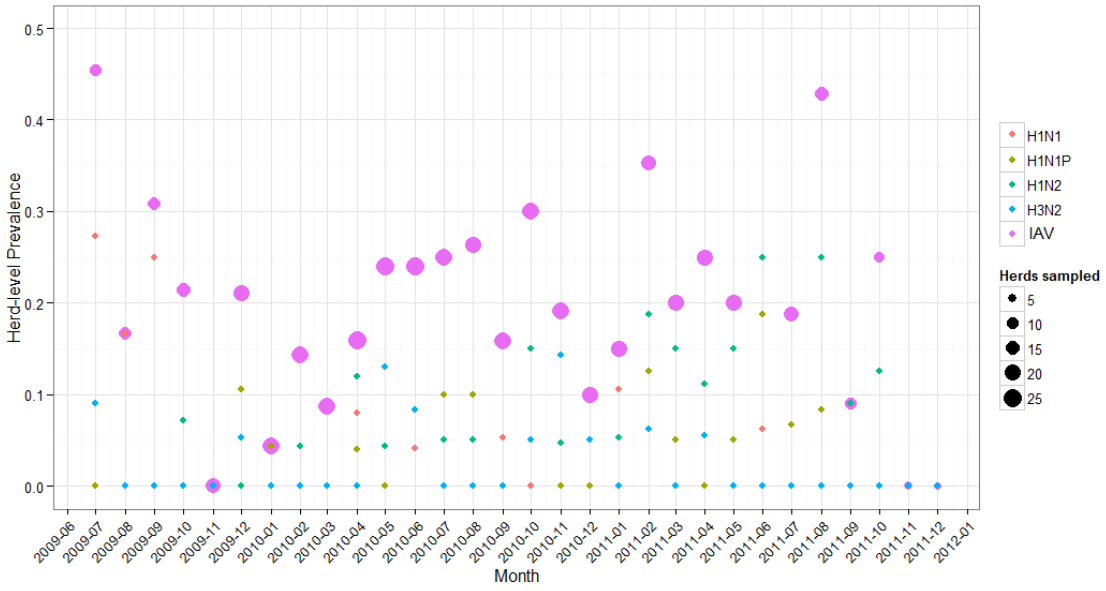
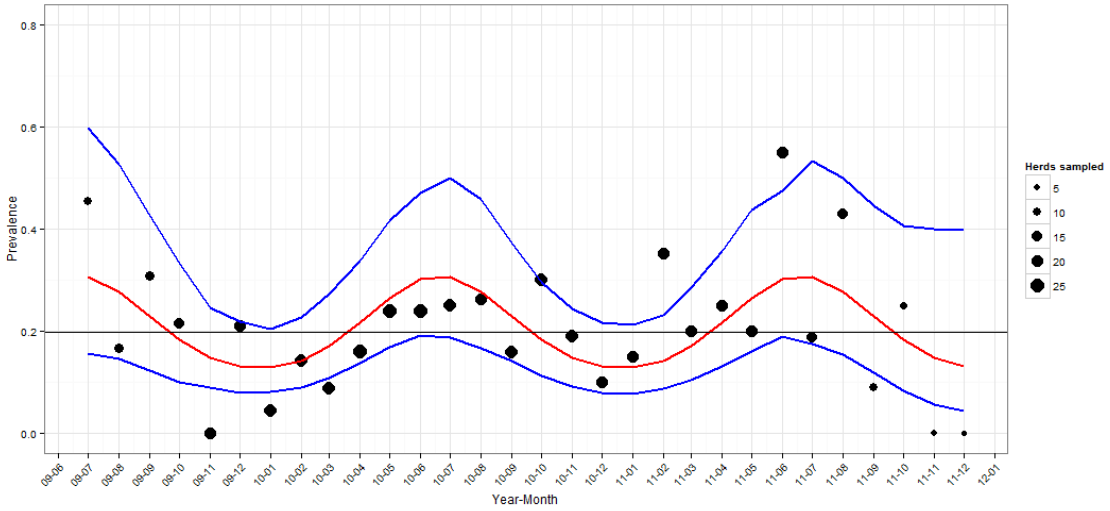


