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The role of gilts in swine Influenza A virus transmission and evaluation of quarantine and vaccination strategy in Danish sow herds

Veterinary Master's thesis (30 ECTS points)

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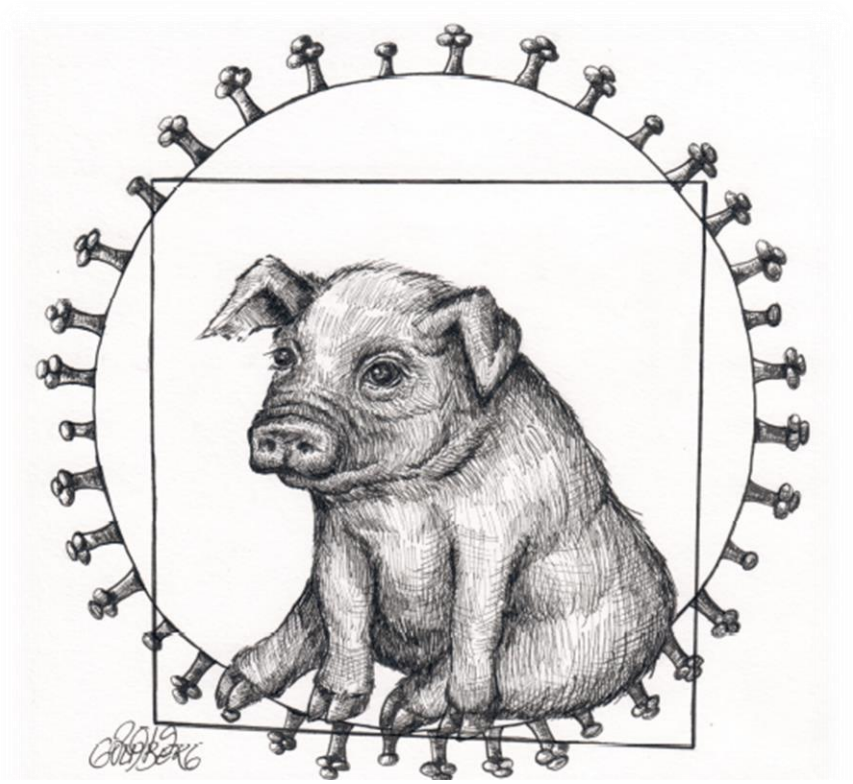

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Preface and acknowledgement

This thesis is written as a final part of our master's degree in Veterinary Medicine at the University of Copenhagen, Department of Veterinary and Animals Science from September 2019 to January 2020. The purpose of the project was to clarify the role of gilts in swine Influenza A virus transmission through a study in ten Danish sow herds by investigating quarantine management, biosecurity measures and vaccination strategy. The ten herds were sampled over a period of three months from September to November 2019.

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Abstract

Swine Influenza A virus (swIAV) is widespread in pig production. Previous studies have shown that replacement gilts contribute to the introduction and/or maintenance of swIAV in herds. There are several ways of introducing new gilts into herds including through quarantine and different vaccination strategies. The objective of this study was to clarify the role of gilts in the transmission of swIAV in Danish sow herds and evaluate the effect of quarantine measures and gilt vaccination.

The study was conducted through cross-sectional studies performed in ten Danish sow herds, including five vaccinated and five unvaccinated herds. Blood- and nasal swab samples of gilts, first parity sows, and piglets were collected in different sections of the production system and analysed for the presence of swIAV and antibodies. An association between the seroprevalence, detection of swIAV, quarantine measures, and vaccination strategy were investigated to identify possible risk factors for swIAV introductions and persistence within the herds.

The results revealed a difference in seroprevalence in gilts of the gestation unit (93% and 69%, $p<0.001$) and farrowing unit (95% and 58%, $p<0.001$) between vaccinated and unvaccinated herds, respectively. Moreover, antibody levels in farrowing units and across all gilt subpopulations were significantly higher in vaccinated herds ($p=0.006$, $p<0.0001$). However, generally no difference in virus prevalence of gilts and piglets was found between vaccinated and unvaccinated herds. Nevertheless, six out of ten herds had virus positive gilts at the end of the quarantine with an overall virus prevalence of 11.5% and virus positive gilts at the end of the quarantine were associated with positive piglets one-week-of-age (RR=2.5, 95%CI [1.03, 6.37], $p=0.047$). Observations and questionnaires about quarantine management and biosecurity indicated that the biosecurity focus was not aimed at protecting the gilts against influenza, but rather aimed at protecting the sow herd from gilts in the quarantine. This suggests the need to focus on biosecurity interventions and proper immunisation of gilts to control swIAV transmission between sow herd, humans, and quarantine.

Keywords: Swine Influenza A virus, gilts, transmission dynamics, influenza risk factors, influenza control, quarantine, biosecurity, influenza vaccination.

List of abbreviations

AP12	*Actinobacillus pleuropneumoniae* serotype 12
Ct value	Cycle threshold
ELISA	Enzyme Linked Immunosorbent Assay
H1av	HA gene from avian-like H1N1
H1N2dk	Influenza A Virus strain isolated in Danish pigs in 2003
H3hu	HA gene related to human influenza virus H3N2.
H1N1pdm09	Influenza A Virus strain causing human pandemic in 2009
HA	Hemagglutinin
HI	Hemagglutination Inhibition
huIAV	Human Influenza A Virus
IAV	Influenza A virus
Ig	Immunoglobulins
M1	Matrix protein 1
M2	Matrix protein 2
McRebel	Management Changes to Reduce Exposure to Bacteria to Eliminate Losses
MDAs	Maternally Derived Antibodies
MHC	Major histocompatibility Complex
MYC	*Mycoplasma hyopneumoniae*
N1av	NA gene from avian-like H1N1
N2sw	NA gene from swine H3N2 or H1N2dk
NA	Neuraminidase
NeuAcα2,3Gal	*N*-acetylneuraminic acid-α2,3-galactose
NP	Nucleoprotein
NS1	Nonstructural protein 1
NS2	Nonstructural protein 2
PA	Polymerase Acid protein
PB1	Polymerase Basic protein 1
PB2	Polymerase Basic protein 2
PRDC	Porcine Respiratory Disease Complex
PRRS (PRRSv)	Porcine Reproductive and Respiratory Syndrome (virus)
R0	Reproduction number
RNA	Ribonucleic Acid
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SA	Sialic Acid
S/N value	Sample-to-negative value
SPC	Summary of Product Characteristics
SPF	Specific patogene free
swIAV	Swine Influenza A virus
VAERD	Vaccine Associated Enhanced Respiratory Disease

1. Introduction

1.1 Characteristics of Influenza A Virus

Influenza A virus belongs to the virus family *Orthomyxoviridae* which contains seven different genera including Influenza A, B, C, and a recently identified type D (1). Common for all influenza types are the enveloped RNA virus and the segmented genome, which contain seven to eight gene segments comprised of negative-strand viral RNA (2) (**Appendix 1**). Hemagglutinin (HA) and neuraminidase (NA) are surface glycoproteins and facilitate viral entry and release, respectively. They are used to characterize Influenza A virus (IAV) subtypes (3). 16 HA and nine NA IAV subtypes have been discovered in aquatic birds (4), the subtypes 17-18 HA and NA 10-11 are found in bats (3) but only H1, H3, N1, and N2 have become enzootic in pig production (5).

1.1.1 Antigenic drift and shift

The lack of RNA polymerase proofreading is responsible for random errors in all IAV proteins and positive selection of mutations, driven by host immunity mainly directed towards the surface proteins, alter antibody binding and promotes antigenic drift (3). The altering of antibody affinity of neutralising antibodies directed against the HA protein makes it difficult for the immune system to recognize and neutralise IAV infections.

Genetic shift is a process of viral reassortment and is less frequent than antigenic drift. It can occur when two IAVs co-infect the same host cell, where the segmented genome allows viral progeny to contain genes from both parental viruses. Antigenic shift occurs when gene-exchange involves the gene-segments HA or NA (2). Reassortment has shown to be important in host shift events and the origin of new subtypes to which the human population is immunological naïve, resulting in pandemic outbreaks (6,7). Swine Influenza A virus (swIAV) and human Influenza A virus (huIAV) both have preferences for the NeuAcα2,6Gal linked sialic acid receptors, whereas the preference of avian Influenza A virus is NeuAcα2,3Gal-receptors (8). Pigs are thought to play a distinctive role in the global epidemiology of human influenza because pigs have receptors for both avian and mammalian IAV, and can therefore act as a mixing vessel for generation of new reassorted subtypes which can cross species barriers (7). However, a study of the distribution of both sialic acid receptors in the pig respiratory tract showed a close similarity to the published data of the human tract, suggesting that humans can also act as a mixing vessel (8).

1.2 Pathogenesis

SwIAV has tissue tropism in the airways and virus replication is limited to epithelial cells of the upper and lower respiratory tract in pigs - the nasal mucosa, ethmoid, trachea, and lungs, and cause acute respiratory tract infection. Virus excretion and transmission occur exclusively from the respiratory route (9). SwIAV has a short incubation time, one to three days (10,11), and virus shedding was detected in experimental studies in naïve pigs from one day post inoculation to five to seven days (9,12,13), but some studies have evidence of maternally derived antibodies (MDA) might contribute to "prolonged IAV shedders" for more than two weeks (14–16).

1.2.1 Clinical signs

SwIAV is widespread in pig production affecting animal welfare and causes negative economic consequences due to reduction of farm productivity and increased medical treatment costs (17–19). Disease can proceed as epizootic or enzootic infections. In an epizootic outbreak swIAV causes high morbidity with rapid recovery, but continuous exposure of naïve piglets can lead to persistence of swIAV in the herd resulting in enzootic disease (11). Clinical findings in experimental swIAV infection studies revealed pneumonia and lower respiratory tract symptoms - dyspnoea, coughing, and abdominal breathing, attended with high fever above 40°C (9,10). Moreover, mild clinical signs as anorexia, coughing, sneezing, nasal/ocular discharge, and conjunctivitis are often observed, and in field studies also correlated with swIAV infections (12,13,15). Sporadic abortion, increased stillborn and weak piglets have also been reported after herd outbreaks and in experimental studies (20). However, swIAV infection can be subclinical without the clinical signs mentioned above (10).

1.2.2 Co-infections and Porcine respiratory disease complex (PRDC)

SwIAV is a primary agent and can initiate clinical disease by itself. SwIAV causes impairment of the immune system through viral destruction of the mucociliary apparatus in the airways clearing the way for opportunistic agents, which can exploit the virulence mechanism of swIAV (21,22). SwIAV and Porcine Reproductive and Respiratory syndrome virus (PRRSv) are the main agents in the PRDC (21,22). Infections with swIAV and either PRRSv or *Mycoplasma hyopneumoniae* extend and causes severe respiratory disease (23). Clinically ill PRRSv positive pigs were also found more likely to be infected with swIAV than PRRSv negative pigs (22).

1.3 Immunology

The innate immune response is a non-specific response and contains a cellular and humoral part, but the first line of defence against swIAV infection in the upper airways is the mucosal surface (12). It consists of a chemical and physical barrier of ciliated epithelial cells and a mucus and mucin layer produced by goblet cells. The mucus contains antimicrobial peptides including decoy sialic acid (SA) which can bind HA and entrap virus and subsequently the mucociliary barrier clear virus (24).

The cellular part consists of macrophages, natural killer cells, $\gamma\delta$ T cells (unconventional T cells), granulocytes, and dendritic cells, whereas the humoral part provides acute phase proteins in the blood, cytokines, and the complement system (22). The neutrophil infiltration is important for the controlling and clearance of acute swIAV infection in the respiratory tract and lungs by phagocytosis and initiating a proinflammatory cytokine response (10,22). There has been detected a tight correlation between virus titre, cytokine response in the airways (interferon-alpha, interleukin-6, tumour necrosis factor-alpha), and the severity of symptoms (10,25). These cytokines attract inflammatory cells to the lung tissue, increase vascular permeability, and induce bronchoconstriction. They are all pyogenic and cause pyrexia with a body temperature above 40℃. Virus replication, cytokine response, and disease peaks 24 hours post infection with swIAV (10,11). Dendritic cells connect the innate and adaptive immune system, when recognizing foreign antigens and present them on the cell surface to CD4+ T helper cells in the lymph nodes (22).

The adaptive immune response can adapt to the specific pathogen - the most important cells are T (T-helper cells (CD4+)), cytotoxic T cells (CD8+), and B cells (plasma B cells, memory B cells). CD4+ T helper cells promote the proliferation of CD8+ T cell into cytotoxic T lymphocytes and help B plasma cells with improving antigen affinity and maturing to memory B cells (26,27). T cells are more directed against conserved regions in the surface and internal proteins. Additionally, cytotoxic T lymphocytes are important in the cell-mediated immune response and viral clearance from the lungs, by initiating cytolysis of virus infected cells when recognizing internal antigen-presenting major histocompatibility complex (MHC) class I (9,12,13,27).

Antibody response is mainly against the swIAV proteins HA, NA, matrix protein (M), and nucleoprotein (NP) (**Appendix 1**), nevertheless, only antibodies towards HA can prevent viral attachment to the host cell and neutralise viral activity (27). NA antibodies can prevent newly

formed viral release. M and NP antibodies can contribute to cytolysis of infected cells. These antibodies can be detected in a haemagglutination inhibition test (HI test) or virus neutralisation test (27). In an experimental study, CD4+ and CD8+ IAV specific reactive T cells as well as neutralizing antibodies were present in the lungs four days post infection. Immunoglobulins G (IgG) and immunoglobulins A (IgA) were detected four to six days post infection and reached the highest levels nine and 15 days post infection, with IgG as the predominant isotype (12,13). Similar responses were found in the airway mucosa with IgA antibodies as the predominant isotype (12), demonstrating that IgA is locally secreted by plasma cells in respiratory mucosal tissue (27). A virus specific IgA response and T cell mediated immune response are thought to be important for protection against reinfection with swIAV because of more cross-reactivity and swIAV entry and infection of mammalian nasal mucosa (12,27).

1.3.1 Maternally derived antibodies (MDAs)

Pigs have an epitheliochorial placenta, three maternal layers and three foetus layers, which prevents placental transfer of immunoglobulins (28). Moreover, the immune system of the piglet is not completely developed, wherefore they rely on maternally derived antibodies (MDAs) from colostrum, when fighting neonatal infections and the presence of MDAs in piglets is estimated to decay within ten weeks (11,16).

Experimental studies have shown, that MDAs originating from both naturally exposed and vaccinated sows protected partly against clinical signs of primary swIAV infection, but were not able to prevent virus replication after weaning (14,29). In the study by Loeffen *et al.*, 2003, pigs with MDAs shed virus longer than pigs without MDA (14).“Prolonged IAV shedders” for more than two weeks were also found in longitudinally field studies (16,30), and swIAV positive piglets one-week-of-age were detected despite MDAs (16).

Experimental studies have also shown that inhibition or delay of antibody and T cell proliferation response of a primary infection were affected by MDAs (11,14,30,31). However, in one study, pigs were nevertheless shown to be protected against a second infection with a homologous virus strain in spite of the impaired immune response (31). The lacking ability of MDAs to control virus replication in the upper airways and the inhibition of the immune response might explain “prolonged IAV shedders” (14).

Maternally-derived antibodies do not prevent transmission of swIAV between neonatal pigs, when the reproduction rate (R0) does not fall below one, and MDAs were shown to increase the likelihood of swIAV persistence in herds, because of a longer duration of epidemic within the batch which benefit transmission to new susceptible piglets (11,32,33).

1.3.2 Vaccines

In Denmark, two commercial inactivated, adjuvanted, whole swIAV vaccines are available on the market. The monovalent Respiporc FLUpan H1N1 containing the subtype H1N1pdm09 and the triple valent Respiporc FLU3 containing the subtypes H3N2, H1N1 and H1N2 (34,35). The vaccines are licensed to be used in pigs from 56 days of age, however, the primary vaccination is recommended after day 96 if there are risk of high levels of MDAs interfering with the vaccine response. According to the manufacturer, the duration of immunity for Respiporc FLU3 is six months if administered after 96 days of age and four months when vaccinating between day 56-96 of age with two injections of 2 ml intramuscular 21 days apart which is defined as a basis vaccination. Furthermore, it is described that a booster vaccination given 14 days prepartum can protect the piglets from clinical symptoms of influenza for at least 33 days after farrowing because of MDAs. The clinical protection of the piglets is not mentioned in the summary of product characteristics (SPC) of Respiporc FLUpan H1N1 and the duration of immunity of this vaccine last for three months (34,35).

The two commercial vaccines available in Denmark cover the predominant subtypes in Danish pig production even though the vaccine strains are 15-19 years old (5,34). The vaccines primarily provide an antibody response against the specific HA in the vaccine by the production of neutralizing serum IgG which can reduce the spreading of swIAV in the lungs and viral replication (34–37). However, the efficacy of the vaccine can depend on the homology between vaccine and herd strain, the levels of antibodies produced, antigenic dose, and adjuvants (13,27,36).

Sow vaccination is used in Denmark to protect sows against influenza, ensure the production of MDAs for the piglets, and provide clinical protection (38). In several studies mentioned previously in chapter 1.3.1, it was suggested that MDAs originating both from naturally exposed and vaccinated sows do not protect the piglets against virus infection and could contribute to swIAV persistence within the herd (11,33). Furthermore, experimentally studies observed a development of vaccine associated enhanced respiratory disease (VAERD) in weaned pigs heterologous challenged in the presence of MDAs. The phenomenon is associated with

the use of vaccine strain with the same hemagglutinin subtype as the challenge strain, but with substantial antigenic drift with no cross-reactivity detected in HI test (39,40).

1.4 Epidemiology

1.4.1 Subtypes in Danish pig production and surveillance

In European pig production, the predominant subtypes are the "avian-like" H1avN1, H3N2sw, the "human like" H1huN2, and the pandemic IAV from 2009 H1N1pdm09 (5,41).

In Denmark, the passive surveillance program for swIAVs was implemented in 2011 and surveillance data from 2018 has shown that H1N2dk and H1N1pdm09 were the most prevalent subtypes circulating in Danish pig production. H1N1pdm09 appeared in Denmark in January 2010 and has since then represented 14-25% of the subtypes detected through the Danish annual swIAV surveillance (5). Several reassortments between the H1N1pdm09 and H1N2dk have been observed. Moreover, the majority of the internal genes of the H1N2dk is now of H1N1pdm09 origin. The avian-like H1avN1 has been declining since 2014 and the subtype H3N2sw has not been detected in Denmark since 2014. The H1huN2 from Europe with human-like HA gene has never been isolated in Danish pig production. A few cases of H3huN2dk have been detected and cause great concern since the Danish pig population has no immunity and no vaccine is available towards the human seasonal H3 protein (5).

1.4.2 Transmission of Swine Influenza A Virus

In order to analyse or evaluate the transmission dynamics of swIAV, a measure of quantification is needed. The reproduction number (R0) is defined as the expected secondary cases in a completely susceptible population. Generally, if R0 >1, the swIAV infection will spread and become enzootic, and if R0 <1, the infection will not spread (42,43). R0 of swIAV is estimated in controlled studies to 10.4-10.6 in unvaccinated pigs (32,42). The primary route of swIAV transmission in pigs is direct contact (43,44), however, indirect transmission via aerosols or contaminated fomites can also contribute to the transmission of swIAV (43).

Influenza A virus can persist in different environmental matrices such as air, water, soil, faeces, and fomites. The main factor that influences the half-life of IAV, regardless of matrix, is temperature. IAV persists 16.5 times longer at temperatures between 7°C and 12°C then at temperatures ≧27°C. Other factors that affect half-life are salinity, pH, and humidity (45).

When pigs sneeze, cough, or breathe, respiratory droplets of multiple sizes are spread into the surrounding area, these droplets are termed aerosols and are divided into large (>5 μm) and smaller aerosols (<5 μm). The size is important as smaller aerosols remain airborne and thereby increase the potential transmission between pens (46). Aerosol transmission can be affected by humidity, temperature, and ultraviolet radiation from sunlight (46–49). Even though these factors influence swIAV transmission, a seasonal pattern has been discussed because of different outcomes of studies, which may be a result of different methods of measuring (antibodies, virus shedding), study design, sample size, active, and passive surveillance (50). However, the sample size of the passive surveillance of swIAV in Denmark increases in the winter months which could indicate a season peak, but the number of positive samples are stable throughout the year, suggesting that swIAV is present in Danish herds year round (5).

Transmission between herds by means of aerosols has been debated. Under experimental and field conditions, swIAV has been detected in airspace 1.6 km away from an infected farm (43), although a new longitudinal study by Chamba Pardo *et al*, 2018**,** did not establish an association between swIAV infection in weaned pigs and farms located within a 1.6 - 4.8 km radius (51). This study indicates a low likelihood of inter-herd transmission by aerosols (51), but long-distance transport of pigs between herds or countries should be considered as a transmission route and therefore, further investigated. It can potentially lead to a spatial dissemination of swIAV (5,43).

1.4.3 Risk factors of swIAV introduction and maintenance in pig herds

Several risk factors for the introduction and maintenance of swIAV in pig herds have been investigated, but very few studies have investigated the role of gilts. However, some studies suggest replacement gilts and piglets with or without maternally derived antibodies are an important reservoir for maintenance of swIAV in pig herds, because weekly batches of new susceptible gilts and piglets will contribute to the persistence within the herd (18,50–53). Risk factors associated with novel swIAV introduction include intake of replacement gilts, lack of quarantine and common biosecurity measures, and human interaction (53–57).

Simon-Grifé *et al.,* 2011 found that open partitions between pens could serve as a risk factor for higher seroprevalence (55). Continuous flow in a section, movement of pigs in the production system, pig density, and herd size were found as risk factors in other studies (33,54). Cross-fostering increase direct contact to other piglets and sows and according to Rose *et al.,* 2013,

specific management practices of sows and piglets, such as cross-fostering can serve as a potential risk factor (30). Indicating that lack of internal biosecurity increases direct contact between pigs which is important for transmission of swIAV.

The infection dynamics in sow herds are complex because of great difference in replacement rate and source, vaccination strategy, previous swIAV infections, pig age and immunity levels (50,52). A sow herd has a rapid turnover of new susceptible piglets and pigs with a diverse level of immunity towards swIAV, which are continuously moved in between production units. The sow replacement rate in Denmark is 45-55% pr. year and is similar to other countries with extensive pig production (50,58). Increased replacement rate has been associated with increased seropositivity in sows (55), and Diaz *et al.*, 2015, found that gilts residing on farms for less than four weeks had significant higher odds (OR 7.9; [1.1-17.1]) of being swIAV positive than replacement gilts residing on farm for more than four weeks (50). The study did not include sows, however, they were included in a Brazilian study which revealed a significant higher sow herd antibody prevalence among Brazilian farms using external replacement gilts (53). Both studies found multiple herds with either co-circulating influenza subtypes or subtype switch over time. Suggesting that replacement gilts contribute to the introduction and/or maintenance of swIAV in the sow herds.

Poor external biosecurity with uncontrolled entrance to the herd for both humans and animals can be a potential risk of swIAV introduction (53,55–57). Biosecurity measures associated with swIAV seroprevalence are bird-proof net, quarantine, and external replacement gilts (53,55). Human-to-swine transmission has also been investigated in a Norwegian study (57). A cross-sectional study among 115 sow herds showed that a preliminary detection of influenza like illness amongst farm personnel was associated with a seropositive farm (OR=4.15 [1.5-11.4] p=0.005). Note that active surveillance data showed that Norwegian pig farms were H1N1pdm09 negative prior to the study (57), indicating that humans was the source of IAV introduction into the herd, supporting other studies that have revealed swIAV zoonotic nature (59,60).

1.4.4 Control measures

Limiting transmission of swIAV in herds by internal biosecurity can be difficult. Transmission of swIAV can occur both under low and medium biosecurity levels (43,61). Allerson *et al.*, 2013, demonstrated that the movement of personnel wearing, swIAV contaminated coveralls, and boots all served as an indirect transmission route between infected and sentinel pigs. However, in this study, changing of clothing and boots and washing of hands and face did not successfully hinder the indirect transmission of influenza (61).

Herd management practices to control direct and indirect transmission of viruses as PRRS and Porcine Circovirus type 2, are described in the literature (62,63), but such practices on swIAV are not well documented (64). Nevertheless, interventions such as banning cross-fostering, early weaning (0-7 days), disposable gloves/overshoes in between sections, extensive cleaning, and disinfection after every batch, are described to decrease swIAV transmission (17,52,64).

Similar interventions are described when controlling PRRS and a set of rules called McRebel is very important when eliminating PRRSv in sow herds. McRebel stands for "Management Changes to Reduce Exposure to Bacteria to Eliminate Losses", and internal biosecurity measures such as less cross-fostering, sectioning, minimise handling of piglets, and all in/all out, are crucial in order to stop recirculation of PRRSv. Furthermore, gilt acclimatisation and immunising is just as important when controlling PRRS (62). These control measures could be implemented in order to limit the direct swIAV transmission between infected and naïve pigs, and they have shown to contribute to the elimination of swIAV in pig herds (17,64).