Figure 6-4: Representation of the periodic IAV transmission infected in growing pig populations superimposed on herd-level IAV prevalence monthly from 2009 to 2011

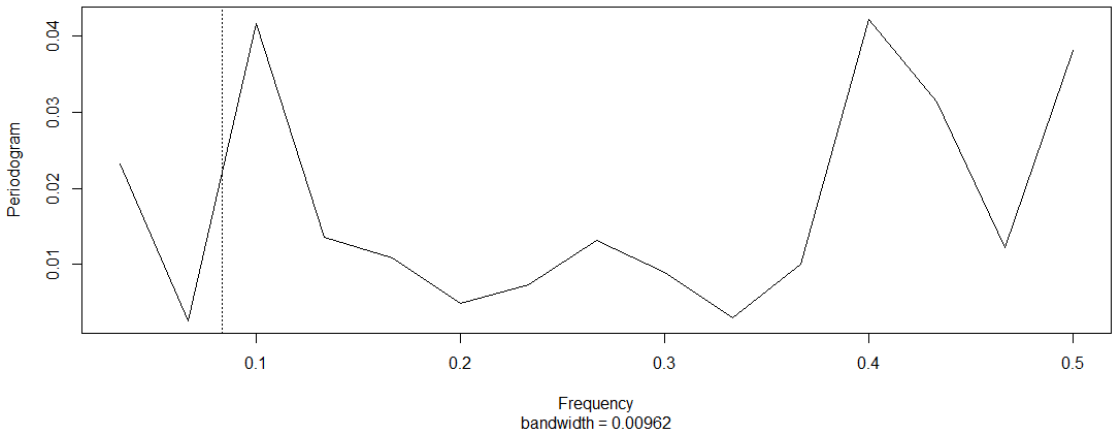


Legend:

The red line expectation (blue lines are corresponding to the 95%confidence interval) of herd-level prevalence estimated by Fourier spectra and with one periodic component. The horizontal line is the average herd-level prevalence across periods of study.

[a]The secular tend of average herd-level prevalence throughout the study was 0.002 (se=0.025), p=0.931

Figure 6-5: the peridogram analysis for Fourier spectra of periodic influenza transmission. The x-axis represents periodic frequency and the y-axis represents a relative variance



Chapter 7 : General discussion and conclusions

General discussion

Influenza A virus (IAV) infection in swine is a concern to producers, veterinarians and the general public. The infection is considered to be one of the top three respiratory diseases in North American swine herds and causes economically respiratory diseases in growing pigs. On-farm assessment of health at the individual pig level for IAV infection involves monitoring respiratory clinical signs (RCS). However, RCS exhibition is not specific to IAV infection. Deriving causality from RCS exhibition to IAV infection requires inductive reasoning using a Bayesian approach (BA). While BA is well-used in veterinary medicine, information regarding IAV epidemiology and control in human medicine is overwhelming, modeling-based knowledge surrounding IAV transmissibility within-herd using RCS as a measure of disease outcome in swine medicine is more limited. Therefore, the aims of this dissertation were to create Bayesian epidemiologic models using inductive reasoning with inverse probability, and to describe and better understand the within-herd transmission of IAV in wean-to-finish pig populations.

This is done by the following 6 chapters:

Chapter 1 provided an overview of relevant literature regarding the principles and concepts of IAV transmission and modeling, leading to the questions addressed in the dissertation. Wealth information is available; however, there are gaps concerning IAV transmission modeling within-herd in growing pig populations. This knowledge could better inform control measure in order to make decisions for controlling, intervening and perhaps eliminating IAV from swine herds based on various conditions of the production system.

Chapter 2 provided an overview of relevant literature regarding BA and concepts that uses in veterinary medicine as similar to inductive reasoning with inverse probability. This chapter summarized when veterinarians are managing patients, veterinarians start with their inference from history and clinical signs to come to an underlying cause using inductive reasoning. In addition, strength of evidence plays an important role in updating from a prior clinical experience to a posterior clinical experience. 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.