Sow vaccination is commonly used as a control measure in the swine industry, including Denmark, to protect the sows against lung infection and clinical symptoms, likewise increasing maternally derived passive protection of the piglets from clinical disease (65). Two different sow vaccination strategies are used: mass sow[1] and pre-farrow[2] vaccination. In order to get optimal protecting of sows and gilts, the vaccine strain has to be homologous to the herd strain (66), however, vaccine derived maternally antibodies might not protect the piglets against swIAV infection (14,31) (Chapter 1.3.1). Therefore, it could be beneficial to eliminate the source of infection through increased external biosecurity measures.

[1] Mass sow vaccination: All sows and gilts are vaccinated at one time with an interval of one to four times a year (38)
[2] Pre-farrow vaccination: All pregnant animals are vaccinated three to five weeks prepartum (66)

As mentioned before, human-to-pig swIAV transmission can serve as a risk factor for novel swIAV subtype introduction. Therefore, farm personnel biosecurity implementations such as vaccination, surgical masks, and gloves could be considered when designing a herd specific or One Health swIAV control program (60,61,66,67). Most importantly, farm personnel with flu-like symptoms must stay at home (41).

There is a lack of scientific data investigating the effect of quarantine areas for the introduction of swIAV and most literature focus on quarantine measures in regard to infections with PRRSv and *Mycoplasma hyopneumoniae* (51). Quarantine areas for replacement gilts before introduction to the herd can be used in order to control swIAV circulation at weaning and/or the herd prevalence of swIAV (52,53). Interestingly, Chamba Pardo *et al.*, 2018, revealed a positive correlation between swIAV detection in gilts upon entry and swIAV positive piglets at weaning, but no significant association between quarantine and swIAV positive piglets at weaning. This suggests that swIAV negative gilts at entry to the herd are important for controlling the introduction of virus (51).

In Denmark, gilts can be introduced to the sow herd in several different ways. Some herds employ quarantine before introduction and some herds have a special vaccination strategy for gilts. Gilt vaccination, decreased introduction frequency, and the use of quarantines, where gilts will recover from infection before moved to the sow herd, can be positive measures of preventing swIAV introduction and maintenance by replacement gilts (52,53). However, the effect of these measures in immunising and limiting the spread of swIAV has not been examined. Therefore, the focus of this project is to evaluate the effect of different quarantine measures and vaccination strategy through a series of cross-sectional studies conducted in ten Danish sow herds.

2. Objective and Hypotheses

The primary objective of this study is to clarify the role of gilts in swine Influenza A virus transmission in Danish sow herds and evaluate the effect of quarantine measures and vaccination strategy. The swIAV status (positive/negative) and the antibody status (positive/negative) was determined based on real-time RT-PCR targeting the matrix protein (M) gene of swIAV and antibody ELISA targeting the nucleoprotein (NP) antigens of IAV, respectively. The study was carried out in ten sow herds, including five herds that applied swIAV vaccination and five herds without any swIAV vaccination.

Hypotheses

H0a: There is no significant difference in section prevalence of virus positive gilts between vaccinated and unvaccinated herds.

H0b: There is no significant difference in prevalence of virus positive pooled samples from piglets in the farrowing unit between vaccinated and unvaccinated herds.

H0c: There is no significant difference in section prevalence of antibody positive gilts between vaccinated and unvaccinated herds.

H0d: There is no significant difference in levels of antibodies in seropositive gilts between vaccinated and unvaccinated herds.

H0e: There is no correlation between virus positive gilts at the end of quarantine and positive piglets.

H0f: There is no correlation between antibody prevalence at the end of quarantine and virus positive gilts or piglets in the herd.

H0g: There is no correlation between levels of antibodies in gilts before and after farrowing and virus positive piglets.

3. Materials and Methods

3.1 Study design

The transmission dynamics was investigated by using a cross-sectional study in ten Danish sow herds, where blood- and nasal swabs were sampled among gilts at five time points: arrival in the quarantine, one week before leaving the quarantine, arrival to the mating unit, one week before and after farrowing to detect antibodies and virus shedding. A total of 80 blood samples and 180 nasal swabs from each herd were collected, with an overall total of 800 blood samples and 1800 nasal swabs.

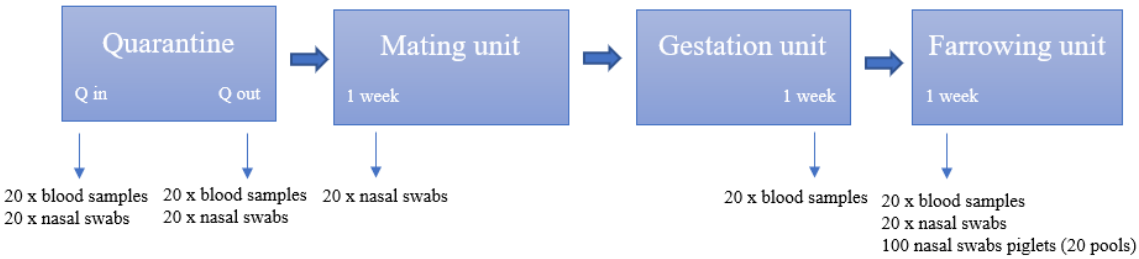


Fig. 1: Study design. Illustration of the cross-sectional study design with the number of samples in one herd. One week after arrival to quarantine, blood samples and nasal swabs were collected from 20 new gilts (Q in). One week before leaving quarantine, blood samples and nasal swabs were collected from 20 gilts (Q out). One week after arrival to the mating unit, 20 nasal swabs were collected from gilts. The last week in the gestation unit, 20 blood samples were collected from gestating gilts. One week after farrowing, blood samples and nasal swabs were collected from 20 first parity sows. In the farrowing unit, five piglets from each sampled first parity sow were nasal swabbed, and the five individual nasal swabs were pooled. A total of 100 nasal swabs were collected and pooled in 20 pools.

The aim was to sample all animals in the herd in one day, but seven out of ten herds had one quarantine and therefore, it was not possible to collect blood samples and nasal swabs from gilts in the beginning and end of quarantine on the same day. Therefore, the sampling was planned according to arrival of new gilts in the beginning of quarantine, and sampling at the end of quarantine was either collected before arrival of new gilts, or the same gilt population was sampled again at the end of the quarantine, resulting in two visits in seven of the herds.

3.2 Sample size

In this study, a sample size of 20 gilts in each sampled section in ten herds was chosen due to time and economic resources. With the sample size of 20 gilts or pooled piglets in a sampled section in each herd, it was possible to detect at least one virus or antibody positive gilt with a section-prevalence of 14.3% (**Appendix 2**).

A repeated cross-sectional study revealed a virus prevalence among gilts and piglets in a vaccinated herd to be 10.7% and in an unvaccinated herd to be 32.1% (50). In order to show a significant difference in virus prevalence between vaccinated and unvaccinated herds with these virus prevalence, a sample size of 59 animals was calculated in Microsoft Excel version 1912 "sample-sizes.xls", using the sheet "diff. proportions" and two-sided formula with a power of 0.8 and an alpha value of 0.05 (68) (**Appendix 2**). Considering this with the sample size of 400 gilts in group one (vaccinated herds) and 400 gilts in group two (unvaccinated herds), and 100 gilts in a sampled section, in each group, it was possible to show a significant difference.

3.3 Selection of herds

Seven herds from Jutland and three herds from Zealand participated in this study. The ten herds were selected by convenience in cooperation with the connected veterinary practitioner after the following criteria: **1)** Herd size at approximately 800 sows or more to ensure enough gilts for sampling. **2)** PRRS stable sow unit or free of PRRS. **3)** The use of quarantine. **4)** Purchase of gilts. **5)** Five sow herds using swIAV vaccination (Respiporc FLU3) and five sow herds with no swIAV vaccination within the last year.

PRRS outbreak can affect the swIAV prevalence (22), therefore, a stable PRRS sow unit was a criterion. This is defined as no clinical symptoms among sows and gilts and PRRSV negative pigs at weaning. In case the herd did not fulfil the criteria, the herd was to be substituted by another herd (69,70). See **Appendix 3 and 4** for detailed sampling list and mapping of the ten herds. Eight out of ten herds were part of the Danish SPF system declaring the herd free of eight specific pathogens. A health status "Blå SPF + MYC + PRRS1", means that antibodies against *Mycoplasma hyopneumoniae* and Danish PRRS (European PRRS virus) have been detected in the yearly SPF surveillance program. If the herd was not part of SPF, the health status was declared unknown (71).

Table 1: An overview of included herds.

The first five herds were unvaccinated against influenza and the last five herds, vaccinated. All the vaccinated herds had the same influenza vaccination strategy of replacement gilts with two vaccination three weeks apart and three yearly mass sow vaccinations. The number of purchased gilts pr. year, the number of gilts purchased pr. year and the age of the gilts when arriving in the quarantine are shown.

Vaccine status	Herd number	Health Status	Herd size	Number of purchased gilts / age	Intake of gilts pr. year
Unvaccinated	1	Blå SPF	1500 sows	600 gilts / 12-20 weeks	5 times
	2	Blå SPF + MYC + AP12	1000 sows	520 gilts / 10-23 weeks	4 times
	3	Blå SPF + AP12	1000 sows	410 gilts / 12-22 weeks	5 times
	4	Blå SPF	930 sows	440 gilts / 13-26 weeks	4 times
	5	Blå SPF + MYC + AP12	1000 sows	514 gilts / 13-19 weeks	6.5 times
Vaccinated	6	Unknown, PRRS1 outbreak	2500 sows	1248 gilts / 12-22 weeks	5 times
	7	Blå SPF + MYC + AP12	1900 sows	936 gilts /14-21 weeks	5 times
	8	Unknown	830 sows	520 gilts / 18-27 weeks	6.5 times
	9	Blå SPF + MYC + AP2,12	1000 sows	500 gilts /4-12 weeks	4 times
	10	Blå SPF + MYC + PRRS1	1050 sows	462 gilts /15-20 weeks	6 times

3.4 Questionnaire and checklist

All information was gathered by using a questionnaire and a checklist based on relevant literature (43,52,53,61,66). The questionnaire was pretested in one herd, not included in the study population, where some questions were modified. The questions were grouped into five parts where the last part about vaccination strategy was left out when answered by unvaccinated herds. The questionnaire included 12 closed questions (Yes/No or multiple choice) and 16 semi open questions (i.e. quantitative variables and description of restrictions after quarantine visit) (**Appendix 5**). The questionnaire was answered on paper by the owner or manager when visiting the herds and later typed into a computer. The checklist was filled out by the authors and included biosecurity measures (i.e. change of clothes and boots in each section), antibiotic use on regular basis, vaccinations, and variables that could explain possible swIAV transmission among pigs (e.g. pig density, housing, and animal flow) and aided the authors in the description of the herds (**Appendix 6**).

3.5 Sample collection

The samples were collected by the authors in the period from September to November 2019. Health status of the herds was considered in the planning, and in cases of sampling for several days in a row, the herd with the highest health status was visited first. A 12-hour quarantine after showering was mandatory.

When more than 20 gilts were present in a section, the sampling was randomized. Gilts were counted in the unit and divided with the sample size, where the outcome was used to count the selection frequency. If the outcome was a decimal number, it was rounded off. If there were less than 20 gilts within a week batch, gilts from the week before were sampled.

The gilts were restrained with a snout break and blood samples were obtained from *vena jugularis* and the blood was stored in vacutainer serum tubes (Becton-Dickinson, Denmark). Nasal swabs were collected from both nostrils with small or large sterile rayon swabs (Medical Wire, UK) depending on the age of the animal. All pigs were restrained to secure a proper swab when the swab was inserted in the nostrils and turned 360 degrees. The sample was preserved in a 5 mL Eppendorf container with 2 mL sterile 0.9% isotonic NaCl and transported to the laboratory of The National Veterinary Institute, Technical University of Denmark (DTU) within 12-48 hours in an electric cooling box under 5-8°C.

3.6 Laboratory tests

All laboratory tests were performed at The National Veterinary Institute, DTU. At arrival, sera were separated by centrifugation at 3000 rpm for 10 min, extracted, and frozen at -20°C until further analysis. All nasal swabs were vortexed and approx. 600 µl of each sample were poured into 1.5 mL Eppendorf tube and stored at -80°C until further analysis. However, one pooled nasal swab from piglets was lost during laboratory analysis.

3.6.1 RNA extraction, real-time RT-PCR

The nasal swabs were centrifuged and 200 uL were transferred to the sample rack and mixed with 400 µl RLT-buffer (QIAGEN, Copenhagen, Denmark) with 2-mercaptoethanol (Merck, Darmstadt, Germany). All pathogen nucleic acids including viral RNA were extracted from all nasal swabs using the Pathogen 96 QIAcube HT Q Protocol version 3 (Qiagen) automated on the Qiacube HT according to instructions from the supplier.

All RNA extractions were subjected to a previous published real-time RT-PCR targeting the matrix gene of IAV to determine if the sample was swIAV positive (72). The matrix protein (MP) segment is highly conserved across subtypes, and MP-assay has a sensitivity of 98.9% and a specificity of 100% when a sample is considered positive at Ct value <36 (72).

In a total volume of 25 µl, all RT-PCRs contained 8 µl RNAse free water, 5x5 µl buffer, 1.5 µM Forward primer SVIP-MP-F, 1.5 µM Reverse primer SVIP-MP-R, 1.5 µM LNA Probe UPL probe no: 104, mM dnTP (nucleotides), 1.5 mM MgCl2, 1 µl Qiagen OneStep Enzyme Mix, and 5 µl of purified RNA. The RT-PCRs were conducted on the Rotor-Gene Q (QIAGEN) using the following thermal profile: 30 min at 50℃ and 15 min at 95℃, after this followed by 45 cycles of 10 s at 95℃, 20s at 60℃, 1 s of 64℃, 1 s of 68℃, and 10 s at 72℃.

3.6.2 Subtyping by Sangers sequencing

The two nasal swabs with the lowest Ct values in the above-mentioned real-time RT-PCR from each herd, was selected for HA and NA sequencing. The HA and NA gene was amplified using a previously published conventional PCR assay as described in a previous study (15). The PCR products were then visualized on a gel, purified and send for Sanger sequencing with the PCR primers at LGCs Genomics (Berlin, Germany). The sequencing data from LGC were proof-read, analysed and investigated for the amino acid sequence identity to the vaccine strains of Respiporc FLU3 as previously described (15).

3.6.3 Serology

Sera were screened for antibodies against the highly conserved nucleoprotein (NP) antigens of IAVs by using the commercial blocking enzyme-linked immunosorbent assay (ELISA) (IDEXX Influenza A Ab Test, IDEXX Laboratories, Inc.) and following the recommended procedure. Samples with a sample-to-negative (S/N) value <0.60 were considered positive for IAV antibodies and samples S/N≥0.60 were considered negative. With the recommended cut off from IDEXX Laboratories, Inc., the sensitivity of the test is 86% and the specificity is 79% (73). In unpublished data it was indicated that the S/N values were inverse correlated with HI titre (74).

3.6.4 Statistical analysis

Descriptive statistics were carried out in Microsoft Excel version 1911 and GraphPad Prism 8 version 8.3.0-538 (75,76) which provided an overview of the results (**Fig. 2-11, table 1, Appendix 7**). Comparison of sample time differences among sampled sections in vaccinated and unvaccinated herds was analysed by using Mann-Whitney U tests in GraphPad Prism 8 version 8.3.0-538 (**Fig. 2).** The independent explanatory variables: vaccination, no vaccination, quarantine in, quarantine out, mating unit, gestation unit, farrowing unit, and piglets were used for statistical analysis. In order to test the hypotheses H0a, H0b, H0c, H0e, and H0f, the pseudo continuous outcome variables S/N value and Ct values were converted into qualitative variables on a dichotomous (binary) scale; virus positive/negative (cut off value Ct <36) and antibody positive/negative (cut off value S/N<60). To compare prevalence of virus and antibody positive gilts between vaccinated and unvaccinated herds, a chi-square test was used in Microsoft Excel version 1912, 2by2.xls (68). If there were five or less animals in a category, the Fisher's Exact test was used in GraphPad Prism 8 version 8.3.0-538. In order to test hypothesis H0f, an antibody prevalence cut off value were needed. This was defined by calculating the herd immunity threshold with a mean reproduction number (R0) of 6.5 based on relevant literature (30,42) (**Appendix 11**).

To test hypotheses H0d and H0g, the normal distribution of S/N value was analysed to determine the type of statistical test. As quantitative data were not normally distributed, a total of seven Mann-Whitney U tests were performed using GraphPad Prism 8 version 8.3.0-538. In all performed tests the null hypothesis was rejected if the p-value was < 0.05.

4. Results

The gilts were sampled on average 8.8 days after arrival to the quarantine with a standard deviation (SD) of 3.9 days. Mean weeks in quarantine were 8.4 days (SD=1.8). On average, gilts of the mating unit were sampled 8.7 days (SD=5.8) after arriving to the sow herd. 1.6 (SD=0.7) weeks before farrowing, the gestating gilts were sampled. First parity sows and piglets were sampled after farrowing 8.4 (SD=5.6) and 8.0 days (SD=4.9), respectively. A comparison of sampling time differences among sampled sections in vaccinated and unvaccinated herds are shown in **Fig 2**. (**Appendix 7 and 8**). Sampling time of gilts in the beginning and at the end of the quarantine (weeks in quarantine) was significantly different in the two groups. In the beginning of the quarantine, gilts were sampled eight and seven days after arrival in vaccinated and unvaccinated herds, respectively (p=0.048). At the end of the quarantine, gilts were sampled 9.6 weeks and 7.2 weeks in vaccinated and unvaccinated herds, respectively (p<0.0001). When sampling in the mating unit, gilts in unvaccinated herds had been in the sow herd significantly longer compared to gilts in vaccinated herds (p<0.0001). However, two vaccinated herds had a separate mating unit site and the gilts were moved to the sow herd after sampling time in the mating unit.

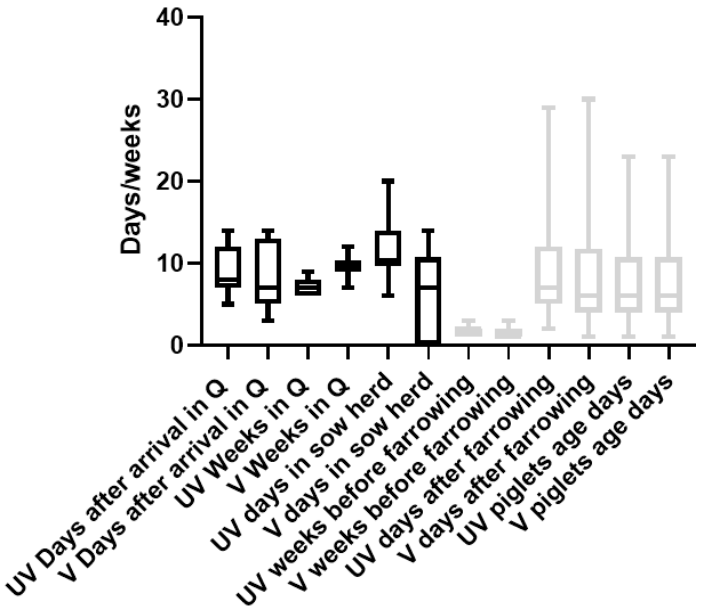


Fig. 2: Comparison of sampling time differences. The box plot shows a comparison of sampling time differences among sampled sections in vaccinated and unvaccinated herds. On the x-axis, different sections in unvaccinated (UV) and vaccinated (V) herds. Q = quarantine. On the y-axis, days or weeks are shown depending on section level. The highlighted boxes show significant differences between unvaccinated and vaccinated herds. Three sampling times were significantly different between the two groups: "days after arrival in quarantine" (p = 0.048), "weeks in quarantine" (p <0.0001), and "days in herd" (p <0.0001) before sampled in the mating unit.

4.1 Subtyping of included herds

Influenza A virus was subtyped in herd 2, 4, 5, 9, and 10. The amino acid identity of the HA and NA gene to the vaccine strains in Respiporc FLU3 was revealed. The HA was identified as H1av in four herds - herd 2, 4, 5,10, and in herd 5, NA from the human influenza season in 1995 was identified. The subtype H1pdm09N1av was found in Herd 9 and H1pdm09 is not included in Respiporc FLU3, for this reason, the HA protein identity cannot be evaluated. Of the other HA proteins identified, the identity to the vaccine strain was between 90-92.4% and identified NA proteins had an identity of 84.7-89.7% to the vaccine strain (**Table 2**).

Table 2: Subtyping

Subtyping of five herds and the amino acid identity of HA and NA to the strains of the vaccines. Two different HA and three different NA were identified - H1av, H1pdm09, N2sw, N2hu95 and N1av. Note, only herd 9 and 10 vaccinated with Respiporc FLU3.

Herds	Subtype	Identity
2	H1avN2sw	HA protein identity to HA haselünne/IDT2617/2003: 92.2-92.4% NA protein identity to NA Bakum/IDT1769/2003(H3N2): 89.5-89.7%
4	H1avN2sw	HA protein identity to HA haselünne/IDT2617/2003: 92% NA protein identity toNA Bakum/IDT1769/2003(H3N2): 89.5%
5	H1avN2hu95	HA protein identity to HA haselünne/IDT2617/2003: 92% NA protein identity to NA Bakum/IDT1769/2003(H3N2): 84.7% NA protein identity to NA Bakum/IDT1833/2000(H1N2): 82.3%
9	H1pdm09N1av	NA protein identity to NA haselünne/IDT2617/2003: 90.4%
10	H1avN2sw	HA protein identity to HA haselünne/IDT2617/2003: 90.25% NA protein identity to NA Bakum/IDT1769/2003(H3N2): 88%

4.2 Results of included herds

4.2.1 Herd 1 (unvaccinated)

This herd was newly started with 1500 sows, sale of seven kg weaned pigs, loose sows in the farrowing unit, and the health status “Blå SPF”. The quarantine was placed next to the sow herd and was inspected in the afternoon following a change of boots and clothes before entry. However, no hand washing was performed but entrance into the sow herd was thereafter prohibited for 12 hours. The gilts were in the quarantine for eight to ten weeks and hereafter a continuous intake of gilts into the sow herd was performed. The quarantine was washed and dried for three days before new gilts entered (**Appendix 9**).

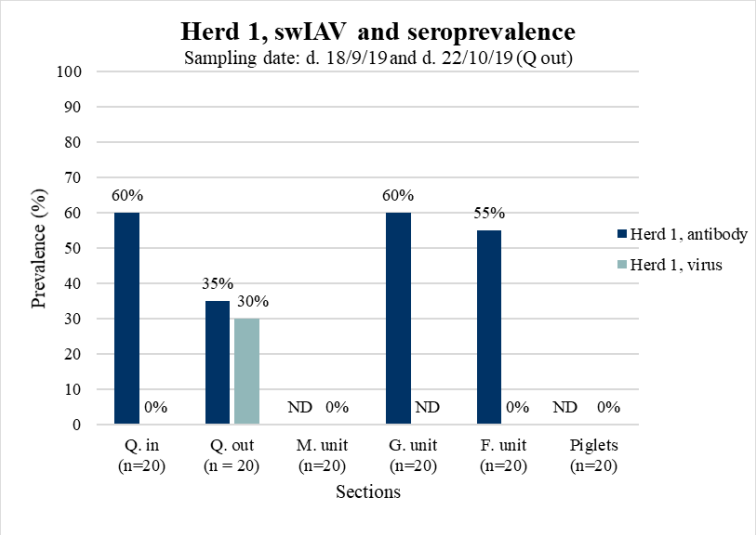


Fig. 3: SwIAV shedding and seroprevalence in herd 1. The prevalence (%) of antibody and virus positive animals is shown on the y-axis and represented on the x-axis are the sections: Quarantine in (Q in), Quarantine out (Q out), Mating unit (M. unit), Gestation unit (G. unit), Farrowing unit (F. unit), and pooled piglets (Piglets). The green columns represent the prevalence of virus positive animals in the particular section. The blue columns represent the prevalence of antibody positive animals in the particular section. Note, antibodies in the mating unit and in piglets were not sampled and virus detection was not performed in the gestation unit (ND = not done).

Fig. 3 illustrates the prevalence of animals testing positive for influenza A virus antibodies (antibody ELISA) or virus (real-time RT-PCR). Seven days after arrival in the quarantine, gilts were sampled. In this herd, sampling was performed at two different days: meaning that the same population of gilts in the beginning of the quarantine were sampled six weeks later, at the end of quarantine. The gilts had an antibody prevalence of 60%, 95%CI [38.5%, 81.5%] in the beginning of quarantine and at the end, 35%, 95%CI [14.1%, 55.9%]. In addition, no virus was detected in the beginning of quarantine, however, 30%, 95%CI [9.9%, 50.1%] of the gilts tested positive for virus at the end of the quarantine-period. The sow herd tested virus negative in all sampled sections, however, in gestating gilts and first parity sows the seroprevalence was 55%, 95%CI [33.2%, 76.8%] and 60%, 95%CI [38.5%, 81.5%], respectively.

The results of the quarantine indicated that swIAV was introduced at the end of the quarantine-period, as virus was detected at this stage and since only 35% of the gilts at the end of the quarantine period were seropositive for IAV which indicated that the majority of the gilts had not yet seroconverted. The fact that virus was only present at the end of the quarantine indicated that swIAV was introduced from the outside and not by incoming gilts, suggesting that the biosecurity measures at the end of the quarantine were not optimal. In addition, the sow herd had a high risk of becoming infected when these gilts were introduced as 40-45% of the gilts

in the herd were seronegative. Moreover, as the seronegative first parity sows were not expected to deliver protective MDAs to the piglets, this age group would also be at great risk of swIAV infections.

4.2.2 Herd 2 (unvaccinated)

This herd had 1000 sows, production of 30 kg pigs and a SPF health status "Blå SPF + MYC + AP12". The quarantine was placed two km from the sow herd, however, in connection with a weaning unit. Additionally, the quarantine had a separate entrance, where change of clothing, boots, and hand wash was required before entering the quarantine when inspected in the afternoon. Entrance into the sow herd was thereafter prohibited for 12 hours. The gilts were in quarantine for six weeks and when the quarantine time expired, a door between the weaning unit and quarantine was opened, making it possible to walk directly from the weaning unit and into the quarantine, while the gilts were waiting to be introduced to the sow herd. All gilts were introduced to the sow herd at the same time. No washing of the quarantine unit was performed between batches of new gilts, but the quarantine was empty before new gilts arrived (**Appendix 9**).

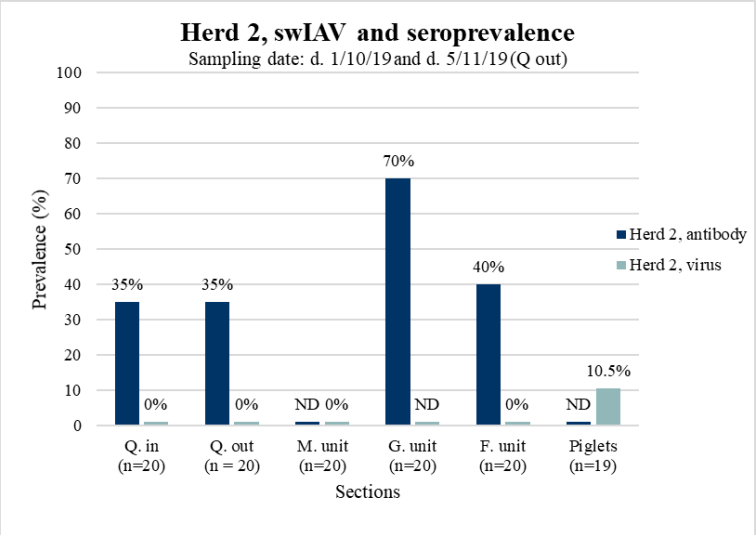


Fig. 4: SwIAV shedding and seroprevalence in herd 2. The prevalence (%) of antibody and virus positive animals is shown on the y-axis and represented on the x-axis are the sections: Quarantine in (Q in), Quarantine out (Q out), Mating unit (M. unit), Gestation unit (G. unit), Farrowing unit (F. unit), and pooled piglets (Piglets). The green columns represent the prevalence of virus positive animals in the particular section. The blue columns represent the prevalence of antibody positive animals in the particular section. Note, antibodies in the mating unit and in piglets were not sampled and virus detection was not performed in the gestation unit (ND = not done).

Fig. 4 illustrates the prevalence of animals testing positive for influenza A virus antibodies (antibody ELISA) or virus (real-time RT-PCR). There were two sampling dates for these gilts: firstly, twelve days after arrival in the quarantine and secondly, the gilt population was sampled at the end of quarantine, five weeks later. During both samplings in the quarantine, 35%, 95%CI [14.1%, 55.9%] of the gilts tested seropositive and no virus was detected. However, a high level of seropositive gilts (70%, 95%CI [49.9%, 90.1%]) was recorded in the gestation unit but no virus was detected in any of the sampled sections of the sow herd. In contrast, 10.5%, 95%CI [0%, 24.3%] of the piglets were positive in the farrowing unit where only 40%, 95%CI [18.5%, 61.5%] of the first parity sows tested seropositive.

No virus was detected in the quarantine and no raise in seroprevalence was observed between the beginning and end of quarantine which indicated that the quarantine was free of swIAV. The biosecurity measures with separate entrance, changing of clothing and boots, and hand wash prevented introduction of swIAV to the quarantine, but the gilts of the quarantine constituted a risk of maintenance of swIAV to the sow herd as only 35% were seropositive. Furthermore, gilts were not moved right after opening the quarantine, wherefore an indirect transmission between the weaning unit and the quarantine could be a potential risk if swIAV was circulating among weaners.

A higher seroprevalence in the gestation unit indicated that the gilts had been exposed to swIAV thereby had seroconverted. Virus was not detected in the mating unit which could indicate that exposure to swIAV might occur in the gestating unit where nasal swabs were not obtained. Positive piglets and a low seroprevalence in the farrowing unit indicated that the gilts were not optimally immunised prior to farrowing, probably resulting in an impaired delivery of MDAs to the piglets which could explain the circulation of swIAV in piglets already at one-week-of-age.

4.2.3 Herd 3 (unvaccinated)

This herd had 1000 sows, a niche-production of Antonius pigs, a SPF health status “Blå SPF + AP12”, Topig Norsvin breeding material, and loose sows in the farrowing unit. The. The quarantine was placed at the same address as the sow herd next to the farrowing unit but with a separate entrance wherein change of clothing and boots, and hand wash was performed before entry. The gilts of the quarantine were inspected in the afternoon and a bath was mandatory before reentering the sow herd. The gilts were in quarantine for eight to nine weeks and all gilts

were moved to the sow herd at the same time. The quarantine section was emptied and washed between batches (**Appendix 9**).

Fig. 5 illustrates the prevalence of animals testing positive for Influenza A virus antibodies (antibody ELISA) or virus (real-time RT-PCR). The gilts were sampled eight days after arrival in the quarantine where a seroprevalence of 70%, 95%CI [49.9%, 90.1%] was detected, however, at the end of quarantine only 25%, 95%CI [6.0%, 44.0%] of the gilts were seropositive. In the gestation unit and farrowing unit, gilts testing antibody positive were 50%, 95%CI [28.1%, 71.9%] and 55%, 95%CI [33.2%, 76.8%], respectively. Nevertheless, in this herd, all sampled gilts and piglets tested negative for swIAV.

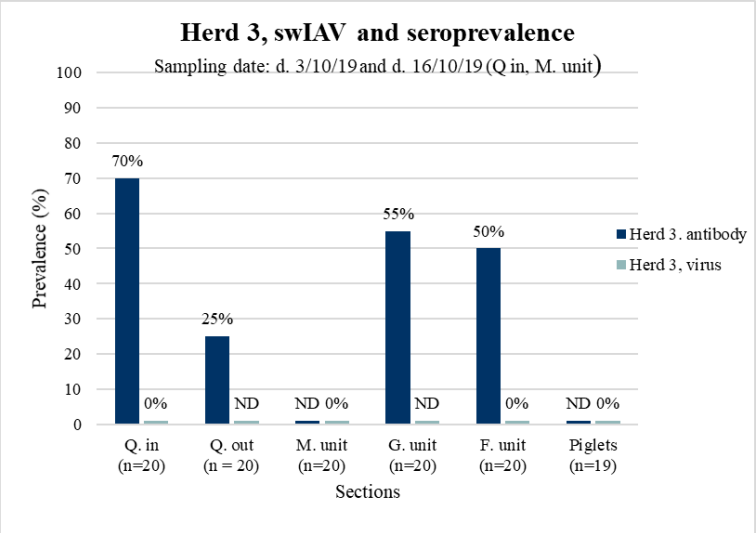


Fig. 5: SwIAV shedding and seroprevalence in herd 3. The prevalence (%) of antibody and virus positive animals is shown on the y-axis and represented on the x-axis are the sections: Quarantine in (Q in), Quarantine out (Q out), Mating unit (M. unit), Gestation unit (G. unit), Farrowing unit (F. unit), and pooled piglets (Piglets). The green columns represent the prevalence of virus positive animals in the particular section. The blue columns represent the prevalence of antibody positive animals in the particular section. Note, antibodies in the mating unit and in piglets were not sampled and virus detection was not performed in the gestation unit (ND = not done).

The high seroprevalence in the beginning of the quarantine indicated that the gilts had been exposed to swIAV before arriving in the quarantine. The antibody prevalence was low at the end of the quarantine which could indicate that swIAV was not circulating in the quarantine at this stage. The strict biosecurity measures performed in this herd could explain why the herd was successful in keeping the quarantine free of swIAV. The seroprevalence in the sow herd could either be explained by a high level of seropositive gilts arriving at the quarantine, which might keep the seroprevalence high within the sow herd, or it could be that a low level of swIAV was circulating within the herd, however at a level lower than what the study design allowed to be detected. Despite the low number of seropositive gilts, no virus was detected in

the piglets, indicating that the herd did not have swIAV circulation in the farrowing unit, where strict management also aided in preventing the transfer of pathogens between litters.

In this herd, swIAV was not detected and the low seroprevalence in gilts would make the herd highly susceptible and vulnerable for swIAV infection as many naive animals could become infected. However, the management in the farrowing unit with no cross-fostering could aid the herd in becoming enzootically infected after swIAV introduction.

4.2.4 Herd 4 (unvaccinated)

This herd had 930 sows, a production of 30 kg pigs, and a "Blå SPF" health status. The quarantine was located 20 m north of the sow herd and consisted of pens within the same section as where the slaughter pigs were housed. The quarantine-period ranged between six to eight weeks. Health inspection of the gilts were performed in the afternoon and entrance into the sow herd was thereafter prohibited for 12 hours. Change of clothing and boots and hand wash was performed before entering the quarantine. The pens were not washed between batches and a continuous intake of gilts into the sow herds was performed (**Appendix 9**).

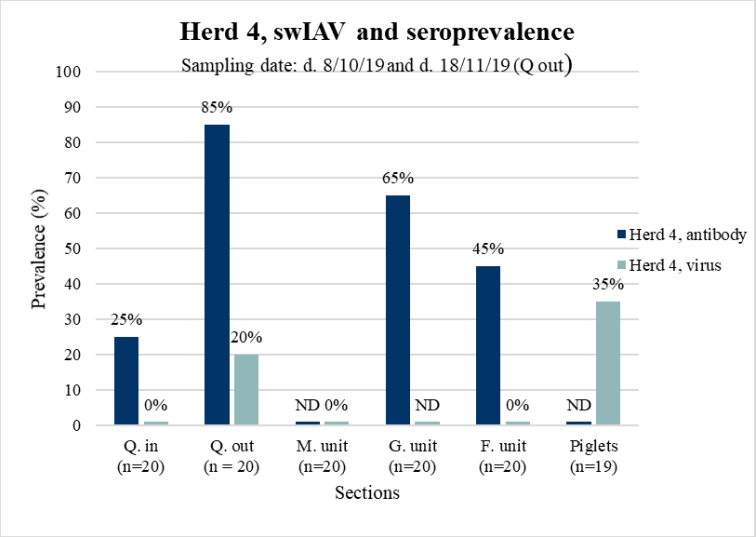


Fig. 6: SwIAV shedding and seroprevalence in herd 4. The prevalence (%) of antibody and virus positive animals is shown on the y-axis and represented on the x-axis are the sections: Quarantine in (Q in), Quarantine out (Q out), Mating unit (M. unit), Gestation unit (G. unit), Farrowing unit (F. unit), and pooled piglets (Piglets). The green columns represent the prevalence of virus positive animals in the particular section. The blue columns represent the prevalence of antibody positive animals in the particular section. Note, antibodies in the mating unit and in piglets were not sampled and virus detection was not performed in the gestation unit (ND = not done).

Fig. 6 illustrates the prevalence of animals testing positive for Influenza A virus antibodies (antibody ELISA) or virus (real-time RT-PCR). Five days after arrival in the quarantine the gilts were sampled where they had a seroprevalence of 25%, 95%CI [6,0%, 44,0%] and tested negative for swIAV. The same population of gilts were sampled five weeks later at the end of the quarantine where a pronounced increase in antibody prevalence (85%, 95%CI [69,4%, 100%]) and virus shedding (20%, 95%CI [2.5%, 37.5%]) was detected. A seroprevalence of 65%, 95%CI [44.1%, 85.9%] and 45%, 95%CI [23.2%, 66.8%] were observed in gestating gilts and first parity sows in the farrowing unit, respectively. In addition, 35%, 95%CI [14.1%, 55.9%] of the pooled piglets were tested positive for swIAV.

The low seroprevalence in the beginning of the quarantine suggested that only few of the gilts were exposed to swIAV in the breeding herd before arriving in quarantine. The virus detection and rise in seroprevalence at the end of the quarantine was clearly correlated with the presence of swIAV in this section. The quarantine gilts were placed beside continuously driven pens with slaughter pigs wherefore direct contact and airborne transmission between gilts and slaughter pigs were possible. The seroprevalence in gestating gilts and first parity sows in the farrowing unit indicated that they still had antibodies from the quarantine, or the gilts in mating and gestating units were exposed to undetectable levels of swIAV. However, the relatively low seroprevalence of first parity sows (45%) posed a risk of low passive transfer of MDAs which probably resulted in a great number of susceptible piglets which correlated with the finding of swIAV circulation in the farrowing unit.

The lack of proper biosecurity measures and poor management in the quarantine (**Appendix 9**) promoted virus circulation, additionally providing a potential source of swIAV transmission into the sow herd.

4.2.5 Herd 5 (unvaccinated)

This herd had 1000 sows, production of 30 kg pigs, and a “Blå SPF MYC + AP12” health status. The quarantine was located 50 m east of the sow herd. New gilts were housed in the quarantine for six to eight weeks. The quarantine had one entry for both personnel and gilts and change of clothing and boots before entering was performed but washing of hands was not possible. The health of gilts was inspected in the afternoon and entrance into the sow herd was thereafter prohibited for 12 hours. The quarantine was not washed between these batches of gilts and all gilts were introduced to the sow herd at the same time (**Appendix 9**).

Fig. 7 illustrates the prevalence of animals testing positive for Influenza A virus antibodies (antibody ELISA) or virus (real-time RT-PCR). 14 days after arriving in the quarantine, the gilts were sampled. The seroprevalence 95-100%, 95%CI [85.5%, 100%] in all investigated sections was found, however, none of the tested gilts were tested positive for swIAV. Nevertheless, a swIAV prevalence of 15%, 95%CI [0%, 30.7%] was found in the piglets.

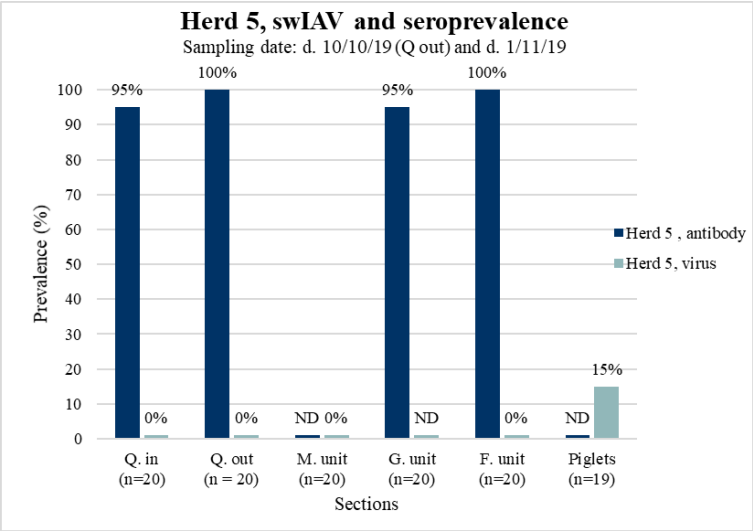


Fig. 7: SwIAV shedding and seroprevalence in herd 5. The prevalence (%) of antibody and virus positive animals is shown on the y-axis and represented on the x-axis are the sections: Quarantine in (Q in), Quarantine out (Q out), Mating unit (M. unit), Gestation unit (G. unit), Farrowing unit (F. unit), and pooled piglets (Piglets). The green columns represent the prevalence of virus positive animals in the particular section. The blue columns represent the prevalence of antibody positive animals in the particular section. Note, antibodies in the mating unit and in piglets were not sampled and virus detection was not performed in the gestation unit (ND = not done).

14 days after arriving to quarantine, the gilts were tested where the high seroprevalence could indicate that the gilts had been exposed to swIAV before arriving, or the gilts were infected when introduced to the quarantine, however no virus was detected. The biosecurity in the quarantine was low with no entrance room for hand wash and changing of clothing, neither was the quarantine washed between these two batches of purchased gilts. Nevertheless, the management of the quarantine resulted in a seroprevalence of 100% at the end of quarantine, no virus detection, and well-immunised gilts before moved into the sow herd, where virus in the gilts was not detected. Even though first parity sows in the farrowing unit had antibodies against swIAV, the piglets were not protected, which could be a cause of low colostrum intake, heterologous MDAs, and MDAs lack of ability to protect against swIAV infections of the upper respiratory tract.

In a herd with such high seroprevalence in all sampled sections without influenza vaccination, it would be expected to find swIAV positive gilts or a decline in seroprevalence, but this was

not the case. The sample size of 20 gilts in a section made it possible to detect swIAV with a prevalence of approx. 14%, if lower, virus would not be detected. The study design could also influence the findings: it was a cross-sectional study, which illustrated the swIAV dynamics the specific day. Furthermore, there were several months in the production system between the sampled subpopulations where virus could have circulated, for instance in the gestating gilts.

4.2.6 Herd 6 (vaccinated)

This herd had 2.500 sows, production of 30 kg pigs, and was not included in the Danish SPF system. The herd had an outbreak of the Danish PRRS in August 2019 and was currently undergoing a PRRS-eradication program. The sow herd was not excluded because the owner and the authors did not observe any PRRS clinical symptoms in the sows prior to both sampling as well as sampling date. The quarantine was located ten km north of the sow herd. Clothing was changed before entering the site, but hand washing was not performed regularly, and sick personnel were allowed to enter the quarantine unit. In the quarantine, health service was carried out in the afternoon and entering the sow herd was prohibited for 12 hours. New gilts were housed in the quarantine for eight to ten weeks, hereafter all gilts were moved to another site ten km away for mating and three weeks prepartum, the gestating gilts were moved to the sow herd. The quarantine section was washed and left to dry for 3-14 days between batches (**Appendix 9**).

Influenza mass sow vaccination with Respiporc FLU3 was performed three times a year with vaccinations scheduled in November, March, and July. The latest mass sow vaccination was thereby performed three months prior to sampling. After quarantine, all gilts received two vaccinations with three weeks apart (**Appendix 10**).

Fig. 8 illustrates the prevalence of animals testing positive for Influenza A virus antibodies (antibody ELISA) or virus (real time RT-PCR). 12 days after arrival in the quarantine, the gilts were sampled where 50%, 95%CI [28.1%, 71.9%] of the new gilts tested positive for swIAV, however, only 15%, 95%CI [0%, 30.7%] tested seropositive. A virus prevalence of 15%, 95%CI [0%, 30.7%] was detected at the end of quarantine where 25%, 95%CI [6,0%, 44.0%] of the gilts tested seropositive. After quarantine, the gilts were moved to the mating unit site, where a swIAV prevalence of 5%, 95%CI [0%, 14.6%] was found. After mating, the gilts were moved to the sow herd where the gestating gilts and first parity sows in the farrowing unit had a seroprevalence of 100%, 95%CI [100%, 100%] and no virus was detected in the piglets.

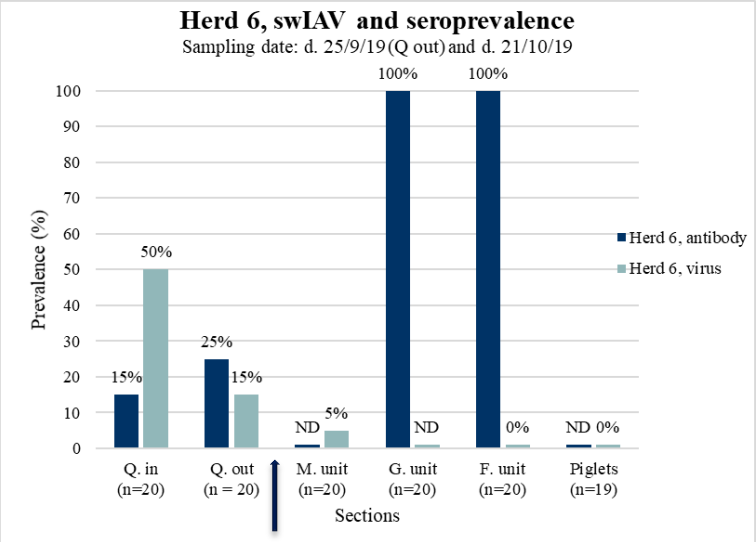


Fig. 8: SwIAV shedding and seroprevalence in herd 6. The prevalence (%) of antibody and virus positive animals is shown on the y-axis and represented on the x-axis are the sections: Quarantine in (Q in), Quarantine out (Q out), Mating unit (M. unit), Gestation unit (G. unit), Farrowing unit (F. unit) and pooled piglets (Piglets). The green columns represent the prevalence of virus positive animals in the particular section. The blue columns represent the prevalence of antibody positive animals in the particular section. Note, antibodies in the mating unit and in piglets were not sampled, and virus detection was not performed in the gestation unit (ND = not done). The arrow marks the time of basis vaccination of gilts.

The presence of swIAV positive gilts in the beginning of the quarantine indicated that either the gilts had brought swIAV from the breeding herd or virus was persistently present in the quarantine unit and infected the gilts when introduced into the quarantine. The vaccination of the gilts against influenza was performed at 24 and 26 weeks of age, meaning that not all gilts in the quarantine were vaccinated before being moved. This correlated to the low seroprevalence observed at the end of quarantine. SwIAV circulated in the quarantine, which should immunise the gilts before leaving, however this was not the case, indicating that the sampled gilts at the end of quarantine were recently infected and had not yet seroconverted. The presence of swIAV in both the beginning and end of the quarantine indicated that the biosecurity measures and management was not performed properly and resulted in a high risk of introducing swIAV into the sow herd.

The high seroprevalence in the gestation and farrowing unit was most likely a result of the current vaccination strategy to which no detection of swIAV in the young piglets could also be attributed. However, no measures were in place to reduce the circulation of swIAV in the quarantine and in the mating unit.

4.2.7 Herd 7 (vaccinated)

The owner had two sow herds but only one of the herds was part of this study. The herd had 1900 sows and a health status "Blå SPF MYC + AP12". The sow herd received gilts from two quarantine sites: one, four km east and the other, two km north-west placed with the weaning unit. The gilts of each quarantine site were housed for six weeks. For this study, samples were only collected from the quarantine four km away which was an extension to the second sow herd. This quarantine had two sections with separate entrances where change of clothing and boots, and hand wash was performed. When the quarantine-time expired, a period with continuous intake of gilts to the sow herd was performed. During this period, none of the above-mentioned biosecurity measures were performed and personnel entered directly from the sow herd. The quarantines were not washed between batches but left empty for seven days (**Appendix 9**).

Influenza mass sow vaccination, with Respiporc Flu3 was performed three times a year with vaccinations scheduled in January, May, and October. The latest vaccination was performed one day prior to sampling. In the beginning of the quarantine, all gilts received two vaccinations three weeks apart, with Respiporc FLU3 (**Appendix 10**).

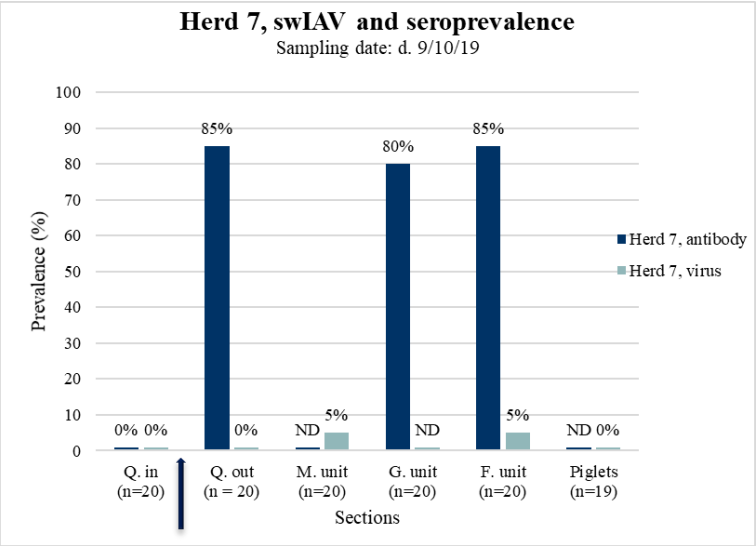


Fig. 9: SwIAV shedding and seroprevalence in herd 7. The prevalence (%) of antibody and virus positive animals is shown on the y-axis and represented on the x-axis are the sections: Quarantine in (Q in), Quarantine out (Q out), Mating unit (M. unit), Gestation unit (G. unit), Farrowing unit (F. unit) and pooled piglets (Piglets). The green columns represent the prevalence of virus positive animals in the particular section. The blue columns represent the prevalence of antibody positive animals in the particular section. Note, antibodies in the mating unit and in piglets were not sampled, and virus detection was not performed in the gestation unit (ND = not done). The arrow marks the time of basis vaccination of gilts.