To investigate the diagnostic test accuracy of using RCS observation as on-farm assessment for IAV infection, Chapter 3 presented the general theory and an application behind Bayesian estimation of diagnostic test accuracy in an absence of the gold standard, in particular, for RCS. In general, the Se and Sp of RCS from posterior distributions were 0.38 (95% Credible interval (CrI): 0.28-0.48) and 0.66 (95%CrI: 0.61-0.71), respectively. However, the Se and Sp of RCS were reduced by vaccination for vaccinated herds. RCS is able to be as a measure of the true disease prevalence, which was estimated to be 0.24 (95%CrI: 0.16-0.30) for a Midwestern USA growing pig populations.

While Chapter 3 provided the low estimate Se and Sp of RCS and inconsistent estimates for Se and Sp in vaccinated herds, Chapter 4 provided useful information as an on-farm assessment of swine health using RCS, especially for IAV infection. With the Bayesian approach used as inductive reasoning, it infers causal direction from RCS exhibition to causes of RCS (from Chapter 2), thus the use of RCS observation for the measurement of clinical disease and disease transmissibility is feasible in growing pig

populations. To reduce misclassification bias (by accounting for imperfect diagnostic test) arising from RCS, Bayesian models with Susceptible-Infectious-Recovered (SIR) epidemic models were implemented. The transmission rate was 1.40 day^{-1} (95%Cridible interval (CrI): 0.42-5.52) and the reproductive number (R) was 4.19 (95%CrI: 1.98-26.29).

Chapter 5 presented stochastic processes for modeling viral transmission dynamics within a herd in conjunction with waning maternal immunity using the Maternally immune-Susceptible-Infectious-Recovered (MSIR) epidemic model to describe the spread of the clinical IAV disease. This Chapter took R estimates from Chapter 4 to be parameters in models. Another parameter such as waning rate of maternal immunity was obtained this Chapter itself. Based on the stochastic-MSIR simulations, an epidemic may or may not occur in a scenario with medium maternal immunity. Interestingly, if an epidemic occurs, an epidemic can occur at any point in time during a wean-to-finish period with more than one epidemic peak. With low maternal immunity, an epidemic can occur. Heterogeneity of maternal immunity in weaned pigs plays a crucial role in enhancing IAV transmission and subclinical IAV infection may be created by waning maternal immunity. Therefore, for swine herd health managements, veterinarians should focus on managements of herd immunity to control IAV transmission in wean-to-finish pigs.

While Chapter 5 provided explanations regrading stochastics dynamics of IAV and demonstrated that viral dynamics in growing pig population is complex, Chapter 6 presented periodic IAV transmission in growing pig populations. As we may know that periodic IAV transmission in human medicine has been well described; however,

periodicity of IAV transmission in swine medicine has not been yet characterized in sufficient details. The average periodic prevalence was 0.20. The absolute IAV intensity was 0.18. The relative IAV intensity was 2.41, implying that prevalence intensity at the periodic peak was 2.41 times higher compared to that at the periodic nadir. IAV prevalence in growing pigs rises and falls periodically on annual basis. The periodic pattern was characterized by increasing in spring-summer (the peak in mid of June) then slightly decline with nadir in fall-winter. Understanding periodic transmission should result in a better design of the optimal control strategies and periodic IAV vaccination and may help veterinarians apply periodic vaccination to growing pig populations.

Chapter 7 provided that general conclusions and links of the main findins in each Chapter of the disssertation **(Figture7-1)**.