Fig. 9 illustrates the prevalence of animals testing positive for Influenza A virus antibodies (antibody ELISA) or virus (real-time RT-PCR). 14 days after arrival in the quarantine, the gilts were sampled where no antibodies against Influenza A were detected. In contrast, the gilts at the end of the quarantine had a seroprevalence of 85%, 95%CI [69.4%, 100%], however, no gilts tested positive for swIAV. In the gestating gilts and first parity sows in the farrowing unit, the same seroprevalence of 80%, 95%CI [62.5%, 97.5%] and 85%, 95%CI [69.4%, 100%] was also found, respectively. One gilt in the mating unit and one first parity sow were found positive for swIAV (5%, 95%CI [0%, 14.6%]), however, no virus was detected in the piglets at one-week-of-age.

In the beginning of the quarantine, the gilts tested negative for swIAV, which indicated that they had not been exposed to swIAV before arriving. The seroprevalence of 85% at the end of the quarantine indicated that the vaccination strategy was effective in stimulating an antibody response. However, it should be noted that not all gilts seroconverted post vaccination, unlike the results of the other vaccinated herds included in this study. A possible explanation for this could be poor management, poor injection technique, incorrect storage of the vaccine or host immune response. Interestingly, the influenza vaccination was administered with three other vaccines (**Appendix 10**). According to the manufacturer the efficacy of the vaccine combined with other vaccines is not investigated.

Mass sow vaccination one day prior to sampling cannot explain the seroprevalence of the gestation and farrowing units, 80% and 85% respectively, but rather, the mass sow vaccination in May or an enzootic circulation of swIAV. As the seroprevalence in first parity sows were 85%, a proportion of piglets would not be clinically protected with MDAs.

The current biosecurity measures of the quarantine seemed to prevent swIAV circulation, but immunisation of the gilts was not optimal meaning that susceptible gilts could be moved to the sow herd where swIAV positive gilts were detected. Interestingly, the site containing the second quarantine was known by the herd veterinarian to be influenza positive, which could serve as a risk factor for introducing virus positive gilts to the sow herd if a high level of biosecurity was not obtained.

4.2.8 Herd 8 (vaccinated)

This herd had 860 sows and a production of seven kg pigs. The health status was unknown, but vaccinations against *Actinobacillus pleuropneumoniae* serotype 2 and Danish PRRS were performed. The sow herd was not excluded because the owner and the authors did not observe any PRRS clinical symptoms in the sows prior to both sampling as well as sampling date. The quarantine was located on a property 400 m from the sow herd as an extension to the weaning unit. The quarantine had a separate entrance, but there was no separate room for changing and handwash. In the quarantine, health service was carried out in the afternoon and entering the sow herd and weaning unit was prohibited for 12 hours. After eight weeks in the quarantine, all the gilts were moved to a continuously driven section next to the quarantine where they stayed until five days prior to mating and in this study, the section was defined as the mating unit. Thereafter, the gilts were introduced weekly into the sow herd (**Appendix 9**).

Influenza mass sow vaccination, with Respiporc FLU3 was performed three times a year with vaccinations scheduled in February, June, and October, and latest vaccination was in week 41, October, three weeks prior to sampling in the sow herd. After quarantine, all gilts received two vaccinations three weeks apart, with Respiporc FLU3 and Respiporc FLUPan (**Appendix 10**).

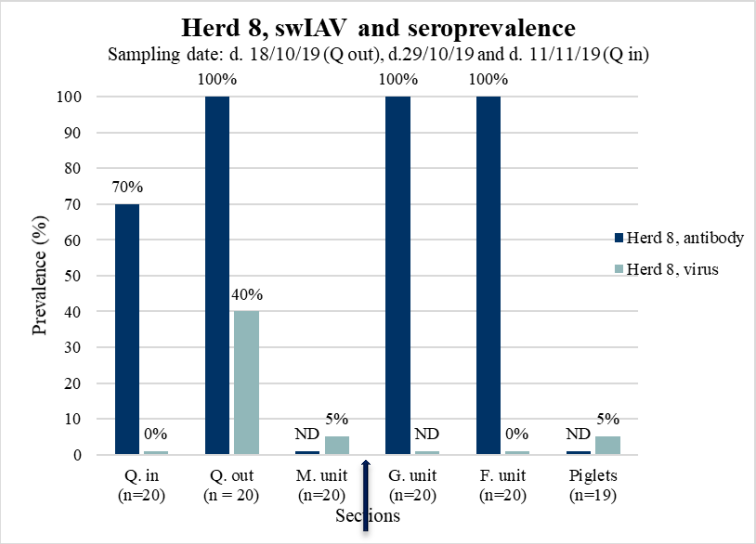


Fig. 10: SwIAV shedding and seroprevalence in herd 8. The prevalence (%) of antibody and virus positive animals is shown on the y-axis and represented on the x-axis are the sections: Quarantine in (Q in), Quarantine out (Q out), Mating unit (M. unit), Gestation unit (G. unit), Farrowing unit (F. unit) and pooled piglets (Piglets). The green columns represent the prevalence of virus positive animals in the particular section. The blue columns represent the prevalence of antibody positive animals in the particular section. Note, antibodies in the mating unit and in piglets were not sampled, and virus detection was not performed in the gestation unit (ND = not done). The arrow marks the time of basis vaccination of gilts.

Fig. 10 illustrates the prevalence of animals testing positive for Influenza A virus antibodies (antibody ELISA) or virus (real-time RT-PCR). The gilts were sampled three days after arriving at the quarantine where they tested swIAV negative but had a seroprevalence of 70%, 95%CI [49.9%, 90.1%]. At the end of the quarantine, 40%, 95%CI [18.5%, 61.5%] of the gilts tested positive for swIAV and the seroprevalence was 100%, 95%CI [100%, 100%]. The high seroprevalence was also detected in gestating gilts and first parity sows in the farrowing unit, however, swIAV was detected in both the mating unit and in piglets (5%, 95%CI [0%, 14.6%]).

The relatively high seroprevalence in the beginning of the quarantine suggested that the gilts had been exposed to Influenza A virus before arriving at the quarantine. At the end of quarantine, gilts shed virus and had a 100% seroprevalence, corresponding to massive swIAV circulation at the end of the quarantine and presented a possible risk of transmission into the mating unit that was placed next to the quarantine. The high seroprevalence at the end of the quarantine was not a result of vaccination but rather natural immunisation, since the vaccination took place in the mating unit.

The massive circulation of swIAV at the end of the quarantine suggested that swIAV was introduced from the outside and not by the purchase of swIAV positive gilts. The fact that swIAV was introduced from the outside could be attributed to poor biosecurity measures in the quarantine and structure of the herd. The presence of swIAV positive gilts at the end of the quarantine represented a risk of spreading the infection into the mating unit wherein swIAV was also detected. Moreover, the weaning unit was located with the quarantine and mating unit, and entrance to these sections was through the weaning unit. This could also be a risk of transmission of swIAV to both units.

The high seroprevalence in the sow herd was probably a result of the vaccination strategy and the fact that all gilts and sows had been vaccinated three weeks prior to sampling. The piglets sampled were of different ages and swIAV was only detected in one 15-days-old litter, whereas no virus was present at one-week-of-age. This could indicate that the MDAs were able to protect the piglets against early infections but as the MDAs waned, infections could be detected.

4.2.9 Herd 9 (vaccinated)

This herd had 1000 sows, production of 30 kg pigs, and a SPF health status "Blå SPF + MYC + AP2 + AP12". The quarantine was an extension to the sow herd and did not have a separate entrance. However, a change of clothing, boots and the use of gloves were mandatory before entering the quarantine. A bath was mandatory after inspecting the quarantine, but personnel had to walk through the herd to enter the showers. The new gilts were between 4-12 weeks of age at arrival and were housed in the quarantine for 11 weeks. The quarantine was washed and dried for five days before the arrival of new gilts. After quarantine, all gilts were moved to a section next to the quarantine, where they stayed until mating. In the study, this section was defined as the end of the quarantine, where the gilts had been for ten days, because of different definitions of a quarantine (**Appendix 9**).

Influenza mass sow vaccination, with Respiporc FLU3 was performed three times a year with vaccinations scheduled in week 10 (Mar.), week 26 (Jun.), and week 44 (Oct.), thereby, the latest vaccination was performed just after sampling day. After quarantine, all gilts received two vaccinations three weeks apart, with Respiporc FLU3 (**Appendix 10**).

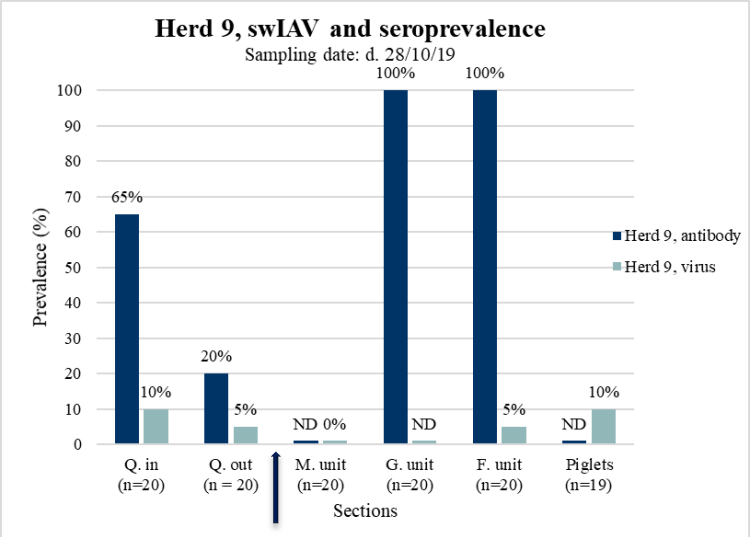


Fig. 11: SwIAV shedding and seroprevalence in herd 9. The prevalence (%) of antibody and virus positive animals is shown on the y-axis and represented on the x-axis are the sections: Quarantine in (Q in), Quarantine out (Q out), Mating unit (M. unit), Gestation unit (G. unit), Farrowing unit (F. unit) and pooled piglets (Piglets). The green columns represent the prevalence of virus positive animals in the particular section. The blue columns represent the prevalence of antibody positive animals in the particular section. Note, antibodies in the mating unit and in piglets were not sampled, and virus detection was not performed in the gestation unit (ND = not done). The arrow marks the time of basis vaccination of gilts.

Fig. 11 illustrates the prevalence of animals testing positive for Influenza A virus antibodies (antibody ELISA) or virus (real time RT-PCR). Five days after arriving at the quarantine, the gilts were sampled where 65%, 95%CI [44.1%, 85.9%] had seroconverted and 10%, 95%CI [0%, 23.2%] were found swIAV positive. Virus was also detected in 5%, 95%CI [0%, 14.6%] of the gilts at the end of the quarantine, however, only 20%, 95%CI [2.5%, 37.6%] were seropositive. The gilts in the gestation unit and the farrowing unit were all 100% seropositive. Despite a high seroprevalence, swIAV shedding was observed in both first parity sows and piglets in the farrowing unit (10%, 95%CI [0%, 23.2%]).

Herd 9 purchased gilts that were 4-12 weeks old, and the seroprevalence in the beginning of the quarantine could reflect the presence of MDAs which was still present until approx. ten weeks of age. As the gilts had been in the quarantine for five days, the swIAV could have been introduced with the arrival of the gilts. Moreover, one gilt was found positive for swIAV at the end of the quarantine, thereby suggesting that swIAV was also introduced from the outside, possibly the sow herd. At the end of the quarantine, the gilts had a very low seroprevalence making them susceptible to infection with herd strain circulating in the sow herd. However, the gilts were vaccinated twice before mating, thereby stimulating an antibody response before entering the farrowing unit where swIAV was circulating. As there were entrance through the herd and no hand wash, the quarantine biosecurity measures in this herd were one of the poorest of the investigated herds, which clearly presented a risk of swIAV circulation in the quarantine (**Appendix 9**).

The latest mass sow vaccination was 4.5 months ago, and it could be questioned if the high seroprevalence among gestating gilts and first parity sows was a result of basis vaccination or virus circulation in gestating gilts and farrowing unit. Despite a high seroprevalence in first parity sows in the farrowing unit, virus was found in piglets. This could be explained by the finding of the influenza subtype H1pdm09N1av (**Table 2**), which is not part of the vaccine Respiporc FLU3 used in this herd. Among other factors, the herd also had a high degree of first parity nursery sows, which could affect the uptake of colostrum and contribute to swIAV dissemination.

4.2.10 Herd 10 (vaccinated)

This herd had 1050 sows, a production of 30 kg pigs, and a SPF health status "Blå SPF + MYC + PRRS DK". The sow herd was not excluded because the owner and the authors did not observe any PRRS clinical symptoms in the sows prior to both sampling as well as sampling date. The quarantine was an extension to the sow herd and consisted of two sections with separate entrances from outside with a room for changing clothing and boots, and hand wash was possible, but there was also an entrance from the sow herd, only used when the quarantine-time expired. In the quarantine, health service was carried out in the afternoon and entering the sow herd was prohibited for 12 hours. The new gilts had a quarantine-time of five to seven weeks, hereafter a continuous intake of gilts into the sow herd was performed and the section was washed and dried before arrival of new gilts (**Appendix 9**).

Influenza mass sow vaccination, with Respiporc FLU3 was performed three times a year with vaccinations scheduled the November 1st, March 1st, and July 1st. Latest vaccination was performed three days prior sampling day. In the beginning of the quarantine, all gilts received two vaccinations, three weeks apart, with Respiporc FLU3 (**Appendix 10**).

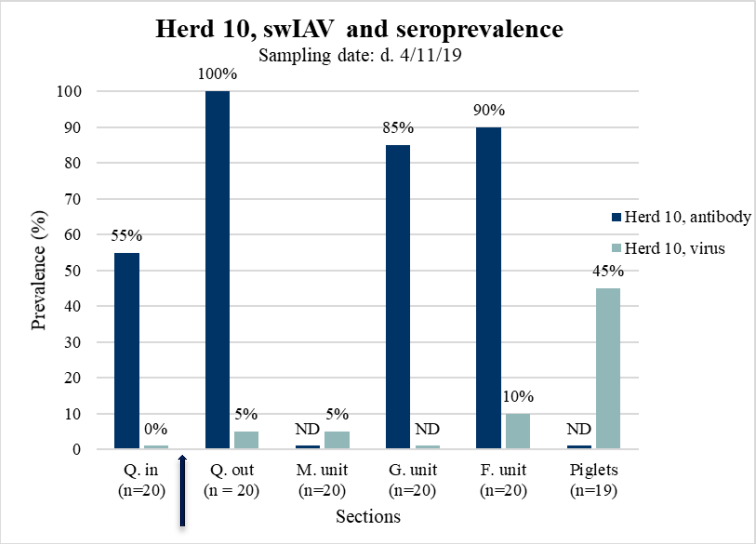


Fig. 12: SwIAV shedding and seroprevalence in herd 10. The prevalence (%) of antibody and virus positive animals is shown on the y-axis and represented on the x-axis are the sections: Quarantine in (Q in), Quarantine out (Q out), Mating unit (M. unit), Gestation unit (G. unit), Farrowing unit (F. unit) and pooled piglets (Piglets). The green columns represent the prevalence of virus positive animals in the particular section. The blue columns represent the prevalence of antibody positive animals in the particular section. Note, antibodies in the mating unit and in piglets were not sampled, and virus detection was not performed in the gestation unit (ND = not done). The arrow marks the time of basis vaccination of gilts.

Fig. 12 illustrates the prevalence of animals testing positive for Influenza A virus antibodies (antibody ELISA) or virus (real-time RT-PCR). Seven days after arriving at the quarantine, the gilts were sampled, where 55%, 95%CI [33.2%, 76.8%] were seropositive and no virus was detected. In contrast, 100%, 95%CI [100%, 100%] of the gilts at the end of the quarantine tested antibody positive and swIAV was detected in one gilt, similarly, one gilt tested positive for swIAV in the mating unit (5%, 95%CI [0%, 14,6%]). In gestating gilts and first parity sows in the farrowing unit, the seroprevalence of 85%, 95%CI [69.4%, 100%] and 90%, 95%CI [76.9%, 100%] was determined, respectively. However, virus shedding was revealed in 10%, 95%CI [0%, 23.2%] of the first parity sows and 45%, 95%CI [23.2%, 66.8%] of the piglets in the farrowing unit.

The seroprevalence in the beginning of the quarantine indicated that the gilt had been exposed to Influenza A virus in the breeding herd. The 100% seroprevalence at the end of the quarantine reflected that the gilts had been vaccinated twice in the quarantine. However, one gilt was found virus positive which suggested virus circulation at the end of quarantine and emphasized that the vaccine does not provide sterile immunity. The influenza vaccination in the quarantine was administered at the same time as two attenuated live vaccines, and according to the manufacturer the efficacy of the vaccine combined with other live vaccines is not investigated. Additionally, the quarantine-time expired three weeks prior to sampling and no biosecurity measures were taken in this period.

The seroprevalence of the gilts in the gestation unit and the farrowing unit did not reflect the vaccination given three days prior to sampling but rather, the effect of the mass sow vaccination performed in July or exposure to swIAV in the mating or gestation period. SwIAV was detected in both the gilts and piglets in the farrowing unit, suggesting that the vaccine did not provide sterile immunity. Moreover, the dissemination of swIAV in the farrowing unit was aided by a large proportion of the lactating sows that were present in two large sections resulting in the mixing of age groups. Furthermore, all personnel had to walk through the farrowing unit before entering the rest of the sow herd.

4.3 Analysis of the hypotheses

Table 3: The prevalence of swIAV and seroprevalence in unvaccinated and vaccinated herds.

The overall prevalence of animals testing positive for Influenza A antibodies or virus in unvaccinated and vaccinated herds in all sections. Number (n) of gilts or pooled piglets.

	Unvaccinated Herds		Vaccinated Herds	
Sections	**Virus prevalence**	**Seroprevalence**	**Virus prevalence**	**Seroprevalence**
Quarantine in	**0%,** (n=100)	**57%** , 95%CI [47.2%, 66.7%], (n=100)	**12%,** 95%CI [5.6%, 18.4%], (n=100)	**41%,** 95%CI [31.4%, 50.6%], (n=100)
Quarantine out	**10%** , 95%CI [4.1%, 15.9%], (n=100)	**56%** , 95%CI [46.3%, 65.7%], (n=100)	**13%,** 95%CI [6.4%, 19.6%], (n=100)	**66%** , 95%CI [56.7%, 75.3%], (n=100)
Mating unit	**0%,** (n=100)	-	**4%** , 95%CI [0.2%, 7.8%], (n=100)	-
Gestation unit	-	**69%** 95%CI [59.9%, 78.1%], (n=100)	-	**93%,** 95%CI [88.0%, 98.0%], (n=100)
Farrowing unit	**0%** ,(n=100)	**58%** , 95%CI [48.3%, 67.7%], (n=100)	**4%,** 95%CI [0.2%, 7.8%], (n=100)	**95%,** 95%CI [90.7%, 99.27%], (n=100)
Piglets	**12.12%** , 95%CI [5.7%, 18.6%], (n=99)	-	**12%** , 95%CI [5.6%, 18.4%], (n=100)	-

H0a: There is no significant difference in section prevalence of virus positive gilts between vaccinated and unvaccinated herds.

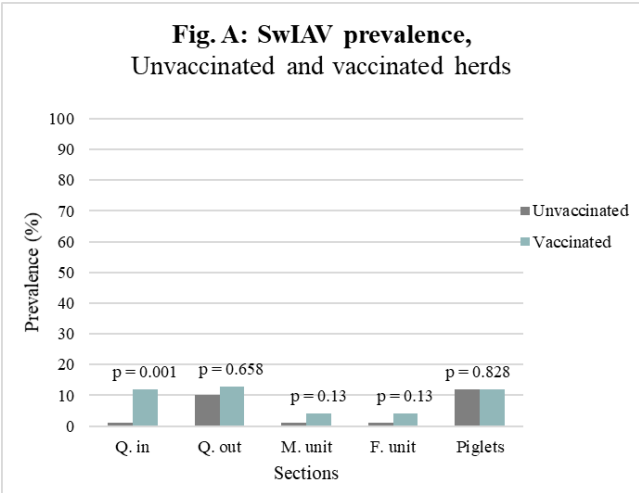
The proportions of virus positive gilts in each sampled section for vaccinated and unvaccinated herds are shown in **Fig. 13A**. To compare the prevalence of virus positive gilts in the quarantine, mating unit, gestation unit, and farrowing unit between vaccinated herds and unvaccinated herds, four chi-square tests were performed (**Appendix 11**). In this study vaccinated herds had a significantly higher proportion of positive gilts at arrival in the quarantine compared to unvaccinated herds, 12% and 0%, respectively ($p<0.001$) (**Fig. 13A**).

H0b: There is no significant difference in prevalence of virus positive pooled samples from piglets in the farrowing unit between vaccinated and unvaccinated herds.

There was no significant difference in virus shedding in piglets between the two groups (12% and 12.12%, $p=0.828$) (**Fig. 13A, Appendix 11**).

H0c: There is no significant difference in section prevalence of antibody positive gilts between vaccinated and unvaccinated herds.

The proportions of antibody positive gilts in each sampled section for vaccinated and unvaccinated herds are shown in **Fig. 13B**. There was a significant difference in prevalence of antibody positive gilts between vaccinated and unvaccinated herds in three out of four sections: quarantine in (41% and 57%, $p=0.034$), gestation unit (93% and 69%, $p <0.001$), and farrowing unit (95% and 58%, $p <0.001$) (**Fig. 13B, Appendix 11**).



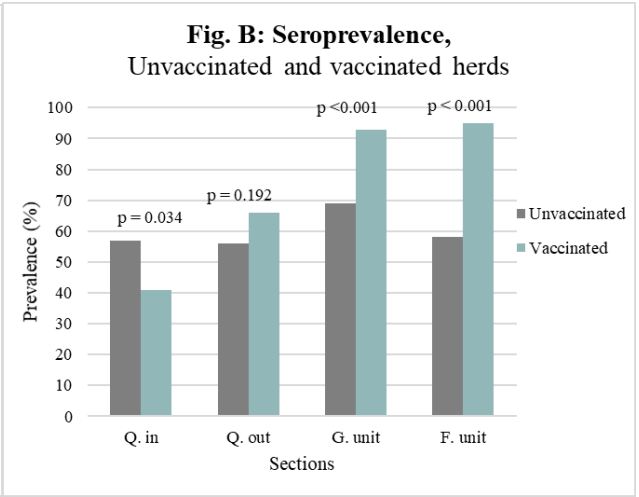


Fig. 13A and 13B: Prevalence of swIAV and antibodies. The overall prevalence of animals testing positive for Influenza A virus (**Fig. 13A**) or antibodies (**Fig. 13B**) in unvaccinated and vaccinated herds in all sections. Grey columns = all unvaccinated herds, green columns = all vaccinated herds. x-axis: sampled sections, Quarantine in (Q. in), Quarantine out (Q. out), Mating unit (M. unit), Farrowing unit (F. unit), Piglets. y-axis: prevalence (%). **Fig. 13A**, swIAV prevalence is illustrated, a significant higher swIAV prevalence in vaccinated herds in the beginning of the quarantine was found (p<0.001). **Fig. 13B**, seroprevalence in the unvaccinated and vaccinated is illustrated, the unvaccinated herds were higher in seroprevalence in the beginning of the quarantine (p=0.034). The gestation unit and farrowing unit revealed a significantly higher seroprevalence in the vaccinated herds (p<0.001) (**Appendix 11**).

H0d: There is no significant difference in levels of antibodies in seropositive gilts between vaccinated and unvaccinated herds.

In vaccinated herds, the S/N values in seropositive first parity sows were significantly lower in the farrowing unit (p=0.006) and when all sections were merged (p<0.0001) (**Appendix 11**). The S/N values of positive gilts are illustrated in **Fig. 14** in unvaccinated and vaccinated herds**.**

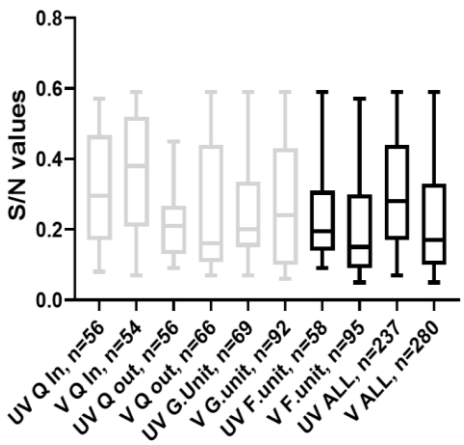


Fig. 14: S/N values in unvaccinated and vaccinated herds. The box plot illustrates S/N values of positive samples (S/N value <0.60) in different sections in unvaccinated (UV) and vaccinated (V) herds. Quarantine in (Q In), Quarantine out (Q out), Gestations unit (G.unit), Farrowing unit (F. unit), all sections (ALL). x-axis: sections and numbers of seropositive gilts i each group (n). y-axis: S/N values. The highlighted boxes show significant differences between unvaccinated and vaccinated herds. In the farrowing unit and when all sections are merged, a significant difference in S/N-values were shown. The prevalence of antibody positive gilts in each section are shown in **Fig. 13B**.

H0e: There is no correlation between virus positive gilts at the end of quarantine and positive piglets.

Herds with virus positive gilts at the end of the quarantine had a significant higher prevalence of virus positive piglets (p=0.047) and the relative risk of having virus positive piglet one-week-of-age was 2.5, 95%CI [1.03, 6.37] (**Appendix 11**).

H0f: There is no correlation between antibody prevalence at the end of quarantine and virus positive gilts or piglets in the herd.

The risk of having positive gilts or/and piglets, when having a seroprevalence under 85% at the end of quarantine was significant lower compared to having a seroprevalence above 85% (RR=0.31, 95%CI [0.15, 0.65], p=0.01) (**Appendix 11**).

H0g: There is no correlation between levels of antibodies in gilts before and after farrowing and virus positive piglets.

In this study, herds with virus positive piglets had lower S/N values in the first parity sows in the farrowing unit (p=0.0039) and no significant difference was found in the gestation unit (**Fig. 15., Appendix 11**)

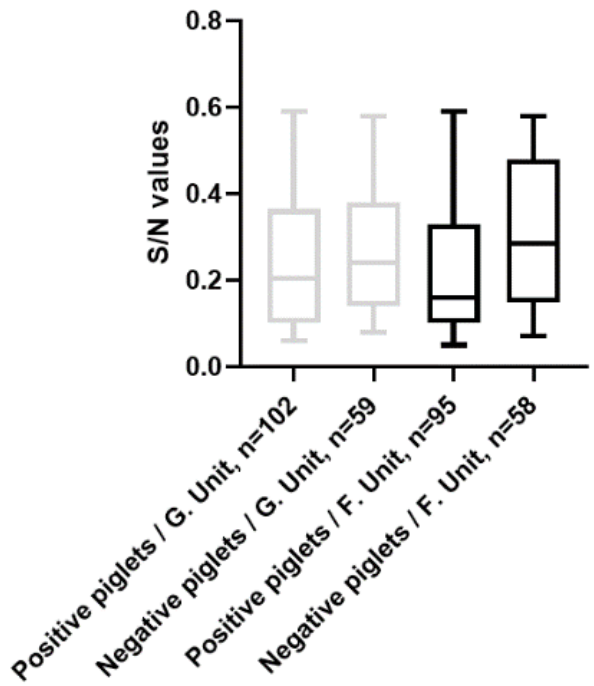


Fig. 15: S/N values in herds with virus positive and negative piglets. The box plot illustrates S/N values of positive samples (S/N value <0.60) in gestation and farrowing units in herds with virus positive piglets and herds with virus negative piglets. x-axis: Number of seropositive gilts (n) in the gestation unit (G. unit) and farrowing unit (F. unit) in herds with positive or negative piglets. y-axis: S/N values. The highlighted boxes show a significant difference between the two groups. The S/N values were significantly different in the farrowing unit between the two groups (p=0.0039) but was not significantly different in the gestating unit.

5. Discussion

5.1 Discussion of study design and methods

In this study, the role of gilts in swIAV transmission was investigated through a cross-sectional study in ten Danish sow herds by investigating quarantine management, biosecurity measures, and vaccination strategy (**Fig. 1**). The chosen cross-sectional study was used to clarify virus and/or antibody prevalence in gilts and exposure of potential risk factors, but it has its limitations. The design illustrated the swIAV dynamics on a specific day, and there were several months in the production system between the sampled subpopulations where virus could have circulated, for instance in the gestating gilts. The causality between an exposure and outcome in a cross-sectional study can be difficult to evaluate, while it is not known if virus positive piglets is a consequence of virus positive gilts or the exposure follows the positive piglets (68). Moreover, a confounding factor as herd management can influence both the virus and antibody status of the gilts and piglets which can either cause an apparent relationship to appear or conceal the true relationship between risk factors and outcome (68).

The aim was to sample all animals in a herd in one day and collect samples within the first week after introduction to a given section or one week before and after farrowing. However, the design of the study did not consider that majority of herds only had one quarantine section and therefore, two to three sampling days were necessary. The observed difference in sampling time in the beginning of the quarantine, at the end of the quarantine, and in the mating unit can be explained by practicality, health status, communicative misunderstandings, and management. In practice, a short period between sampling points is often necessary and accepted when conducting cross-sectional studies (68). The ten herds were sampled over three months from September to November 2019 why a seasonal variance in swIAV dynamics could affect virus status in the specific herd and among herds (50,77)

In order to obtain descriptive features about risk factors of quarantine management and vaccination strategy, a questionnaire was designed. Although precautions to minimise questionnaire bias, such as pretesting, information bias might still occur. The questionnaire was answered by the herd owner or manager whom might not participate in the daily management of the quarantine. The prestige bias is when a respondent answers what he believes to be the right answer, and could have occurred under these circumstances (68) (**Appendix 5**).

We acknowledge that our results do not represent swIAV infection dynamics and vaccination strategy across Danish herds given our herd selection bias, since only a limited number of herds was conveniently selected, and only herds above 800 sows participated to ensure enough gilts for the sample size. Nevertheless, this study provides descriptive knowledge about the role of gilts in swIAV transmission throughout the production cycle in sow herds (**Table 2, Appendix 3**).

5.2 Discussion of results

5.2.1 Quarantine management and biosecurity

Only a few studies have investigated the role of gilts in swIAV transmission (50,78) and found gilts important for the transmission dynamics of swIAV. In this study, gilts in seven out of ten herds tested virus positive, indicating that gilts are part of the transmission dynamics in Danish sow herds (**Appendix 7**).

The insight in the quarantine management and biosecurity measures in the present study can provide knowledge about risk factors that could contribute to virus infection of gilts at the end of the quarantine. Generally, all quarantines in the ten herds were inspected in the afternoon, change of boots and clothing was performed, but hand wash was not carried out in few of them (**Appendix 9**). Six out of ten quarantines were an extension of the sow herd or weaning unit. In four of them, the quarantine-time was expired when sampling, meaning that the personnel moved between sections without any biosecurity precautions even though the gilts had the highest health status. This could be the source of virus positive gilts at the end of the quarantine in two of these herds (**Fig. 11 and 12**). In one herd, slaughter pigs were housed in a pen beside the gilts of the quarantine leading to constant direct transmission of swIAV, as slaughter pigs were continuously transferred to the quarantine which was also supported with the finding of 20% virus prevalence at the end of the quarantine (**Fig. 6**). A significantly higher swIAV prevalence in vaccinated herds in the beginning of the quarantine ($p=0.01$) was discovered (**Fig. 13A, Table 3**). The presence of swIAV positive gilts in the beginning of the quarantine indicated that either the gilts were bringing swIAV from the breeding herd, staff members were bringing virus from the sow herd, or virus was persistently present in the quarantine unit, thereby infecting the gilts when introduced into the quarantine.

According to the health regulation of SPF, the purpose of a quarantine is to prevent transmission of infection to the herd caused by purchase animals (79). This was consistent with the

observations in all ten herds, nevertheless, this mindset might not compatible with the prevention of swIAV infection in gilts at the end of the quarantine. The biosecurity focus of the personnel was not aimed at protecting the gilts against influenza, but rather aimed at protecting the sow herd from pathogens carried by the gilts in the quarantine (**Appendix 9**).

This mindset could explain why six out of ten herds had virus positive gilts at the end of the quarantine, as a consequence, five of these sow herds tested positive for swIAV in gilts or piglets. This can indicate that virus positive gilts at the end of quarantine pose a risk of introduction of influenza to the sow herd, which is consistent with previous findings (33,50–53). In an American study, gilts testing positive for swIAV at entry to the sow herd were associated with the probability of having swIAV positive piglets at weaning (RR=1.67, $p<0.001$) (51). Present study, the correlation between positive gilts at the end of quarantine and positive piglet one-week-of-age was investigated. A similar correlation was found meaning that the risk of having virus positive piglets when having virus positive gilts at the end of the quarantine was 2.5, 95% CI [1.03, 6.37] times higher compared to herds with virus negative gilts at the end of quarantine. Interestingly, this association was found although the animals were housed in different places in the production system. Furthermore, Diaz *et al.*, 2015, found that gilts residing for more than four weeks cleared the swIAV infection before farrowing. This suggests that replacement gilts contribute to the introduction of swIAV and lack of internal biosecurity promote to the enzootic circulation of swIAV within the herd (50–52,78). This risk of introducing swIAV through replacement gilts could be minimised by decreasing the introduction frequency (17,52). On average, the ten herds had 2.5 months between purchase of gilts. Four out of the ten herds had a weekly introduction of new gilts to the sow herd after quarantine-time expired wherein three of them had virus positive gilts or piglets. A case report applied a temporary halt of introduction of new gilts for six months and changed the introduction frequency from two months to four months in order to provide a required break to stop transmission of disease to new gilts. This introduction strategy combined with high internal biosecurity successfully eliminated swIAV in a sow herd of 1200 sows (17).

5.2.2 Immunisation of gilts

The immunisation of gilts at the end of the quarantine is important because incoming gilts lack immunity against the circulating swIAV in the herd. Naïve gilts will become infected as soon as they enter the herd and contribute to the persistence of swIAV (50,78). Herd immunity is the ability of a group of animals to resist becoming infected or to minimize the effect of an infection in severity and incidence (80). Five out of ten herds had a seroprevalence between

85% and 100% at the end of the quarantine (**Appendix 7**). A high seroprevalence at the end of the quarantine is necessary in order to control the high reproduction number of influenza (42,80), however, this study found that the risk of having positive gilts and/or piglets in the herd, when having a relatively low seroprevalence under 85% at the end of the quarantine, was not significantly higher, in fact it had a protecting effect (RR = 0.31, 95%CI [0.15, 0.65]). Nevertheless, three out of five herds with a seroprevalence above 85% had virus positive gilts at the end of the quarantine, indicating the importance of virus status of the gilts at this point of time (50,51,78), suggesting that this finding was likely a result of virus positive gilts at the end of the quarantine and not a protecting effect of low seroprevalence (**Appendix 7**).

Vaccinating herds had the possibility of optimal immunising of the gilts before moving them, however three out of five vaccinated herds did not achieve this due to the vaccination strategy, where the gilts were vaccinated at the age of 24 and 26 weeks (**Fig. 8, 10 and 11**). This is consistent with this study finding no difference in seroprevalence between vaccinated and unvaccinated herds in the end of the quarantine. However, the vaccinated herds had a significant higher seroprevalence in the gestation unit ($p<0.001$) and farrowing unit ($p<0.001$) (**Fig. 13B, Table 3**), which could be explained by either vaccination or confounding bias and interactions such as herd size, management, age, circulating swIAV in the gestation unit, which was not investigated in this study (33,54,55,78).

Considering the significantly higher seroprevalence in gestation and farrowing units in vaccinated herds, it was expected that the semiquantitative S/N values (74), was lower in the gestation and farrowing unit (27,37). This study found a difference in antibody levels in the farrowing unit and across all gilt subpopulations between vaccinated and unvaccinated herds, but not in the gestation unit, despite the use of excessive vaccination programs in vaccinated herds. Nevertheless, three out of the five vaccinated herds mass sow vaccinated more than four months ago and therefore, gilts in these herds were not included in the mass sow vaccination and the antibody levels detected were from the basis vaccination, which can explain the antibody level deviation in this group (**Fig. 14, Appendix 10**) (34). However, virus detection in gestating gilts was not performed, therefore, virus circulation in the gestation unit could influence the results by equalising the difference in antibody levels between the two groups. The marked difference in antibody levels in the farrowing unit can be explained by vaccination or swIAV circulation.

5.2.3 The effect of vaccination on viral shedding

The vaccine Respiporc FLU3 is a whole inactivated vaccine and does not provide sterile immunity to the vaccinated gilts, however, it can reduce the viral replication in the lungs and clinical signs (34,36,37). Furthermore, prefarrow vaccination provide clinical protection to piglets, but MDAs do not protect the piglets against infections (34). Studies suggested that the degree of clinical protection and virus shedding of the piglets depends on the level of MDAs (18,29). Moreover, the samples were collected from the nasal cavity where vaccine derived IgG is not present (12). However, studies indicate that vaccination reduces IAV infections in pigs (42,51,52). Observation of clinical signs were not part of this study. Nevertheless, the correlation between vaccination and virus shedding was investigated due to the correlation that had been detected between virus titre, cytokine response in the airways, and the severity of symptoms (10,25). This study found no correlation between high antibody levels in first parity sows and the protection of piglets from swIAV infection, as the antibody levels were significantly higher in the farrowing unit with virus positive piglets. A possible explanation of the marked difference in antibody levels in the farrowing unit could be an immune boost due to virus circulation in the farrowing unit.

The present study found no significant difference in swIAV prevalence after vaccination between vaccinated and unvaccinated herds (**Fig. 13A, Table 3**). The above-mentioned clinical and viral protection is based on the assumption that vaccine and herd strain are compatible. This was not the case in herd 9 where the subtype H1pdm09N1av was identified in one sample from the farrowing unit (**Fig 11, Table 2**). Additionally, it was not possible to sequence samples from herd 6, 7, and 8, - these herds could potentially vaccinate with the wrong vaccine. The sequencing and amino acid identity of the HA and NA gene to the vaccine strains revealed a genetic diversity of the subtype in herd 10. The viral drift could have a negative impact on the vaccine efficacy, but the impact of genetic diversity needs to be further investigated as the location of changes in the HA protein might be more important than the total number of changes (27,38). The whole inactivated vaccine induces only serum antibodies and do not activate the endogenous pathway of antigen presentation and is therefore unable to induce cellular and mucosal cross-reactivity against difference antigenic variants (27,81). Additionally, MDAs do not protect against virus infection in piglets and can induce vaccine associated enhanced respiratory disease in weaners (VAERD), but this is only observed in experimental studies (39,40).

Respiporc FLU3 contains subtypes from 2000 and 2003 (34) but cover the predominant subtypes circulation in Danish pig production (5). The drift of swIAV is slower than huIAV, not

because of a lower amino acid substitution rate, but because of a lower selective immune pressure in the pig population as a result of limited level of pre-existing immunity (82,83). Moreover the adjuvants of the inactivated vaccine promote a high homologous hemagglutination inhibiting antibodies which can protect against heterologous strains resulting in a broader protection (27). In all five vaccinated herds, the influenza basis vaccination of the gilts was combined with other vaccines and in one herd administered together with live PRRS vaccine (**Appendix 10**). The efficacy of the vaccine when using this combined vaccine procedure is not investigated according to SPC of Respiporc FLU3 and the passive protection of the piglets are achieved when using prefarrow vaccination strategy, which was not the case in the vaccinated herds that were studied (34). However, mass sow and prefarrow vaccination have shown to reduce swIAV infections in piglets at weaning compared to no vaccination (51,52).

Furthermore, seven out of ten herds had *Mycoplasma hyopneumonia* or/and PRRS which both are important in the PRDC together with swIAV, and these co-infections can result in a more pronounced disease in co-infected animals (21) (**Table 1**). Additionally, in our study three of the swIAV vaccinated herds had PRRS and PRRSv positive pigs have shown to more likely infected with swIAV than PRRSv negative pigs, therefore these herds could have higher odds of being swIAV positive (22). Co-infections, vaccination strategy and herd management procedures, such as continuous flow in a section, movement of pigs in the production system, pig density, herd size, and cross-fostering act as risk factors for swIAV transmission (30,33,54). Indicating, lack of internal biosecurity can affect the swIAV transmission dynamics and potentially influence the results of vaccination. This study suggests that the Respiporc FLU3 vaccine is not the solution when controlling swIAV but can be part of the solution where improving the external and internal biosecurity might be as important as vaccination.

The recommendations for controlling swIAV transmission in Danish sow herds based on the results of this study are as follows: Improving biosecurity in the quarantine thereby protecting the quarantine gilts against influenza from the sow herd and personnel. SwIAV testing of gilts and optimal immunisation before introduction to the sow herd, decreased introduction frequency, implementing strict sectioning all in/all out, optimal flow of animals in the production system, limited cross-fostering, and vaccination of the personnel.

6. Conclusion

The results of this study provide unique data on the role of gilts in swIAV transmission and quarantine biosecurity measures in Danish sow herds. This knowledge could contribute to the understanding of swIAV transmission in Danish herds. The seroprevalence in gestation and farrowing units, antibody levels in farrowing units and across all gilt subpopulation were significantly higher in vaccinated herds, however, generally no difference in virus prevalence in gilts and piglets was found between vaccinated and unvaccinated herds. Moreover, virus positive gilts at the end of the quarantine were associated with virus positive piglets one-week-of-age. This indicates the need to focus on biosecurity interventions to control swIAV transmission in replacement gilts. The improvement of biosecurity at the end of the quarantine is necessary to prevent swIAV circulation between sow herd, humans, and quarantine. Furthermore, immunisation of gilts with a vaccine strain compatible with the herd strain before introduction to the sow herd is important to reduce contribution of naïve gilts to the persistence of swIAV in the sow herd.

7. Perspective

The ideal swIAV vaccine should induce a broad immunity and overcome MDAs interfering. Novel vaccines have been investigated and tested including intranasal live attenuated vaccine, recombinant protein vaccine, vector vaccine, and DNA vaccine. Recently, the intranasal live attenuated vaccine became available in the US market (38). The immune response promoted by the live attenuated influenza vaccine is more likely mucosal and T cell mediated antibodies and are less likely to interfere with MDAs (81). Vaccination of neonatal piglets and weaners with live attenuated influenza vaccine inoculated intranasal in a single dose has shown to provide a greater cross-protection against variant strains without inducing VAERD and reducing viral shedding (81,84). Majority of novel vaccines cannot yet compete with the current commercially inactivated vaccines considering both cost and safety. A major concern with live vaccines is the risk of reassortment and the development of novel reassortant swIAVs (27). Vaccines have never been able to stand alone when controlling diseases in pig production, therefore, with the knowledge from this study, further research on which biosecurity factors that most effectively could reduce swIAV transmission might be just as important as novel vaccines.

In one herd, the subtype H1avN2hu95 was found with the NA from the human influenza season in 1995 (**Table 2**). Through the Danish passive surveillance, a spill over of human seasonal influenza virus has been discovered in Danish pig herds (85). This indicates that pigs are susceptible to human influenza virus and new subtypes can emerge (5,85,86). Pigs might also act as a reservoir for older human influenza HA-genes and reenter human population a decade after, causing high morbidity in children, because of the lack of exposure and antibody formation (87). H1N1pdm09 is now circulating in humans and pigs and has shown to transmit between them (59,60). New reassorted subtypes originated from H1N1pdm09 in Danish pig herds might have a zoonotic potential and can increase the risk to public health, because of H1N1pdm09 zoonotic character. However, in this study, none of the staff members were vaccinated against influenza (**Appendix 9**). Considering, the zoonotic character of Influenza A virus and the history of pandemic outbreaks, education and communication about protecting pigs and humans against IAV infections should be a high priority.

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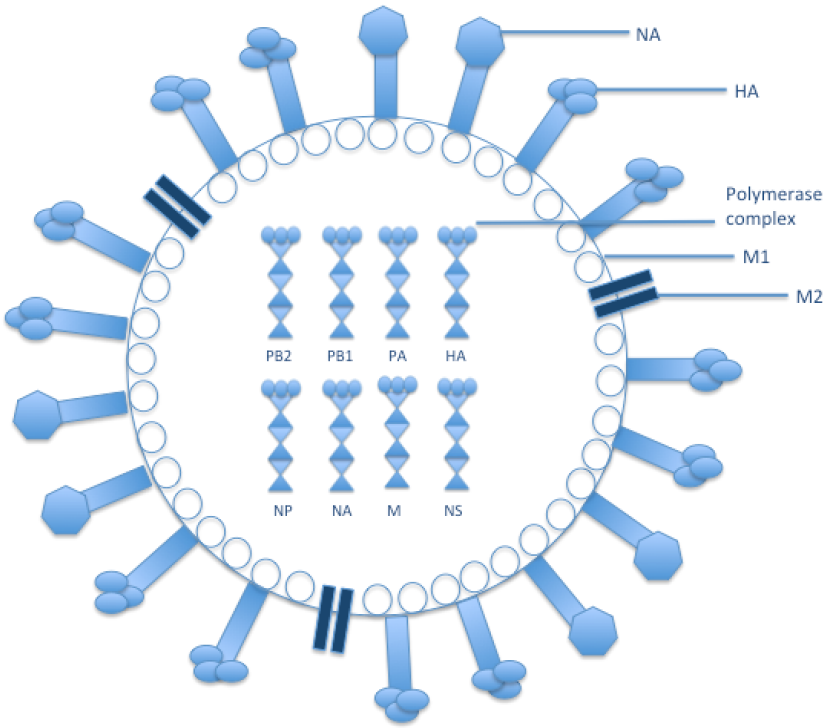
9. Appendix

Appendix 1 - Structure of Influenza A virus



Structure of IAV. The IAV's are polymorphic (spherical or filamentous shape) and are approximately 80–120 nm in diameter. IAV contains 8 gene-segments encoding the following viral proteins: hemagglutinin (HA), neuraminidase (NA), matrix protein 1 and 2 (M1, M2), non-structural protein 1 and 2 (NEP1, NEP2), nucleoprotein (NP) and the 3P-polymerase complex (PB2, PB1, PA) (2).

Appendix 2 - Sample size

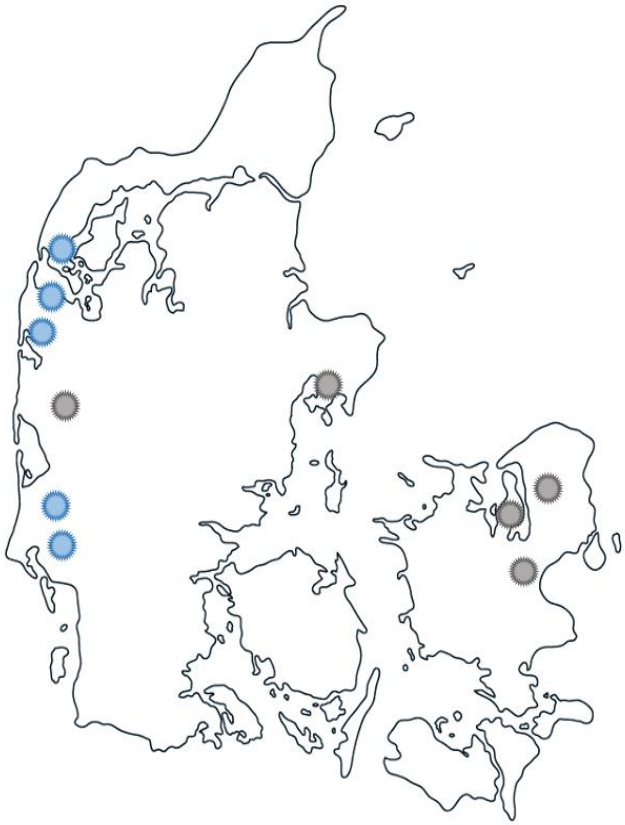
Sample size to detect disease	
Population size (N)	500
Assumed prevalence (p)	0.143
Number of detectable cases (d)	71.5
Probability of finding at least one (P)	0.95
Required sample size (n)	19.07
Rounded sample size (n)	20

Sample size - detection of disease. A population size of 500 gilts had been chosen since an average herd with 1000 sows purchases approx. 500 gilts per year. With a prevalence of 14.3%, at least one positive sample can be detected with the sample size of 20 gilts in a sampled section in each herd.

Test for relationship between disease a			
The simple formula (two-sided)			
Estimated response in group 1 (p1)			0.11
Estimated response in group 2 (p2)			0.32
Confidence level (1-alpha)			0.95
Z(1-alpha)			1.96
Power (1-beta)			0.8
Z(beta)			0.84
Intermediate calculations:			
q1 = 1 - p1	0.89	p = (p1 + p2),	0.22
q2 = 1 - p2	0.68	q = 1 - p	0.79
Required sample (from each group) (n)			58.9
Rounded sample (from each group) (n)			59

Sample size (two-sided) – difference in prevalence between vaccinated and unvaccinated herds. With the sample size of 59 animals, a difference in prevalence between the two groups can be detected, when the prevalence in group one (vaccinated) is estimated to 10.7% and the prevalence in group two (unvaccinated) is estimated to 32.1%.

Appendix 3 - Mapping of the ten herds



Mapping of the ten herds. Grey = unvaccinated herds, Blue = vaccinated herds.