Conclusions

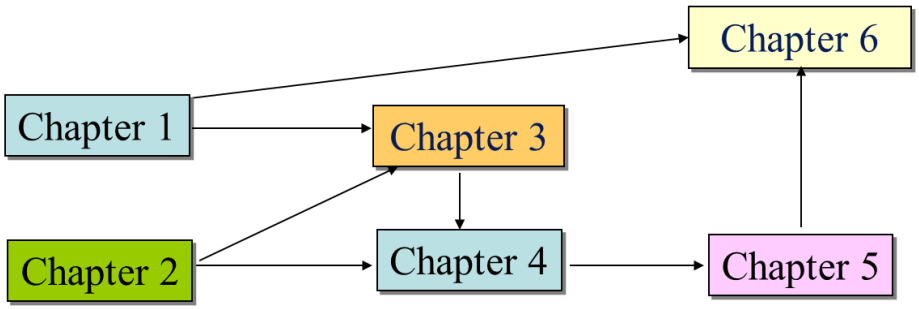
IAV often has been described as an occasional outbreak with a time-limited impact on herd health but its transmission within herds is very progressive. After IAV introduction into a swine herd, on-farm assessment may be made and its accuracy needs to be identified. On-farm assessment for IAV infection uses respiratory clinical signs (RCS) and behavioral observations. The dissertation showed that the usage of RCS as measured of disease transmission is potential. However, RCS has to take into consideration a misclassification of diseases outcome because accuracy of its diagnostic test is low. An implementation of Bayesian approach can incorporate such misclassification, leading to estimating parameters of interest such as transmission rate and the reproductive number. By using a Bayesian approach, inductive reasoning applies inverse probability to make inference from effects to causes. Therefore, such approach

allows us to use clinical diseases making inference to the cause(s) of such clinical diseases, and thus being able to measure disease transmission.

Altogether the work presented in the dissertation not only provided science-based information but also generated "modelling-driven research questions" on IAV transmission control and management to mitigate devastation of clinical IAV diseases, and perhaps eliminate IAV infection from swine populations. "Modelling-driven solution" can assist swine veterinarians to make decision on appropriate intervention strategies with/without accounting for seasonal IAV transmission, to reduce production losses, and to decrease IAV transmission within herds. "Modeling-driven experiment" can also be tested to prove a hypothesis that veterinarians may have, for instance, if seasonal vaccination in growing pigs may help improve productivity in growing pigs. Performing a simulation study may follow a flowchart as presented in **Appendix1**.