Appendix 4 - Detailed sampling list

Week	Week day / date	Herd	Sections	Sample to DTU laboratory
38	Wed. d. 18/9/19	Herd 1	Q in (A) + C, D, E	Fri. d. 20/9/19
39	Wed d. 25/9/19	Herd 6	Q out (B)	Thu. d. 26/9/19
40	Tue. d. 1/10/19	Herd 2	Q in (A) + C, D, E	Wed. d. 2/10/19
40	Thu. d. 3/10/19	Herd 3	Q out (B) + D, E	Fri. d. 4/10/19
41	Tue. d. 8/10/19	Herd 4	Q in (A) + C, D, E	Thu. d. 10/10/19
41	Wed. d. 9/10/19	Herd 7	A, B, C, D, E	Thu. d. 10/10/19
41	Thu. d. 10/10/19	Herd 5	Q out (B)	Thu. d. 10/10/19
42	Wed. d. 16/10/19	Herd 3	Q out (A) + C	Wed. d. 16/10/19
42	Fri. d. 18/10/19	Herd 8	Q out (B)	Fri. d. 18/10/19
43	Mon. d. 21/10/19	Herd 6	Q in (A) + C, D, E	Tue. d. 22/10/19
43	Tue. d. 22/10/19	Herd 1	Q out (B)	Tue. d. 22/10/19
44	Mon. d. 28/10/19	Herd 9	A, B, C, D, E	Wed. d. 30/10/19
44	Tue. d. 29/10/19	Herd 8	C, D, E	Wed. d. 30/10/19
44	Fri. d. 1/11/19	Herd 5	Q in (A) + C, D, E	Fri d. 1/11/19
45	Mon. d. 4/11/19	Herd 10	A, B, C, D, E	Tue. d. 5/11/19
45	Tue. d. 5/11/19	Herd 2	Q out (B)	Tue. d. 5/11/19
46	Mon. d. 11/11/19	Herd 8	Q in (A)	Tue. d. 5/11/19
47	Mon. d. 18/11/19	Herd 4	Q out (B)	Mon. d. 18/11/19

Detailed sampling list. Sampling date for each herd and sections. Quarantine in (Q in, A), Quarantine out (Q out, B), Mating unit (C), Gestation unit (D), Farrowing unit (F). The day the samples were delivered and prepare for analysis are listed to the right.

Appendix 5 – Questionnaire, quarantine management and biosecurity

Spørgeskema vedrørende karantæne- og vaccinationsstrategi mod influenza		
Chr. Nr.: **Sundhedsstatus:** **Udfyldt af:**		
Er alle medarbejdere vaccineret mod influenza indenfor det seneste år	Ja	Nej
Polte rekruttering		
Hvor mange polte indkøbes pr. år		
Alder ved indsættelse i karantænen		uger
Antal gange, der er indkøbt polte det seneste år		gange
Er poltene vaccineret for influenza inden ankomst	Ja	Nej
Hvis ja, hvilken vaccine Antal gange	Ja	Nej
Antal leverandører de seneste 2 år		
Karantænestalden		
Antallet af karantænestalde og sektioner	stalde	sektioner
Er der separat indgang til karantænestalden	Ja	Nej
Er karantænestalden placeret på en anden ejendom Hvis ja, hvor mange km væk	Ja	Nej
Er karantænen **altid** helt tom for dyr før nye dyr indsættes	Ja	Nej
Står karantænen tom mellem hold af nye polte Hvis ja, hvor længe	Ja	Nej
Vaskes og udtørres karantænen **altid** mellem hold af nye polte	Ja	Nej
Polte i karantænen		
Tages **alle** polte ud af karantænen samtidigt	Ja	Nej
Hvor lang er karantænetiden		uger
Hvornår på dagen tilses poltene i karantænestalden	Morgen/middag/eftermiddag	
Er der restriktioner omkring færdsel i besætningen efter besøg i karantænestalden Hvis ja, beskriv kort	Ja	Nej
Vaccinationsstrategi		
Hvornår efter indsættelse vaccineres poltene **første** gang og anden **gang** for influenza med Respiporc FLU3		
Vaccineres poltene **2 gange** for influenza inden udgang af karantænestalden	Ja	Nej
Hvilke måneder blitzes soholdet		
Hvor ofte blitzes soholdet		
Hvornår har I sidst blitz vaccineret soholdet		
Medtages polte/gylte altid i blitz af soholdet	Ja	Nej
Medtages slagtesøer altid i blitz af soholdet	Ja	Nej

Questionnaire, English version

Questionnaire about quarantine management, biosecurity measures, and vaccination strategy		
Influenza vaccination of staff members?	Yes	No
Gilt recruitment		
Number of purchased gilts pr. year		
Gilt age when arriving in quarantine		weeks
The number of gilt purchases in the past year		
Vaccination of gilts against Influenza A before arrival? If yes, which vaccine and times	Yes	No
Number of suppliers over the past 2 years		
The Quarantine unit		
Number of quarantine units and sections	Units	Sections
Is there separate entrance to the quarantine	Yes	No
Is the quarantine placed on another site away from the sow herd?	Yes	No
Is the quarantine completely empty of animals before a new batch?	Yes	No
Is the quarantine empty between batches	Yes	No
Is the quarantine washed and dried between batches	Yes	No
Gilts in quarantine		
Are all gilts moved from the quarantine at the same time	Yes	No
How long is the quarantine-time?		weeks
In a working day, when are the quarantine gilts inspected	Morning/Noon/Afternoon	
Any restrictions for the personnel after leaving the quarantine If yes, describe	Yes	No
Vaccination strategy		
In the quarantine, when are the gilts basis vaccinated		
Are the gilts vaccinated twice before leaving the quarantine	Yes	No
Mass sow vaccination, when		
Mass sow vaccination frequency		times
The last mass sow vaccination		
Are all gilts in the sow herd included in mass sow vaccination	Yes	No
Are slaughter sows included in mass sow vaccination	Yes	No
Other vaccines administered with Respiporc Flu3		

Appendix 6 - Checklist

Checklist		
Chr. Nr.: **Owner:**		
Vaccinated herd	Yes	No
Internal/external biosecurity		
Change of boot in/out of herd	Yes	No
Change of clothing in/out of herd	Yes	No
Change of clothing between sections		
Quarantine unit	Yes	No
Mating unit	Yes	No
Gestation unit	Yes	No
Farrowing unit	Yes	No
Change of boot between sections		
Quarantine unit	Yes	No
Mating unit	Yes	No
Gestation unit	Yes	No
Farrowing unit	Yes	No
Bird proof net	Yes	No
Rodent control	Yes	No
Vaccination strategy and treatments		
Other vaccinations		
Regular treatments with antibiotics		
Quarantine unit		
Quarantine maintenance	Yes	No
All in/all out	Yes	No
Number of sections		
Mating unit		
Are gilts housed away from the sows	Yes	No
Fixed are free	Fixed	Free
Number of animals pr. pen and section		
Gestation unit		
Are gilts housed away from the sows	Yes	No
Number of animals pr. pen and section		
Farrowing unit		
Sectioning?	Yes	No
Cross-fostering after 48 hours	Yes	No
Weaning of all piglets from the same week	Yes	No
Weaned piglets in farrowing unit	Yes	No
When cross-fostering - is sow or piglets moved	sow	piglets

Appendix 7 - Results from each herd

		Unvaccinated Herds					Vaccinated Herds				
		Herd 1	Herd 2	Herd 3	Herd 4	Herd 5	Herd 6	Herd 7	Herd 8	Herd 9	Herd 10
Quarantine in	Virus positiv	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	50% (10/20)	0% (0/20)	0% (0/20)	10% (2/20)	0% (0/20)
	Antibody positiv	60% (12/20)	35% (7/20)	70% (14/20)	25% (5/20)	95% (19/20)	15% (3/20)	0% (0/20)	70% (14/20)	65% (13/20)	55% (11/20)
Ourantine out	Virus positive	30% (6/20)	0% (0/20)	0% (0/20)	20% (4/20)	0% (0/20)	15% (3/20)	0% (0/20)	40% (8/20)	5% (1/20)	5% (1/20)
	Antibody positiv	35% (7/20)	35% (7/20)	25% (5/20)	85% (17/20)	100% (20/20)	25% (5/20)	85% (17/20)	100% (20/20)	20% (4/20)	100% (20/20)
Mating unit	Virus positiv	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	5% (1/20)	5% (1/20)	5% (1/20)	0% (0/20)	5% (1/20)
Gestation unit	Antibody positiv	60% (12/20)	70% (14/20)	55% (11/20)	65% (13/20)	95% (19/20)	100% (20/20)	80% (16/20)	100% (20/20)	100% (20/20)	85% (17/20)
Farrowing house	Virus positiv	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	5% (1/20)	0% (0/20)	5% (1/20)	10% (2/20)
	Antibody positiv	55% (11/20)	40% (8/20)	50% (10/20)	45% (9/20)	100% (20/20)	100% (20/20)	85% (17/20)	100% (20/20)	100% (20/20)	90% (18/20)
Piglets, pooled	Virus positiv	0% (0/20)	10% (2/20)	0% (0/20)	35% (7/20)	15% (3/20)	0% (0/20)	0% (0/20)	5% (1/20)	10% (2/20)	45% (9/20)

Herd 1

Sampling date: d. 18/9/19				Sampling date: d. 22/10/19				Sampling date: d. 18/9/19			Sampling date: d. 18/9/19			Sampling date: d. 18/9/19				Sampling date: d. 18/9/19		
Quarantine in	PCR, ct-value	Antibody (S/N)	Days in Q	**Ourantine out**	PCR, ct-value	Antibody (S/N)	Weeks in Q	**Mating unit**	PCR, ct-value	Comment	**Gestation unit**	Antibody (S/N)	WBF	**Farrowing unit**	PCR, ct-value	Antibody (S/N)	DAF	**Piglets, pooled**	PCR, ct-value	Age, days
1A1	0	0,36	7	**1B1**	0	0,88	6	**1C1**	0	10 days in herd	**1D1**	0,39	1	**1E1**	0	0,64	5	**1EP1**	0	5
1A2	0	0,76	7	**1B2**	31,08	0,95	6	**1C2**	0	10 days in herd	**1D2**	0,94	1	**1E2**	0	0,64	5	**1EP2**	0	5
1A3	0	2,35	7	**1B3**	0	0,69	6	**1C3**	0	10 days in herd	**1D3**	0,19	1	**1E3**	0	0,3	5	**1EP3**	0	5
1A4	0	0,74	7	**1B4**	0	0,1	6	**1C4**	0	10 days in herd	**1D4**	1,15	1	**1E4**	0	0,58	5	**1EP4**	0	5
1A5	0	0,18	7	**1B5**	0	0,21	6	**1C5**	0	10 days in herd	**1D5**	0,7	1	**1E5**	0	0,37	5	**1EP5**	0	5
1A6	0	0,15	7	**1B6**	31,1	0,97	6	**1C6**	0	10 days in herd	**1D6**	0,84	1	**1E6**	0	2,2	5	**1EP6**	0	5
1A7	0	0,68	7	**1B7**	0	0,14	6	**1C7**	0	10 days in herd	**1D7**	1,18	1	**1E7**	0	0,11	5	**1EP7**	0	5
1A8	0	0,49	7	**1B8**	30,92	0,64	6	**1C8**	0	10 days in herd	**1D8**	0,55	1	**1E8**	0	0,49	5	**1EP8**	0	5
1A9	0	0,37	7	**1B9**	0	0,8	6	**1C9**	0	10 days in herd	**1D9**	0,47	1	**1E9**	0	0,63	5	**1EP9**	0	5
1A10	0	0,49	7	**1B10**	0	1,04	6	**1C10**	0	10 days in herd	**1D10**	0,57	1	**1E10**	0	0,64	5	**1EP10**	0	5
1A11	0	0,68	7	**1B11**	32,02	0,83	6	**1C11**	0	10 days in herd	**1D11**	0,35	1	**1E11**	0	0,44	5	**1EP11**	0	5
1A12	0	0,18	7	**1B12**	30,46	0,56	6	**1C12**	0	10 days in herd	**1D12**	0,78	2	**1E12**	0	0,33	8	**1EP12**	0	8
1A13	0	1,29	7	**1B13**	0	0,23	6	**1C13**	0	10 days in herd	**1D13**	0,12	2	**1E13**	0	0,35	8	**1EP13**	0	8
1A14	0	0,27	7	**1B14**	0	0,71	6	**1C14**	0	10 days in herd	**1D14**	1,93	2	**1E14**	0	0,36	8	**1EP14**	0	8
1A15	0	1,09	7	**1B15**	31,78	0,69	6	**1C15**	0	10 days in herd	**1D15**	0,21	2	**1E15**	0	0,67	10	**1EP15**	0	10
1A16	0	0,54	7	**1B16**	0	0,6	6	**1C16**	0	10 days in herd	**1D16**	0,13	2	**1E16**	0	0,52	10	**1EP16**	0	10
1A17	0	0,7	7	**1B17**	0	0,31	6	**1C17**	0	10 days in herd	**1D17**	0,11	2	**1E17**	0	0,73	12	**1EP17**	0	12
1A18	0	0,13	7	**1B18**	0	0,77	6	**1C18**	0	10 days in herd	**1D18**	0,68	2	**1E18**	0	0,18	15	**1EP18**	0	15
1A19	0	0,39	7	**1B19**	0	0,47	6	**1C19**	0	10 days in herd	**1D19**	0,37	2	**1E19**	0	0,69	15	**1EP19**	0	15
1A20	0	0,17	7	**1B20**	0	0,84	6	**1C20**	0	10 days in herd	**1D20**	0,1	2	**1E20**	0	0,72	13	**1EP20**	0	13
Prevalence	0% (0/20)	60% (12/20)			30% (6/20)	35% (7/20)			0% (0/20)			60% (12/20)			0% (0/20)	55% (11/20)			0% (0/20)	

Herd 2

Sampling date: d. 1/10/19				Sampling date: d. 5/11/19				Sampling date: d. 1/10/19			Sampling date: d. 1/10/19			Sampling date: d. 1/10/19				Sampling date: d. 1/10/19		
Quarantine in	PCR, ct-value	Antibody (S/N)	Days in Q	**Ourantine out**	PCR, ct-value	Antibody (S/N)	Weeks in Q	**Mating unit**	PCR, ct-value	Comments	**Gestation unit**	Antibody (S/N)	WBF	**Farrowing unit**	PCR, ct-value	Antibody (S/N)	DAF	**Piglets, pooled**	PCR, ct-value	Age, days
2A1	0	0,93	12	**2B1**	0	0,6	7	**2C1**	0	14 days in herd	**2D1**	0,2	1	**2E1**	0	0,81	2	**2EP1**	0	2
2A2	0	0,82	12	**2B2**	0	0,99	7	**2C2**	0	14 days in herd	**2D2**	0,39	1	**2E2**	0	0,73	2	**2EP2**	0	2
2A3	0	1,02	12	**2B3**	0	0,67	7	**2C3**	0	14 days in herd	**2D3**	0,89	1	**2E3**	0	0,26	2	**2EP3**	0	2
2A4	0	0,96	12	**2B4**	0	0,52	7	**2C4**	0	14 days in herd	**2D4**	0,38	1	**2E4**	0	0,44	2	**2EP4**	0	2
2A5	0	0,63	12	**2B5**	0	0,81	7	**2C5**	0	14 days in herd	**2D5**	0,16	1	**2E5**	0	0,54	2	**2EP5**	24,42	2
2A6	0	1,01	12	**2B6**	0	1,45	7	**2C6**	0	14 days in herd	**2D6**	0,73	1	**2E6**	0	0,8	7	**2EP6**	0	7
2A7	0	0,73	12	**2B7**	0	0,38	7	**2C7**	0	14 days in herd	**2D7**	0,38	1	**2E7**	0	0,53	9	**2EP7**	0	9
2A8	0	0,96	12	**2B8**	0	0,76	7	**2C8**	0	14 days in herd	**2D8**	0,57	1	**2E8**	0	0,75	6	**2EP8**	0	6
2A9	0	0,95	12	**2B9**	0	0,63	7	**2C9**	0	14 days in herd	**2D9**	0,26	1	**2E9**	0	0,92	10	**2EP9**	0	10
2A10	0	0,28	12	**2B10**	0	0,12	7	**2C10**	0	14 days in herd	**2D10**	0,68	1	**2E10**	0	0,84	14	**2EP10**	-----	------
2A11	0	1,16	12	**2B11**	0	0,48	7	**2C11**	0	14 days in herd	**2D11**	0,6	2	**2E11**	0	0,84	13	**2EP11**	0	13
2A12	0	0,12	12	**2B12**	0	0,79	7	**2C12**	0	14 days in herd	**2D12**	0,5	2	**2E12**	0	0,74	15	**2EP12**	0	15
2A13	0	0,75	12	**2B13**	0	0,68	7	**2C13**	0	14 days in herd	**2D13**	0,54	2	**2E13**	0	0,84	15	**2EP13**	0	15
2A14	0	0,49	12	**2B14**	0	0,83	7	**2C14**	0	14 days in herd	**2D14**	0,13	2	**2E14**	0	0,94	12	**2EP14**	0	12
2A15	0	0,29	12	**2B15**	0	0,21	7	**2C15**	0	14 days in herd	**2D15**	0,61	2	**2E15**	0	0,46	19	**2EP15**	0	19
2A16	0	0,79	12	**2B16**	0	0,38	7	**2C16**	0	14 days in herd	**2D16**	0,59	2	**2E16**	0	0,35	21	**2EP16**	0	21
2A17	0	0,77	12	**2B17**	0	0,79	7	**2C17**	0	14 days in herd	**2D17**	0,22	2	**2E17**	0	0,85	29	**2EP17**	30,78	8
2A18	0	0,18	12	**2B18**	0	0,78	7	**2C18**	0	14 days in herd	**2D18**	0,18	2	**2E18**	0	0,8	17	**2EP18**	0	17
2A19	0	0,55	12	**2B19**	0	0,48	7	**2C19**	0	14 days in herd	**2D19**	0,54	2	**2E19**	0	0,59	17	**2EP19**	0	17
2A20	0	0,41	12	**2B20**	0	0,88	7	**2C20**	0	14 days in herd	**2D20**	0,62	2	**2E20**	0	0,4	17	**2EP20**	0	17
Prevalence	0% (0/20)	35% (7/20)			0% (0/20)	35% (7/20)			0% (0/20)			70% (14/20)			0% (0/20)	40% (8/20)			10,5% (2/19)	

Herd 3

Sampling date: d. 16/10/19				Sampling date: d. 3/10/19				Sampling date: d. 16/10/19			Sampling date: d. 3/10/19				Sampling date: d. 3/10/19				Sampling date: d. 3/10/19		
Quarantine in	PCR, ct-value	Antibody (S/N)	Days in Q	**Ourantine out**	PCR, ct-value	Antibody (S/N)	Weeks in Q	**Mating unit**	PCR, ct-value	Comments	**Gestation unit**	Antibody (S/N)	WBF	**Farrowing unit**	PCR, ct-value	Antibody (S/N)	DAF	**Piglets, pooled**	PCR, ct-value	Age, days
3A1	0	0,49	8	**3B1**	0	0,81	9	**3C1**	0	10 days in herd	**3D1**	0,73	1	**3E1**	0	1,04	5	**3EP1**	0	5
3A2	0	0,55	8	**3B2**	0	0,75	9	**3C2**	0	10 days in herd	**3D2**	0,58	1	**3E2**	0	0,63	2	**3EP2**	0	2
3A3	0	0,51	8	**3B3**	0	0,8	9	**3C3**	0	10 days in herd	**3D3**	0,26	1	**3E3**	0	0,83	5	**3EP3**	0	5
3A4	0	0,87	8	**3B4**	0	0,14	9	**3C4**	0	10 days in herd	**3D4**	0,24	1	**3E4**	0	0,65	5	**3EP4**	0	5
3A5	0	0,55	8	**3B5**	0	0,83	9	**3C5**	0	10 days in herd	**3D5**	0,97	1	**3E5**	0	0,38	5	**3EP5**	0	5
3A6	0	0,17	8	**3B6**	0	0,33	9	**3C6**	0	10 days in herd	**3D6**	0,25	1	**3E6**	0	0,5	5	**3EP6**	0	5
3A7	0	0,38	8	**3B7**	0	0,9	9	**3C7**	0	10 days in herd	**3D7**	0,88	2	**3E7**	0	0,11	5	**3EP7**	0	5
3A8	0	0,14	8	**3B8**	0	0,62	9	**3C8**	0	10 days in herd	**3D8**	0,58	2	**3E8**	0	0,91	5	**3EP8**	0	5
3A9	0	0,78	8	**3B9**	0	0,89	9	**3C9**	0	10 days in herd	**3D9**	0,32	2	**3E9**	0	0,19	5	**3EP9**	0	5
3A10	0	0,65	8	**3B10**	0	0,9	9	**3C10**	0	10 days in herd	**3D10**	0,44	2	**3E10**	0	0,15	5	**3EP10**	0	5
3A11	0	0,33	8	**3B11**	0	0,87	9	**3C11**	0	10 days in herd	**3D11**	0,63	2	**3E11**	0	0,89	7	**3EP11**	0	7
3A12	0	0,79	8	**3B12**	0	0,75	9	**3C12**	0	10 days in herd	**3D12**	0,96	2	**3E12**	0	0,53	7	**3EP12**	0	7
3A13	0	0,26	8	**3B13**	0	0,97	9	**3C13**	0	10 days in herd	**3D13**	0,91	2	**3E13**	0	0,57	10	**3EP13**	0	10
3A14	0	0,19	8	**3B14**	0	0,58	9	**3C14**	0	10 days in herd	**3D14**	0,38	2	**3E14**	0	0,69	10	**3EP14**	0	10
3A15	0	0,71	8	**3B15**	0	0,84	9	**3C15**	0	10 days in herd	**3D15**	1	2	**3E15**	0	0,18	10	**3EP15**	0	10
3A16	0	0,09	8	**3B16**	0	0,87	9	**3C16**	0	10 days in herd	**3D16**	0,52	2	**3E16**	0	0,47	10	**3EP16**	0	10
3A17	0	0,43	8	**3B17**	0	0,8	9	**3C17**	0	10 days in herd	**3D17**	0,96	2	**3E17**	0	0,82	10	**3EP17**	0	10
3A18	0	0,73	8	**3B18**	0	0,62	9	**3C18**	0	10 days in herd	**3D18**	0,86	2	**3E18**	0	0,31	10	**3EP18**	0	10
3A19	0	0,15	8	**3B19**	0	0,97	9	**3C19**	0	10 days in herd	**3D19**	0,42	2	**3E19**	0	1	8	**3EP19**	0	8
3A20	0	0,29	8	**3B20**	0	0,91	9	**3C20**	0	10 days in herd	**3D20**	0,39	2	**3E20**	0	0,74	10	**3EP20**	0	10
Prevalence	0% (0/20)	70% (14/20)			0% (0/20)	25% (5/20)			0% (0/20)			55% (11/20)			0% (0/20)	50% (10/20)			0% (0/20)	

Herd 4

Sampling date: d. 8/10/19				Sampling date: d. 18/11/19				Sampling date: d. 8/10/19			Sampling date: d. 8/10/19			Sampling date: d. 8/10/19				Sampling date: d. 8/10/19		
Quarantine in	PCR, ct-value	Antibody (S/N)	Days in Q	**Ourantine out**	PCR, ct-value	Antibody (S/N)	Weeks in Q	**Mating unit**	PCR, ct-value	Comments	**Gestation unit**	Antibody (S/N)	WBF	**Farrowing unit**	PCR, ct-value	Antibody (S/N)	DAF	**Piglets, pooled**	PCR, ct-value	Age, days
4A1	0	0,91	5	**4B1**	0	0,63	6	**4C1**	0	6 days in herd	**4D1**	0,64	1	**4E1**	0	0,9	3	**4EP1**	0	3
4A2	0	0,17	5	**4B2**	0	0,12	6	**4C2**	0	6 days in herd	**4D2**	0,43	1	**4E2**	0	0,74	3	**4EP2**	0	3
4A3	0	0,98	5	**4B3**	33,75	0,47	6	**4C3**	0	6 days in herd	**4D3**	0,33	1	**4E3**	0	0,59	3	**4EP3**	0	3
4A4	0	0,98	5	**4B4**	0	0,61	6	**4C4**	0	6 days in herd	**4D4**	0,67	1	**4E4**	0	0,74	3	**4EP4**	0	3
4A5	0	0,53	5	**4B5**	0	0,34	6	**4C5**	0	6 days in herd	**4D5**	0,75	1	**4E5**	0	0,31	3	**4EP5**	0	3
4A6	0	0,84	5	**4B6**	0	0,86	6	**4C6**	0	6 days in herd	**4D6**	0,45	1	**4E6**	0	0,26	3	**4EP6**	22,71	3
4A7	0	0,93	5	**4B7**	34,04	0,54	6	**4C7**	0	6 days in herd	**4D7**	0,34	1	**4E7**	0	0,16	3	**4EP7**	0	3
4A8	0	1,05	5	**4B8**	0	0,09	6	**4C8**	0	6 days in herd	**4D8**	0,44	1	**4E8**	0	0,81	5	**4EP8**	0	5
4A9	0	0,64	5	**4B9**	0	0,1	6	**4C9**	0	6 days in herd	**4D9**	0,75	1	**4E9**	0	0,61	5	**4EP9**	0	5
4A10	0	0,76	5	**4B10**	0	0,33	6	**4C10**	0	6 days in herd	**4D10**	0,73	1	**4E10**	0	0,38	12	**4EP10**	0	12
4A11	0	0,94	5	**4B11**	0	0,14	6	**4C11**	0	6 days in herd	**4D11**	0,52	1	**4E11**	0	0,77	12	**4EP11**	24,39	12
4A12	0	1,04	5	**4B12**	0	0,3	6	**4C12**	0	6 days in herd	**4D12**	0,3	2	**4E12**	0	0,28	10	**4EP12**	24,73	10
4A13	0	0,66	5	**4B13**	35,22	0,14	6	**4C13**	0	6 days in herd	**4D13**	0,64	2	**4E13**	0	0,75	13	**4EP13**	23,82	13
4A14	0	0,47	5	**4B14**	0	0,12	6	**4C14**	0	6 days in herd	**4D14**	0,29	2	**4E14**	0	0,59	14	**4EP14**	25,46	14
4A15	0	0,45	5	**4B15**	0	0,35	6	**4C15**	0	6 days in herd	**4D15**	0,51	2	**4E15**	0	0,65	12	**4EP15**	0	12
4A16	0	0,91	5	**4B16**	0	0,09	6	**4C16**	0	6 days in herd	**4D16**	0,57	2	**4E16**	0	0,88	14	**4EP16**	0	14
4A17	0	0,57	5	**4B17**	0	0,09	6	**4C17**	0	6 days in herd	**4D17**	0,46	2	**4E17**	0	0,68	12	**4EP17**	0	12
4A18	0	0,89	5	**4B18**	0	0,16	6	**4C18**	0	6 days in herd	**4D18**	0,68	2	**4E18**	0	0,17	14	**4EP18**	26,81	14
4A19	0	0,77	5	**4B19**	33,07	0,16	6	**4C19**	0	6 days in herd	**4D19**	0,45	2	**4E19**	0	0,48	16	**4EP19**	0	16
4A20	0	0,86	5	**4B20**	0	0,23	6	**4C20**	0	6 days in herd	**4D20**	0,52	2	**4E20**	0	0,65	16	**4EP20**	23,61	16
Prevalence	0% (0/20)	25% (5/20)			20% (4/20)	85% (17/20)			0% (0/20)			65% (13/20)			0% (0/20)	45% (9/20)			35% (7/20)	

Herd 5

Sampling date: d. 1/11/19				Sampling date: d. 10/10/19				Sampling date: d. 1/11/19			Sampling date: d. 1/11/19			Sampling date: d. 1/11/19				Sampling date: d. 1/11/19		
Quarantine in	PCR, ct-value	Antibody (S/N)	Days in Q	**Ourantine out**	PCR, ct-value	Antibody (S/N)	Weeks in Q	**Mating unit**	PCR, ct-value	Comments	**Gestation unit**	Antibody (S/N)	WBF	**Farrowing unit**	PCR, ct-value	Antibody (S/N)	DAF	**Piglets, pooled**	PCR, ct-value	Age, days
5A1	0	0,46	14	**5B1**	0	0,22	8	**5C1**	0	20 days in herd	**5D1**	0,13	1 WBF	**5E1**	0	0,43	5	**5EP1**	0	5
5A2	0	0,38	14	**5B2**	0	0,23	8	**5C2**	0	20 days in herd	**5D2**	0,1	1 WBF	**5E2**	0	0,17	5	**5EP2**	0	5
5A3	0	0,64	14	**5B3**	0	0,13	8	**5C3**	0	20 days in herd	**5D3**	0,27	1 WBF	**5E3**	0	0,2	5	**5EP3**	0	5
5A4	0	0,37	14	**5B4**	0	0,26	8	**5C4**	0	20 days in herd	**5D4**	0,07	1 WBF	**5E4**	0	0,12	5	**5EP4**	0	5
5A5	0	0,3	14	**5B5**	0	0,44	8	**5C5**	0	20 days in herd	**5D5**	0,33	1 WBF	**5E5**	0	0,2	6	**5EP5**	0	6
5A6	0	0,27	14	**5B6**	0	0,37	8	**5C6**	0	20 days in herd	**5D6**	3,14	2 WBF	**5E6**	0	0,27	6	**5EP6**	0	6
5A7	0	0,17	14	**5B7**	0	0,16	8	**5C7**	0	20 days in herd	**5D7**	0,27	2 WBF	**5E7**	0	0,2	5	**5EP7**	0	5
5A8	0	0,25	14	**5B8**	0	0,22	8	**5C8**	0	20 days in herd	**5D8**	0,15	2 WBF	**5E8**	0	0,1	2	**5EP8**	0	2
5A9	0	0,51	14	**5B9**	0	0,13	8	**5C9**	0	20 days in herd	**5D9**	0,12	2 WBF	**5E9**	0	0,21	19	**5EP9**	29,99	19
5A10	0	0,47	14	**5B10**	0	0,17	8	**5C10**	0	20 days in herd	**5D10**	0,09	2 WBF	**5E10**	0	0,24	5	**5EP10**	0	5
5A11	0	0,23	14	**5B11**	0	0,17	8	**5C11**	0	20 days in herd	**5D11**	0,43	2 WBF	**5E11**	0	0,42	8	**5EP11**	0	8
5A12	0	0,33	14	**5B12**	0	0,21	8	**5C12**	0	20 days in herd	**5D12**	0,2	2 WBF	**5E12**	0	0,18	7	**5EP12**	0	7
5A13	0	0,38	14	**5B13**	0	0,11	8	**5C13**	0	20 days in herd	**5D13**	0,33	2 WBF	**5E13**	0	0,33	12	**5EP13**	0	12
5A14	0	0,15	14	**5B14**	0	0,11	8	**5C14**	0	20 days in herd	**5D14**	0,14	2 WBF	**5E14**	0	0,25	7	**5EP14**	0	7
5A15	0	0,17	14	**5B15**	0	0,16	8	**5C15**	0	20 days in herd	**5D15**	0,16	3 WBF	**5E15**	0	0,21	13	**5EP15**	0	13
5A16	0	0,64	14	**5B16**	0	0,11	8	**5C16**	0	20 days in herd	**5D16**	0,16	3 WBF	**5E16**	0	0,25	16	**5EP16**	31,95	16
5A17	0	0,09	14	**5B17**	0	0,24	8	**5C17**	0	20 days in herd	**5D17**	0,08	3 WBF	**5E17**	0	0,24	7	**5EP17**	23,67	7
5A18	0	0,08	14	**5B18**	0	0,13	8	**5C18**	0	20 days in herd	**5D18**	0,09	3 WBF	**5E18**	0	0,09	14	**5EP18**	0	14
5A19	0	0,15	14	**5B19**	0	0,1	8	**5C19**	0	20 days in herd	**5D19**	0,09	3 WBF	**5E19**	0	0,17	13	**5EP19**	0	13
5A20	0	0,17	14	**5B20**	0	0,14	8	**5C20**	0	20 days in herd	**5D20**	0,07	3 WBF	**5E20**	0	0,47	11	**5EP20**	0	11
Prevalence	0% (0/20)	95% (19/20)			0% (0/20)	100% (20/20)			0% (0/20)			95% (19/20)			0% (0/20)	100% (20/20)			15% (3/20)	

Herd 6

Sampling date: d. 21/10/19				Sampling date d. 25/9/19				Sampling date: d. 21/10/19			Sampling date: d. 21/10/19			Sampling date: d. 21/10/19				Sampling date: d. 21/10/19		
Quarantine in	PCR, ct-value	Antibody (S/N)	Days in Q	**Ourantine out**	PCR, ct-value	Antibody (S/N)	Weeks in Q	**Mating unit**	PCR, ct-value	Comment	**Gestation unit**	Antibody (S/N)	WBF	**Farrowing unit**	PCR, ct-value	Antibody positiv	DAF	**Piglets, pooled**	PCR, ct-value	Age, days
6A1	0	0,94	13	**6B1**	32,27	0,53	9	**6C1**	32,76	another site	**6D1**	0,13	1	**6E1**	0	0,48	5	**6EP1**	0	5
6A2	0	1,07	13	**6B2**	0	0,1	9	**6C2**	0	another site	**6D2**	0,3	1	**6E2**	0	0,14	4	**6EP2**	0	4
6A3	30,43	0,9	13	**6B3**	0	0,6	9	**6C3**	0	another site	**6D3**	0,1	1	**6E3**	0	0,27	4	**6EP3**	0	4
6A4	0	0,86	13	**6B4**	0	0,69	9	**6C4**	0	another site	**6D4**	0,21	1	**6E4**	0	0,12	6	**6EP4**	0	6
6A5	31,2	0,39	13	**6B5**	0	0,46	9	**6C5**	0	another site	**6D5**	0,08	1	**6E5**	0	0,49	11	**6EP5**	0	3
6A6	31,21	0,84	13	**6B6**	0	0,65	9	**6C6**	0	another site	**6D6**	0,11	1	**6E6**	0	0,52	5	**6EP6**	0	5
6A7	0	0,66	13	**6B7**	0	0,67	9	**6C7**	0	another site	**6D7**	0,2	1	**6E7**	0	0,48	7	**6EP7**	0	7
6A8	31,07	0,9	13	**6B8**	31,78	0,59	9	**6C8**	0	another site	**6D8**	0,09	1	**6E8**	0	0,5	6	**6EP8**	0	6
6A9	29,59	1,02	13	**6B9**	0	0,62	9	**6C9**	0	another site	**6D9**	0,24	1	**6E9**	0	0,13	7	**6EP9**	0	7
6A10	29,85	0,95	13	**6B10**	0	1,54	9	**6C10**	0	another site	**6D10**	0,14	1	**6E10**	0	0,09	4	**6EP10**	0	4
6A11	0	0,74	13	**6B11**	0	0,65	9	**6C11**	0	another site	**6D11**	0,14	1	**6E11**	0	0,16	5	**6EP11**	0	5
6A12	0	0,52	13	**6B12**	0	0,78	9	**6C12**	0	another site	**6D12**	0,11	1	**6E12**	0	0,57	6	**6EP12**	0	6
6A13	30,9	0,89	13	**6B13**	0	0,75	9	**6C13**	0	another site	**6D13**	0,12	1	**6E13**	0	0,08	6	**6EP13**	0	6
6A14	0	0,53	13	**6B14**	0	0,77	9	**6C14**	0	another site	**6D14**	0,25	1	**6E14**	0	0,11	3	**6EP14**	0	3
6A15	0	0,91	13	**6B15**	0	0,96	9	**6C15**	0	another site	**6D15**	0,17	1	**6E15**	0	0,07	7	**6EP15**	0	7
6A16	30,66	0,99	13	**6B16**	0	0,96	9	**6C16**	0	another site	**6D16**	0,11	1	**6E16**	0	0,16	5	**6EP16**	0	5
6A17	29,71	0,89	13	**6B17**	0	0,64	9	**6C17**	0	another site	**6D17**	0,15	1	**6E17**	0	0,3	5	**6EP17**	0	5
6A18	0	0,96	13	**6B18**	0	0,76	9	**6C18**	0	another site	**6D18**	0,15	1	**6E18**	0	0,08	4	**6EP18**	0	4
6A19	0	1,37	13	**6B19**	31,18	0,9	9	**6C19**	0	another site	**6D19**	0,3	1	**6E19**	0	0,07	3	**6EP19**	0	3
6A20	32,12	0,84	13	**6B20**	0	0,47	9	**6C20**	0	another site	**6D20**	0,14	1	**6E20**	0	0,1	4	**6EP20**	0	4
Prevalence	50% (10/20)	15% (3/20)			15% (3/20)	25% (5/20)			5% (1/20)			100% (20/20)			0% (0/20)	100% (20/20)			0% (0/20)	

Herd 7

Sampling date: d. 9/10/19				Sampling date: d. 9/10/19				Sampling date: d. 9/10/19				Sampling date: d. 9/10/19				Sampling date: d. 9/10/19			
Quarantine in	PCR, ct-value	Antibody (S:N)	Days in Q	**Ourantine out**	PCR, ct-value	Antibody (S:N)	Weeks in Q	**Mating unit**	PCR, ct-value	Comment		**Gestation unit**	Antibody (S:N)	WBF		**Farrowing unit**	PCR, ct-value	Antibody (S:N)	DAF	**Piglets, pooled**	PCR, ct-value	Age, days
7A1	0	0,87	14	**7B1**	0	0,15	10	**7C1**	0	7 days in herd		**7D1**	0,2	1		**7E1**	0	0,1	24	**7EP1**	0	6
7A2	0	0,99	14	**7B2**	0	0,41	10	**7C2**	0	7 days in herd		**7D2**	0,27	1		**7E2**	0	0,24	5	**7EP2**	0	5
7A3	0	0,88	14	**7B3**	0	0,57	10	**7C3**	0	7 days in herd		**7D3**	0,13	1		**7E3**	0	0,15	2	**7EP3**	0	2
7A4	0	1,03	14	**7B4**	0	0,12	10	**7C4**	0	7 days in herd		**7D4**	0,2	1		**7E4**	0	0,17	6	**7EP4**	0	6
7A5	0	0,9	14	**7B5**	0	0,2	10	**7C5**	0	7 days in herd		**7D5**	0,16	1		**7E5**	0	0,34	3	**7EP5**	0	3
7A6	0	0,98	14	**7B6**	0	0,64	10	**7C6**	33,34	7 days in herd		**7D6**	0,25	1		**7E6**	33,07	0,56	3	**7EP6**	0	3
7A7	0	0,96	14	**7B7**	0	0,15	10	**7C7**	0	7 days in herd		**7D7**	0,76	1		**7E7**	0	0,17	2	**7EP7**	0	2
7A8	0	0,87	14	**7B8**	0	0,46	10	**7C8**	0	7 days in herd		**7D8**	0,24	1		**7E8**	0	0,26	2	**7EP8**	0	2
7A9	0	0,79	14	**7B9**	0	0,4	10	**7C9**	0	7 days in herd		**7D9**	0,68	1		**7E9**	0	0,76	4	**7EP9**	0	4
7A10	0	0,9	14	**7B10**	0	0,51	10	**7C10**	0	7 days in herd		**7D10**	0,58	1		**7E10**	0	0,52	5	**7EP10**	0	5
7A11	0	0,81	14	**7B11**	0	0,56	10	**7C11**	0	7 days in herd		**7D11**	0,51	1		**7E11**	0	0,21	3	**7EP11**	0	3
7A12	0	0,97	14	**7B12**	0	0,19	10	**7C12**	0	7 days in herd		**7D12**	0,19	1		**7E12**	0	0,26	5	**7EP12**	0	5
7A13	0	0,89	14	**7B13**	0	0,27	10	**7C13**	0	7 days in herd		**7D13**	0,62	1		**7E13**	0	0,33	3	**7EP13**	0	3
7A14	0	0,76	14	**7B14**	0	0,76	10	**7C14**	0	7 days in herd		**7D14**	0,1	1		**7E14**	0	0,12	4	**7EP14**	0	4
7A15	0	0,91	14	**7B15**	0	0,23	10	**7C15**	0	7 days in herd		**7D15**	0,28	1		**7E15**	0	0,54	4	**7EP15**	0	4
7A16	0	0,78	14	**7B16**	0	0,74	10	**7C16**	0	7 days in herd		**7D16**	0,3	1		**7E16**	0	0,19	6	**7EP16**	0	6
7A17	0	0,94	14	**7B17**	0	0,24	10	**7C17**	0	7 days in herd		**7D17**	0,76	1		**7E17**	0	0,76	7	**7EP17**	0	7
7A18	0	1,02	14	**7B18**	0	0,13	10	**7C18**	0	7 days in herd		**7D18**	0,58	1		**7E18**	0	0,39	7	**7EP18**	0	7
7A19	0	0,87	14	**7B19**	0	0,27	10	**7C19**	0	14 days in herd		**7D19**	0,38	1		**7E19**	0	0,92	29	**7EP19**	0	7
7A20	0	0,9	14	**7B20**	0	0,23	10	**7C20**	0	14 days in herd		**7D20**	0,33	2		**7E20**	0	0,3	30	**7EP20**	0	6
Prevalence	0% (0/20)	0% (0/20)			0% (0/20)	85% (17/20)			5% (1/20)				80% (16/20)				5% (1/20)	85% (17/20)			0% (0/20)	

Herd 8

Sampling date: d. 11/11/19				Sampling date: d. 18/10/19				Sampling date: d. 29/10/19				Sampling date: d. 29/10/19				Sampling date: d. 29/10/19				Sampling date		
Quarantine in	PCR, ct-value	Antibody (S:N)	Days in Q	**Ourantine out**	PCR, ct-value	Antibody (S:N)	Weeks in Q	**Mating unit**	PCR, ct-value	Comment		**Gestation unit**	Antibody (S:N)	WBF		**Farrowing unit**	PCR, ct-value	Antibody (S:N)	DAF	**Piglets, pooled**	PCR, ct-value	Age, days
8A1	0	0,8	3	**8B1**		33,99	0,23	7	**8C1**	0	another site	**8D1**	0,07	1		**8E1**	0	0,09	8	**8EP1**	0	8
8A2	0	0,32	3	**8B2**		33,54	0,12	7	**8C2**	0	another site	**8D2**	0,08	1		**8E2**	0	0,09	5	**8EP2**	0	5
8A3	0	0,78	3	**8B3**		0	0,09	7	**8C3**	0	another site	**8D3**	0,07	1		**8E3**	0	0,06	9	**8EP3**	0	9
8A4	0	0,19	3	**8B4**		0	0,14	7	**8C4**	0	another site	**8D4**	0,14	1		**8E4**	0	0,11	6	**8EP4**	0	6
8A5	0	0,75	3	**8B5**		34,43	0,12	7	**8C5**	0	another site	**8D5**	0,07	1		**8E5**	0	0,09	8	**8EP5**	0	8
8A6	0	0,32	3	**8B6**		0	0,21	7	**8C6**	0	another site	**8D6**	0,1	2		**8E6**	0	0,08	8	**8EP6**	0	8
8A7	0	0,75	3	**8B7**		0	0,3	7	**8C7**	0	another site	**8D7**	0,09	2		**8E7**	0	0,08	9	**8EP7**	0	9
8A8	0	0,1	3	**8B8**		0	0,11	7	**8C8**	0	another site	**8D8**	0,08	2		**8E8**	0	0,09	2	**8EP8**	0	2
8A9	0	0,7	3	**8B9**		0	0,17	7	**8C9**	0	another site	**8D9**	0,26	2		**8E9**	0	0,12	2	**8EP9**	0	2
8A10	0	0,12	3	**8B10**		33,82	0,11	7	**8C10**	0	another site	**8D10**	0,13	2		**8E10**	0	0,09	2	**8EP10**	0	2
8A11	0	0,07	3	**8B11**		0	0,1	7	**8C11**	0	another site	**8D11**	0,08	2		**8E11**	0	0,09	2	**8EP11**	0	2
8A12	0	0,56	3	**8B12**		35,07	0,16	7	**8C12**	0	another site	**8D12**	0,06	3		**8E12**	0	0,07	10	**8EP12**	0	10
8A13	0	0,27	3	**8B13**		0	0,47	7	**8C13**	0	another site	**8D13**	0,17	3		**8E13**	0	0,05	11	**8EP13**	0	11
8A14	0	0,52	3	**8B14**		34,79	0,12	7	**8C14**	0	another site	**8D14**	0,09	3		**8E14**	0	0,1	14	**8EP14**	0	14
8A15	0	0,21	3	**8B15**		0	0,35	7	**8C15**	0	another site	**8D15**	0,1	3		**8E15**	0	0,06	14	**8EP15**	0	14
8A16	0	0,46	3	**8B16**		0	0,2	7	**8C16**	35,27	another site	**8D16**	0,07	3		**8E16**	0	0,07	17	**8EP16**	0	17
8A17	0	0,32	3	**8B17**		34,48	0,16	7	**8C17**	0	another site	**8D17**	0,23	3		**8E17**	0	0,13	15	**8EP17**	34,9	15
8A18	0	0,56	3	**8B18**		0	0,15	7	**8C18**	0	another site	**8D18**	0,07	3		**8E18**	0	0,09	15	**8EP18**	0	15
8A19	0	0,66	3	**8B19**		33,91	0,12	7	**8C19**	0	another site	**8D19**	0,09	3		**8E19**	0	0,1	15	**8EP19**	0	15
8A20	0	0,2	3	**8B20**		0	0,11	7	**8C20**	0	another site	**8D20**	0,08	3		**8E20**	0	0,06	1	**8EP20**	0	1
Prevalence	0% (0/20)	70% (14/20)			40% (8/20)	100% (20/20)			5% (1/20)				100% (20/20)				0% (0/20)	100% (20/20)			5% (1/20)	

Herd 9

Sampling date: d. 28/10/19				Sampling date: d. 28/10/19				Sampling date: d. 28/10/19			Sampling date: d. 28/10/19			Sampling date: d. 28/10/19				Sampling date: d. 28/10/19		
Quarantine in	PCR, ct-value	Antibody positiv	days in Q	**Ourantine out**	PCR, ct-value	Antibody (S/N)	Weeks in Q	**Mating unit**	PCR, ct-value	Comment	**Gestation unit**	Antibody (S/N)	WBF	**Farrowing unit**	PCR, ct-value	Antibody (S/N)	DAF	**Piglets, pooled**	PCR, ct-value	Age, days
9A1	0	0,72	5	**9B1**	0	0,74	12	**9C1**	0	mating unit	**9D1**	0,14	1	**9E1**	0	0,13	4	**9EP1**	0	4
9A2	0	0,45	5	**9B2**	0	0,66	12	**9C2**	0	mating unit	**9D2**	0,36	1	**9E2**	0	0,23	4	**9EP2**	0	4
9A3	0	0,95	5	**9B3**	0	0,08	12	**9C3**	0	mating unit	**9D3**	0,17	1	**9E3**	0	0,34	3	**9EP3**	0	3
9A4	0	0,59	5	**9B4**	0	0,8	12	**9C4**	0	mating unit	**9D4**	0,1	1	**9E4**	0	0,1	2	**9EP4**	0	2
9A5	0	0,15	5	**9B5**	0	0,57	12	**9C5**	0	mating unit	**9D5**	0,16	1	**9E5**	0	0,12	6	**9EP5**	0	6
9A6	0	0,25	5	**9B6**	34,42	0,8	12	**9C6**	0	mating unit	**9D6**	0,21	2	**9E6**	0	0,13	7	**9EP6**	0	7
9A7	35,13	0,48	5	**9B7**	0	0,67	12	**9C7**	0	gestation unit	**9D7**	0,43	2	**9E7**	0	0,41	-	**9EP7**	0	6
9A8	0	0,15	5	**9B8**	0	0,85	12	**9C8**	0	gestation unit	**9D8**	0,1	2	**9E8**	0	0,5	-	**9EP8**	0	7
9A9	0	0,11	5	**9B9**	0	0,76	12	**9C9**	0	gestation unit	**9D9**	0,23	2	**9E9**	0	0,08	-	**9EP9**	0	7
9A10	0	0,48	5	**9B10**	0	0,77	12	**9C10**	0	gestation unit	**9D10**	0,25	2	**9E10**	0	0,12	14	**9EP10**	0	14
9A11	0	0,46	5	**9B11**	0	0,33	12	**9C11**	0	gestation unit	**9D11**	0,08	2	**9E11**	0	0,13	13	**9EP11**	0	13
9A12	0	0,13	5	**9B12**	0	0,62	12	**9C12**	0	gestation unit	**9D12**	0,11	3	**9E12**	0	0,08	9	**9EP12**	28,7	9
9A13	35,05	0,67	5	**9B13**	0	0,79	12	**9C13**	0	gestation unit	**9D13**	0,24	3	**9E13**	33,51	0,06	8	**9EP13**	0	8
9A14	0	0,85	5	**9B14**	0	0,64	12	**9C14**	0	gilt unit	**9D14**	0,24	3	**9E14**	0	0,1	8	**9EP14**	0	8
9A15	0	0,82	5	**9B15**	0	0,77	12	**9C15**	0	gilt unit	**9D15**	0,43	3	**9E15**	0	0,12	-	**9EP15**	0	21
9A16	0	0,66	5	**9B16**	0	0,77	12	**9C16**	0	gilt unit	**9D16**	0,2	3	**9E16**	0	0,12	-	**9EP16**	0	21
9A17	0	0,54	5	**9B17**	0	0,16	12	**9C17**	0	gilt unit	**9D17**	0,23	3	**9E17**	0	0,2	23	**9EP17**	0	23
9A18	0	0,52	5	**9B18**	0	0,74	12	**9C18**	0	gilt unit	**9D18**	0,1	3	**9E18**	0	0,45	-	**9EP18**	0	14
9A19	0	0,57	5	**9B19**	0	0,67	12	**9C19**	0	gilt unit	**9D19**	0,32	3	**9E19**	0	0,1	-	**9EP19**	0	14
9A20	0	0,74	5	**9B20**	0	0,69	12	**9C20**	0	gilt unit	**9D20**	0,31	3	**9E20**	0	0,1	-	**9EP20**	28,95	14
Prevalence	10% (2/20)	65% (13/20)			5% (1/20)	20% (4/20)			0% (0/20)			100% (20/20)			5% (1/20)	100% (20/20)			10% (2/20)	