Figure

Figure 7-1: Representation of the connections of the dissertation



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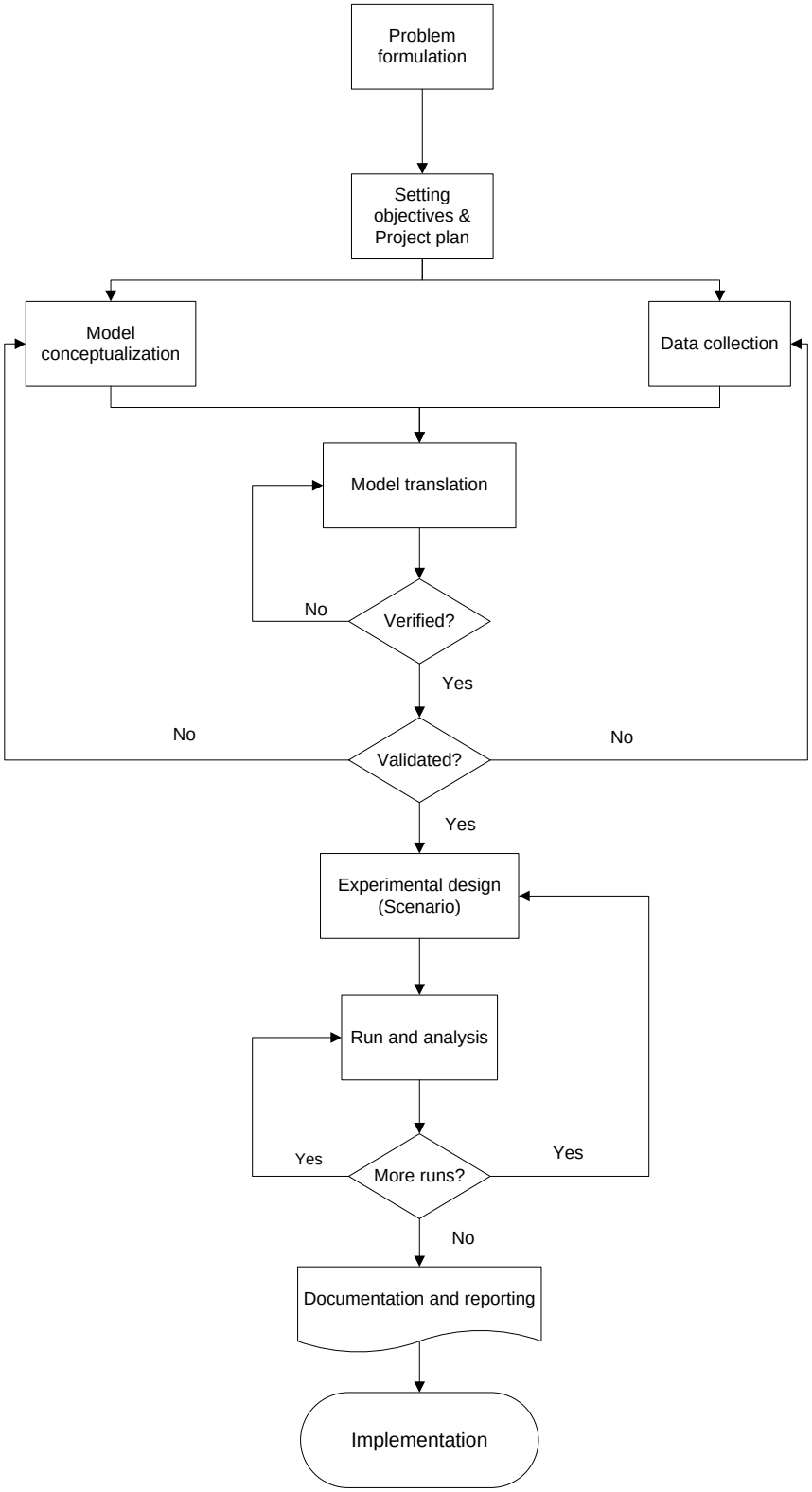
Appendix:

Appendix 1: Steps in a simulation study (Banks, 2000; Banks et al., 2010)



Appendix 2: Other works

Douglas Marthaler, **Nitipong Homwong**, Kurt Rossow, Marie Culhane, Sagar Goyal, James Collins, Jelle Matthijnssens, Max Ciarlet, 2014. Rapid detection and high occurrence of porcine rotavirus A, B, and C by RT-qPCR in diagnostic samples. **J of Virological Methods**. (209): 30-34.