Herd 10

Sampling date: d. 4/11/19				Sampling date: d. 4/11/19				Sampling date: d. 4/11/19			Sampling date: d. 4/11/19			Sampling date: d. 4/11/19				Sampling date		
Quarantine in	PCR, ct-value	Antibody (S/N)	Days in Q	**Ourantine out**	PCR, ct-value	Antibody (S/N)	Weeks in Q	**Mating unit**	PCR, ct-value	Comment	**Gestation unit**	Antibody (S/N)	WBF	**Farrowing unit**	PCR, ct-value	Antibody (S/N)	DAF	**Piglets, pooled**	PCR, ct-value	Age, days
10A1	0	0,85	7	**10B1**	0	0,09	10	**10C1**	0	7 days in herd	**10D1**	0,36	1	**10E1**	0	0,16	1	**10EP1**	33,01	1
10A2	0	0,1	7	**10B2**	0	0,08	10	**10C2**	0	7 days in herd	**10D2**	0,1	1	**10E2**	0	0,15	1	**10EP2**	0	1
10A3	0	0,83	7	**10B3**	0	0,07	10	**10C3**	0	7 days in herd	**10D3**	0,62	1	**10E3**	0	0,29	1	**10EP3**	0	1
10A4	0	0,73	7	**10B4**	0	0,1	10	**10C4**	0	7 days in herd	**10D4**	0,7	1	**10E4**	0	0,42	2	**10EP4**	0	2
10A5	0	0,7	7	**10B5**	0	0,22	10	**10C5**	0	7 days in herd	**10D5**	0,1	1	**10E5**	0	0,33	12	**10EP5**	0	12
10A6	0	0,57	7	**10B6**	0	0,45	10	**10C6**	0	7 days in herd	**10D6**	0,62	1	**10E6**	0	0,11	16	**10EP6**	27,6	16
10A7	0	0,23	7	**10B7**	0	0,1	10	**10C7**	0	7 days in herd	**10D7**	0,14	1	**10E7**	0	0,69	15	**10EP7**	0	15
10A8	0	0,35	7	**10B8**	0	0,24	10	**10C8**	0	7 days in herd	**10D8**	0,3	1	**10E8**	0	0,14	15	**10EP8**	0	15
10A9	0	0,24	7	**10B9**	0	0,16	10	**10C9**	0	7 days in herd	**10D9**	0,15	2	**10E9**	0	0,15	16	**10EP9**	0	16
10A10	0	0,2	7	**10B10**	0	0,1	10	**10C10**	33,82	7 days in herd	**10D10**	0,31	2	**10E10**	34,63	0,1	16	**10EP10**	27,2	16
10A11	0	0,73	7	**10B11**	0	0,08	10	**10C11**	0	7 days in herd	**10D11**	0,2	2	**10E11**	0	0,2	15	**10EP11**	29,65	15
10A12	0	0,48	7	**10B12**	0	0,08	10	**10C12**	0	7 days in herd	**10D12**	0,57	2	**10E12**	0	0,07	16	**10EP12**	22,26	16
10A13	0	0,87	7	**10B13**	0	0,09	10	**10C13**	0	7 days in herd	**10D13**	0,24	2	**10E13**	0	0,14	5	**10EP13**	27,67	5
10A14	0	0,33	7	**10B14**	0	0,08	10	**10C14**	0	7 days in herd	**10D14**	0,31	2	**10E14**	0	0,42	11	**10EP14**	28,2	11
10A15	0	0,07	7	**10B15**	0	0,09	10	**10C15**	0	7 days in herd	**10D15**	0,5	3	**10E15**	0	0,34	9	**10EP15**	0	9
10A16	0	0,66	7	**10B16**	0	0,13	10	**10C16**	0	7 days in herd	**10D16**	0,58	3	**10E16**	0	0,85	7	**10EP16**	33,05	7
10A17	0	0,08	7	**10B17**	0	0,29	10	**10C17**	0	7 days in herd	**10D17**	0,38	3	**10E17**	0	0,57	3	**10EP17**	0	3
10A18	0	0,22	7	**10B18**	0	0,29	10	**10C18**	0	14 days in herd	**10D18**	0,43	3	**10E18**	0	0,4	6	**10EP18**	0	6
10A19	0	0,87	7	**10B19**	31,98	0,25	10	**10C19**	0	14 days in herd	**10D19**	0,15	3	**10E19**	33,81	0,1	15	**10EP19**	33,01	15
10A20	0	0,72	7	**10B20**	0	0,09	10	**10C20**	0	14 days in herd	**10D20**	0,65	3	**10E20**	0	0,16	6	**10EP20**	0	6
Prevalence	0% (0/20)	55% (11/20)			5% (1/20)	100% (20/20)			5% (1/20)			85% (17/20)			10% (2/20)	90% (18/20)			45%% (9/20)	

Appendix 8 - Mann Whitney U test, Sampling time differences

Mann-Whitney test	
Table Analyzed	Data 8
Column B	Days in Q v
vs.	vs.
Column A	uv Days in Q
Mann Whitney test	
P value	0.0487
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	10850 , 9250
Mann-Whitney U	4200
Difference between medians	
Median of column A	8.000, n=100
Median of column B	7.000, n=100
Difference: Actual	-1.000
Difference: Hodges-Lehmann	-1.000

Mann-Whitney test	
Table Analyzed	Data 8
Column D	v Weeks in Q
vs.	vs.
Column C	Weeks in Q uv
Mann Whitney test	
P value	<0.0001
Exact or approximate P value?	Exact
P value summary	****
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column C,D	6250 , 13850
Mann-Whitney U	1200
Difference between medians	
Median of column C	7.000, n=100
Median of column D	10.00, n=100
Difference: Actual	3.000
Difference: Hodges-Lehmann	3.000

pboard (Ctrl+C) Mann-Whitney test

Table Analyzed	Data 8
Column F	v days in sow herd
vs.	vs.
Column E	uv days in sow herd
Mann Whitney test	
P value	<0.0001
Exact or approximate P value?	Exact
P value summary	****
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column E,F	12800 , 7300
Mann-Whitney U	2250
Difference between medians	
Median of column E	10.00, n=100
Median of column F	7.000, n=100
Difference: Actual	-3.000
Difference: Hodges-Lehmann	-6.500

Mann-Whitney test	
Table Analyzed	Data 8
Column L	v piglets age days
vs.	vs.
Column K	uv piglets age days
Mann Whitney test	
P value	>0.9999
Exact or approximate P value?	Exact
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
Sum of ranks in column K,L	10050 , 10050
Mann-Whitney U	5000
Difference between medians	
Median of column K	6.000, n=100
Median of column L	6.000, n=100
Difference: Actual	0.000
Difference: Hodges-Lehmann	0.000

	Mann-Whitney test	
1	Table Analyzed	Data 8
2		
3	Column J	v days after farrowing
4	vs.	vs.
5	Column I	uv days after farrowing
6		
7	**Mann Whitney test**	
8	P value	0.1752
9	Exact or approximate P value?	Exact
10	P value summary	ns
11	Significantly different (P < 0.05)?	No
12	One- or two-tailed P value?	Two-tailed
13	Sum of ranks in column I,J	10603 , 9497
14	Mann-Whitney U	4447
15		
16	**Difference between medians**	
17	Median of column I	7.000, n=100
18	Median of column J	6.000, n=100
19	Difference: Actual	-1.000
20	Difference: Hodges-Lehmann	-1.000
21		

	Mann-Whitney test	
1	Table Analyzed	Data 8
2		
3	Column H	v weeks before farrowing
4	vs.	vs.
5	Column G	uv weeks before farrowing
6		
7	**Mann Whitney test**	
8	P value	0.6976
9	Exact or approximate P value?	Exact
10	P value summary	ns
11	Significantly different (P < 0.05)?	No
12	One- or two-tailed P value?	Two-tailed
13	Sum of ranks in column G,H	10195 , 9905
14	Mann-Whitney U	4855
15		
16	**Difference between medians**	
17	Median of column G	2.000, n=100
18	Median of column H	1.000, n=100
19	Difference: Actual	-1.000
20	Difference: Hodges-Lehmann	0.000

Appendix 9 - Quarantine measures

Quarantine management and biosecurity measures. An overview of management and biosecurity measures of the quarantine in unvaccinated and vaccinated herds.

Quarantine management and biosecurity measures	Unvaccinated Herds					Vaccinated Herds				
	Herd 1	Herd 2	Herd 3	Herd 4	Herd 5	Herd 6	Herd 7	Herd 8	Herd 9	Herd 10
Influenza vaccination of staff members?	4 vacc. / 3 no vacc.	No	No	No	No	No	No	No	No	No
Vaccination of gilts against Influenza A before arrival?	No	No	No	No	No	No	No	No	No	No
The number of quarantines and sections.	1 quarantine/ 1 section	1 quarantine/ 2 sections	1 quarantine/ 2 sections	1 quarantine/ 1 section	1 quarantine/ 1 section	1 quarantine/ 1 section	2 quarantines/ 2 sections	1 quarantine/ 1 section	1 quarantines/ 1 section	2 quarantines/ 1 section
Is there seperate entrance to the quaratine?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
Is the quarantine placed on aother site away from the sow herd?	No	Yes 2 km	No	No	No	Yes, 10 km	Yes, 4-6 km	Yes, 400 m	No	No
Is the quarantine completely emty of animals before a new batch?	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
Is the quarantine emty between batches?	Yes, 3 days	Yes, 0-14 days	Yes, 2-10 days	No	Yes, 1- 14 days	Yes, 3-14 days	Yes, 7 days	Yes, 1-7 days	Yes, 5 days	Yes, 5-10 days
Is the quarantine washed and dried between batches?	Yes	No	No	No	Yes	Yes	No	Yes	Yes	Yes
Are all gilts moved from the quarantine at the same time?	No	Yes	Yes	No	Yes	Yes	No	Yes	Yes	No
How long is the quarantine-time?	8-10 weeks	6 weeks	8-9 weeks	6 weeks	6-8 weeks	8-10 weeks	6 weeks	8 weeks	11 weeks	5-7 weeks
In a working day, when are the quarantine gilts inspected?	afternoon	afternoon	afternoon	afternoon	afternoon	afternoon	afternoon	afternoon	afternoon	afternoon
Any restrictions for the personnel after leaving the quarantine?	12 h quarantine	12 h quarantine	Bath	12 h quarantine, bath	12 h quarantine	Bath	12 h quarantine	Bath	Change boots/clothes	12 h qaurantine, bath

Appendix 10 - Vaccination strategy

Vaccinated Herds	Herd 6	Herd 7	Herd 8	Herd 9	Herd 10
In the quarantine, when are the gilts basis vaccinated?	Age 24 and 26 weeks	21 days, 35 days	Age 26 and 28 weeks	Age 26 and 28 weeks	2 days, 23 days
Are the gilts vaccinated twice before leaving the quarantine?	No	Yes	No	No	Yes
Mass sow vaccination, when?	Mar., July, Nov.	Jan, May, Sep (Oct.)	Feb., June, Oct.	Week 10 (Mar.), 26 (Jun.), 44 (Oct.)	Mar. 1st., July 1.st., Nov. 1st.
Mass sow vaccination frequency?	3 times	3 times	3 times	3 times	3 times
The last mass sow vaccination	July, 2019	Oct 8th., 2019	week 41, Oct., 2019	week 26. Oct, 2019	July 1st, 2019
Are all gilts in the sow herd included in mass sow vaccination?	Yes	Yes	Yes	Yes	Yes
Are slaughter sows included in mass sow vaccination?	No	Yes	Yes	Yes	Yes
Other vaccines administered with Respiporc Flu3?	Porcilis Ery+Parvo+Lepto	Erybac Uno, Porcilis Glässer, Porcilis PCV M Hyo	Respiporc FluPan, Parvoruvax	Porcilis Ery+Parvo+Lepto, Porcilis Glässer	Porcilis PRRS, Porcilis PCV

Influenza vaccination strategy. An overview of influenza vaccination strategy in vaccinated herds.

Appendix 11 - Tests of hypotheses

H0a: There is no significant difference in section prevalence of virus positive gilts between vaccinated and unvaccinated herds

Quarantine in: Fisher's exact test

Table Analyzed	H0aqi		
P value and statistical significance			
Test	Fisher's exact test		
P value	0.0003		
P value summary	***		
One- or two-sided	Two-sided		
Statistically significant (P < 0.05)?	Yes		
Effect size	**Value**	**95% CI**	
Relative Risk	Infinity	2.000 to Infinity	
Reciprocal of relative risk	0.000	0.000 to 0.5000	
Odds ratio	Infinity	3.326 to Infinity	
Reciprocal of odds ratio	0.000	0.000 to 0.3007	
Methods used to compute CIs			
Relative Risk	Koopman asymptotic score		
Odds ratio	Baptista-Pike		
Data analyzed	**Column A**	**Column B**	**Total**
Row 1	12	88	100
Row 2	0	100	100
Total	12	188	200

Quarantine out: Fisher's exact test

Table Analyzed	H0aqu		
P value and statistical significance			
Test	Fisher's exact test		
P value	0.6584		
P value summary	ns		
One- or two-sided	Two-sided		
Statistically significant (P < 0.05)?	No		
Effect size	**Value**	**95% CI**	
Relative Risk	1.300	0.6102 to 2.783	
Reciprocal of relative risk	0.7692	0.3594 to 1.639	
Odds ratio	1.345	0.5446 to 3.241	
Reciprocal of odds ratio	0.7436	0.3085 to 1.836	
Methods used to compute CIs			
Relative Risk	Koopman asymptotic score		
Odds ratio	Baptista-Pike		
Data analyzed	**Column A**	**Column B**	**Total**
Row 1	13	87	100
Row 2	10	90	100
Total	23	177	200
Percentage of row total	**Column A**	**Column B**	
Row 1	13.00%	87.00%	
Row 2	10.00%	90.00%	

Mating unit: Fisher's exact test

Table Analyzed	H0am		
P value and statistical significance			
Test	Fisher's exact test		
P value	0.1212		
P value summary	ns		
One- or two-sided	Two-sided		
Statistically significant (P < 0.05)?	No		
Effect size	**Value**	**95% CI**	
Relative Risk	Infinity	1.062 to Infinity	
Reciprocal of relative risk	0.000	0.000 to 0.9417	
Odds ratio	Infinity	1.000 to Infinity	
Reciprocal of odds ratio	0.000	0.000 to 1.000	
Methods used to compute CIs			
Relative Risk	Koopman asymptotic score		
Odds ratio	Baptista-Pike		
Data analyzed	**Column A**	**Column B**	**Total**
Row 1	4	96	100
Row 2	0	100	100
Total	4	196	200

Farrowing unit: Fisher's exact test

Table Analyzed	H0af		
P value and statistical significance			
Test	Fisher's exact test		
P value	0.1212		
P value summary	ns		
One- or two-sided	Two-sided		
Statistically significant (P < 0.05)?	No		
Effect size	**Value**	**95% CI**	
Relative Risk	Infinity	1.062 to Infinity	
Reciprocal of relative risk	0.000	0.000 to 0.9417	
Odds ratio	Infinity	1.000 to Infinity	
Reciprocal of odds ratio	0.000	0.000 to 1.000	
Methods used to compute CIs			
Relative Risk	Koopman asymptotic score		
Odds ratio	Baptista-Pike		
Data analyzed	**Column A**	**Column B**	**Total**
Row 1	4	96	100
Row 2	0	100	100
Total	4	196	200

H0b: There is no significant difference in prevalence of virus positive pooled samples from piglets in the farrowing unit between vaccinated and unvaccinated herds.

Piglets:

Chi-square test

		Disease +	Disease -	Sum
Exposure	+	12	88	100
	-	12	88	100
	Sum	24	176	200

Note: While all measures can be calculated not all of them makes sense for a given study

Yates Corrected Chi-Square		0.047		
P-Value		0.828		
Measures of Association:			95	% confidence limits
			Lower	Upper
Prevalence:	P	0.120	0.075	0.165
Relative Risk	RR	1.000	0.472	2.118
Odds ratio	OR	1.000	0.426	2.347
Population relative risk	RRpop	1.000		
Population odds ratio	ORpop	1.000		

H0c: There is no significant difference in section prevalence of antibody positive gilts between vaccinated and unvaccinated herds.

Quarantine in: Chi-square test

		Disease +	Disease -	Sum
Exposure	+	41	59	100
	-	57	43	100
	Sum	98	102	200

Note: While all measures can be calculated not all of them makes sense for a given study

Yates Corrected Chi-Square		4.502		
P-Value		0.034		
Measures of Association:			95	% confidence limits
			Lower	Upper
Prevalence:	P	0.490	0.421	0.559
Relative Risk	RR	0.719	0.538	0.962
Odds ratio	OR	0.524	0.299	0.919
Population relative risk	RRpop	0.860		
Population odds ratio	ORpop	0.725		

Quarantine out: Chi-square test

		Disease +	Disease -	Sum
Exposure	+	66	34	100
	-	56	44	100
	Sum	122	78	200

Note: While all measures can be calculated not all of them makes sense for a given study

Yates Corrected Chi-Square		1.702		
P-Value		0.192		
Measures of Association:			95	% confidence limits
			Lower	Upper
Prevalence:	P	0.610	0.542	0.678
Relative Risk	RR	1.179	0.942	1.474
Odds ratio	OR	1.525	0.861	2.702
Population relative risk	RRpop	1.089		
Population odds ratio	ORpop	1.229		

Gestation unit: Chi-square test

		Disease		
		+	-	Sum
Exposure	+	93	7	100
	-	69	31	100
	Sum	162	38	200

Note: While all measures can be calculated, not all of them makes sense for a given study.

Yates Corrected Chi-Square		17.186		
P-Value		0.000		
Measures of Association:			95	% confidence limits
			Lower	Upper
Prevalence:	P	0.810	0.756	0.864
Relative Risk	RR	1.348	1.169	1.553
Odds ratio	OR	5.969	2.482	14.352
Population relative risk	RRpop	1.174		
Population odds ratio	ORpop	1.915		

Farrowing unit: Fisher's exact test

Table Analyzed	H0cF		
P value and statistical significance			
Test	Fisher's exact test		
P value	<0.0001		
P value summary	****		
One- or two-sided	Two-sided		
Statistically significant (P < 0.05)?	Yes		
Effect size	**Value**	**95% CI**	
Relative Risk	1.638	1.399 to 1.979	
Reciprocal of relative risk	0.6105	0.5053 to 0.7146	
Odds ratio	13.76	5.286 to 33.33	
Reciprocal of odds ratio	0.07268	0.03001 to 0.1892	
Methods used to compute CIs			
Relative Risk	Koopman asymptotic score		
Odds ratio	Baptista-Pike		
Data analyzed	**Column A**	**Column B**	**Total**
Row 1	95	5	100
Row 2	58	42	100
Total	153	47	200

H0d: There is no significant difference in levels of antibodies in seropositive gilts between vaccinated and unvaccinated herds.

Mann Whitney U test

Mann-Whitney test	
Table Analyzed	Positive Antibodies
Column B	V Q In
vs.	vs.
Column A	UV Q In
Mann Whitney test	
P value	0.1529
Exact or approximate P value?	Exact
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	2869 , 3237
Mann-Whitney U	1273
Difference between medians	
Median of column A	0.2950, n=56
Median of column B	0.3800, n=54
Difference: Actual	0.08500
Difference: Hodges-Lehmann	0.05000

Prism computed an exact P value (0.1529), which takes into account
ties among values. Note that most other programs do not compute
exact P values when there are tied values, but would instead report
an approximate P value (0.1528).

Mann-Whitney test	
Table Analyzed	Positive Antibodies
Column D	V Q out
vs.	vs.
Column C	UV Q out
Mann Whitney test	
P value	0.9072
Exact or approximate P value?	Exact
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
Sum of ranks in column C,D	3421 , 4082
Mann-Whitney U	1825
Difference between medians	
Median of column C	0.2100, n=56
Median of column D	0.1600, n=66
Difference: Actual	-0.05000
Difference: Hodges-Lehmann	0.000

Prism computed an exact P value (0.9072), which takes into account
ties among values. Note that most other programs do not compute
exact P values when there are tied values, but would instead report
an approximate P value (0.9079).

Mann-Whitney test	
Table Analyzed	Positive Antibodies
Column H	V F.unit
vs.	vs.
Column G	UV F.unit
Mann Whitney test	
P value	0.0067
Exact or approximate P value?	Exact
P value summary	**
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column G,H	5183 , 6598
Mann-Whitney U	2038
Difference between medians	
Median of column G	0.1950, n=58
Median of column H	0.1500, n=95
Difference: Actual	-0.04500
Difference: Hodges-Lehmann	-0.04000

Prism computed an exact P value (0.0067), which takes into account
ties among values. Note that most other programs do not compute
exact P values when there are tied values, but would instead report
an approximate P value (0.0070).

Mann-Whitney test	
Table Analyzed	Positive Antibodies
Column F	V G.unit
vs.	vs.
Column E	UV G.Unit
Mann Whitney test	
P value	0.4851
Exact or approximate P value?	Exact
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
Sum of ranks in column E,F	5794 , 7247
Mann-Whitney U	2969
Difference between medians	
Median of column E	0.2000, n=69
Median of column F	0.2400, n=92
Difference: Actual	0.04000
Difference: Hodges-Lehmann	-0.02000

Prism computed an exact P value (0.4851), which takes into account
ties among values. Note that most other programs do not compute
exact P values when there are tied values, but would instead report
an approximate P value (0.4846).

Mann-Whitney test	
Table Analyzed	Positive Antibodies
Column J	V ALL
vs.	vs.
Column I	UV ALL
Mann Whitney test	
P value	<0.0001
Exact or approximate P value?	Approximate
P value summary	****
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column I,J	71109 , 62278
Mann-Whitney U	23218
Difference between medians	
Median of column I	0.2800, n=237
Median of column J	0.1700, n=279
Difference: Actual	-0.1100
Difference: Hodges-Lehmann	-0.07000

H0e: There is no correlation between virus positive gilts at the end of quarantine and positive piglets

Fisher's exact test

Table Analyzed	H0e		
P value and statistical significance			
Test	Fisher's exact test		
P value	0.0467		
P value summary	*		
One- or two-sided	Two-sided		
Statistically significant (P < 0.05)?	Yes		
Effect size	**Value**	**95% CI**	
Relative Risk	2.533	1.035 to 6.373	
Reciprocal of relative risk	0.3947	0.1569 to 0.9658	
Odds ratio	2.822	1.025 to 7.144	
Reciprocal of odds ratio	0.3544	0.1400 to 0.9757	
Methods used to compute CIs			
Relative Risk	Koopman asymptotic score		
Odds ratio	Baptista-Pike		
Data analyzed	**Column A**	**Column B**	**Total**
Row 1	19	101	120
Row 2	5	75	80
Total	24	176	200
Percentage of row total	**Column A**	**Column B**	
Row 1	15.83%	84.17%	
Row 2	6.25%	93.75%	
Percentage of column total	**Column A**	**Column B**	
Row 1	79.17%	57.39%	
Row 2	20.83%	42.61%	
Percentage of grand total	**Column A**	**Column B**	
Row 1	9.50%	50.50%	
Row 2	2.50%	37.50%	

H0f: There is no correlation between antibody prevalence at the end of quarantine and virus positive gilts or piglets in the herd.

Herd immunity threshold Qc = 1-(1/R0) = 1-(1/6.5) = 85%

Chi-square test

		Disease		
		+	-	Sum
Exposure	+	8	92	100
	-	26	74	100
	Sum	34	166	200

Yates Corrected Chi-Square		10,241		
P-Value		0,001		
Measures of Association:			95	% confidence limits
			Lower	Upper
Prevalence:	P	0,170	0,118	0,222
Relative Risk	RR	0,308	0,146	0,646
Odds ratio	OR	0,247	0,106	0,579
Population relative risk	RRpop	0,654		
Population odds ratio	ORpop	0,583		

H0g: There is no correlation between levels of antibodies in gilts before and after farrowing and virus positive piglets.

Mann Whitney test

Mann-Whitney test	
Table Analyzed	Data 12
Column D	Negative piglets / G levels, n=59
vs.	vs.
Column C	Positive piglets / G levels,n=102
Mann Whitney test	
P value	0.2035
Exact or approximate P value?	Exact
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
Sum of ranks in column C,D	7899 , 5142
Mann-Whitney U	2646
Difference between medians	
Median of column C	0.2050, n=102
Median of column D	0.2400, n=59
Difference: Actual	0.03500
Difference: Hodges-Lehmann	0.03000

Mann-Whitney test	
Table Analyzed	Data 12
Column F	Negative piglets / F level,n=58
vs.	vs.
Column E	Positive piglets / F level, n=95
Mann Whitney test	
P value	0.0039
Exact or approximate P value?	Exact
P value summary	**
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column E,F	6552 , 5229
Mann-Whitney U	1992
Difference between medians	
Median of column E	0.1600, n=95
Median of column F	0.2850, n=58
Difference: Actual	0.1250
Difference: Hodges-Lehmann	0.06000