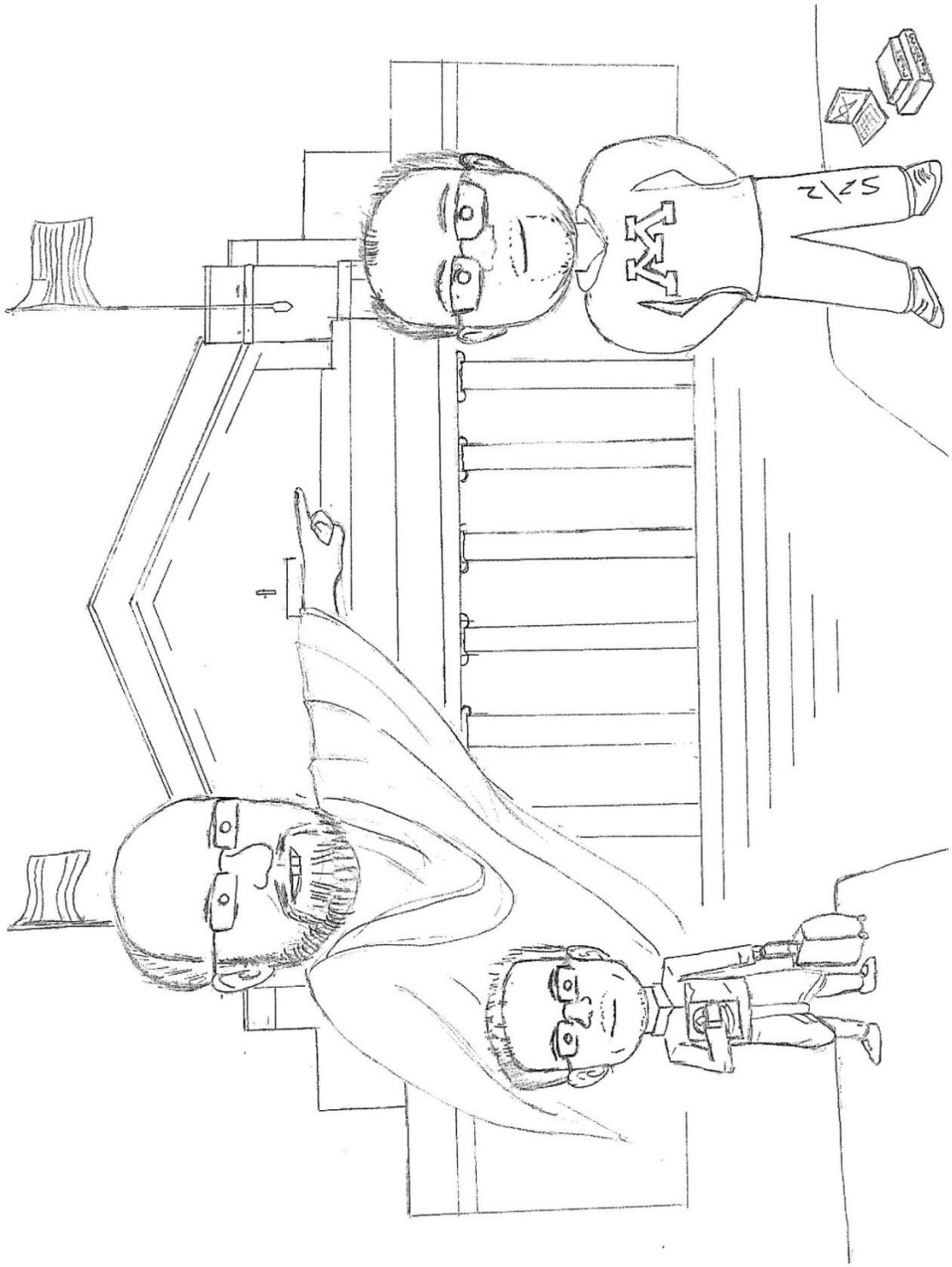
Nitipong Homwong, Matthew C. Jarvis, Ham Ching Lam, Andres Diaz, Albert Rovira, Martha Nelson, and Douglas Marthaler. Characterization and evolution of porcine deltacoronavirus in the United States. 2015. **Preventive Veterinary Medicine**. http://dx.doi.org/10.1016/j.prevetmed.2015.11.001.

Luiza R. Roos, Eduardo Fano, **Nitipong Homwong**, Brian Payne, Maria Pieters, A model to investigate the optimal seeder-to-naïve ratio for successful natural *Mycoplasma hyopneumoniae* gilt exposure prior to entering the breeding herd. 2016. **Veterinary Microbiology**. doi:10.1016/j.vetmic.2016.01.008

Nitipong Homwong, Stephanie Rossow, Andres Diaz, Max Ciarlet, Douglas Marthaler. Three level Mixed-effects logistic regression model reveals complex epidemiology of swine rotaviruses in diagnostic samples from North America, **Submitted to PLOS ONE**.

Luiza R. Roos, **Nitipong Homwong**, Maria Pieters. Diagnostic sensitivity and specificity of laryngeal swab samples as an *In Vivo* diagnostics tool for detection of *Mycoplasma hyopneumoniae* in gilts**. In prepare.**

Appendix 3:



Appendix 4: Notes