Introduction of replacement gilts to PRRS-positive sow herds

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Preface

This paper is a veterinary master's thesis written in 2015 as the last part of my veterinary education. The work lasted approximately 5 months, corresponding to 30 ECTS points.
The composition of the thesis is that chapter 1 – the introduction chapter – is a literature study, which prepares the reader for the following chapters containing the experimental study (referred to as 'the Gilt Project').

The project's main supervisor was professor Lars Erik Larsen, National Veterinary Institute, DTU. Co-supervisors were chief scientist Charlotte Sonne Kristensen, SEGES Danish Pig Research Centre, and senior adviser Charlotte Kristiane Hjulsager, National Veterinary Institute, DTU.

I would like to thank my supervisors for their great help and advice throughout the process. Also a big thank-you to the participating farmers and the veterinarians from Odder Dyreklinik and Hyovet.

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Abstract

Porcine reproductive and respiratory syndrome virus (PRRSV) is an important disease, which creates problems in the swine industry worldwide, including Denmark. After infection with PRRSV, or vaccination with a modified-live vaccine (MLV) against PRRSV, the pigs will contract a prolonged viremia, and will thus be able to transmit virus to PRRS-naïve pigs. Danish recommendations for PRRS-positive farms are to introduce replacement gilts for a quarantine period of 12 weeks after exposure to a wildtype virus or vaccination with an MLV. In some herds it is practical impossible to manage a quarantine for 12 weeks. Therefore there is a need for alternative ways to introduce replacement gilts which secure that the sow herd remains PRRS stable. Hopefully this study will lead to optimised guidelines in how to introduce replacement gilts to a PRRS-positive sow herd.

The objective of the present study was to investigate the use of PRRS MLV and quarantine facilities with the PRRSV status of replacement gilts at first insemination. Furthermore, the study aimed to look at antibody levels, relative to the time from vaccination with PRRS MLV, and the age of the animals when vaccinated.

The study was a cross-sectional study, with 69 PRRS-positive sow herds included. Five blood samples from replacement gilts were taken at each farm, and analysed by RT-qPCR, ELISA and IPMA. A questionnaire regarding information about gilt recruitment strategy, vaccination strategy, and more was filled out.

The study divided each group of gilts on each farm into ‘stable’ (n=63) or ‘unstable’ (n=6). Being stable was defined by being negative by RT-qPCR and positive by ELISA. The study found no viremic gilts by RT-qPCR, but found 6 farms with gilts not having antibodies by ELISA against PRRSV, thus being unstable.

The study concluded that there was no significant difference (α=0.05) between the stable and unstable groups regarding the use of quarantine, duration of quarantine and gilt recruitment strategy of replacement gilts, but found a tendency towards the use of quarantine resulting in stable gilts. Furthermore, the study found no significant relation between age when vaccinated and the level of antibodies. The time from vaccination to when the blood samples were taken, in relation to the level of antibodies, could not be investigated due to a lack of data on the age of the gilts when the blood was sampled.

A higher degree of viremic replacement gilts was expected before the beginning of the study. Estimated time from vaccination to insemination (when blood samples were taken) was on average 18 weeks, and compared to the duration of viremia after vaccination with MLV measured in a collection of studies of average 4 weeks, it was not surprising that no gilts were viremic. 6 (8.7%) farms had ELISA-negative replacement gilts, meaning the gilts were not immunised against PRRSV at first insemination. All 6 farms were vaccinating with an MLV, and the finding was thus surprising. When being naïve in regard to PRRSV in a PRRS-positive sow herd, there is a risk of infection with PRRSV, getting viremia and shedding virus.

The present study showed that replacement gilts are not viremic at first insemination, and that a small proportion of gilts might not be immunised against PRRSV at first insemination. This study does not change the existing guidelines in how to introduce replacement gilts to a PRRS-positive sow herd.

Keywords: PRRS, MLV, viremia, quarantine, replacement gilts, gilt recruitment.

Resumé

Porcine reproductive and respiratory syndrome virus (PRRSV) hos svin er en betydningsfuld sygdom, som skaber problemer i svineproduktion over hele verden, inklusiv Danmark. Efter naturlig infektion med PRRSV eller vaccination med en modificeret levende vaccine (MLV) mod PRRSV vil svin få længerevarende viræmi og vil være i stand til at smitte PRRS-naive dyr. De danske anbefalinger for PRRS-positive besætninger, er at vaccinere dem med en MLV eller eksponere dem for vildtype virus i besætningen, efterfulgt af en 12 ugers karantæneperiode. Denne praksis er dog ikke mulig at udføre i alle besætninger, og derfor er der brug for alternative metoder til at introducere nye polte, som sikrer at sobesætningen forbliver PRRS-stabil. Forhåbentlig vil dette studie føre til optimerede anbefalinger til hvordan man skal introducere nye polte i PRRS-positive besætninger.

Formålet med dette studie var at undersøge brugen af PRRS MLV og karantænefaciliteter med PRRSV-status af nye polte ved første løbning. Derudover undersøgte studiet antistofniveauer relateret til tid fra vaccination med PRRS MLV til blodprøver blev taget, og poltenes alder ved vaccination.

Studiet var et tværsnitsstudie med 69 inkluderede besætninger. Der blev taget 5 blodprøver fra nye polte i hver besætning, og et spørgeskema vedrørende polterekrutteringsstrategi, vaccinationsstrategi m.m. blev udfyldt. Blodprøverne blev analyseret ved RT-qPCR, ELISA og IPT.

Studiet opdelte besætningernes nye polte i henholdsvis stabile (n=63) og ikke stabile (n=6). Stabile polte blev defineret som at være negative ved RT-qPCR og positive i ELISA. Studiet fandt ingen viræmiske polte ved RT-qPCR, men fandt ELISA-negative polte i 6 besætninger.

Studiet fandt ingen signifikant forskel (α=0.05) mellem de stabile og de ustabile grupper mht. karantæne, karantænelængde og polterekrutteringsstrategi, men fandt dog en tendens til at der var en sammenhæng mellem at bruge karantæne og have stabile polte. Der fandtes ingen signifikant forskel mellem antistofniveauer og poltenes alder ved vaccination. Sammenhæng mellem tid fra vaccination til udtagelse af blodprøver, kunne ikke undersøges pga. manglende data på poltenes alder ved blodprøvetagning.

Det var forventet at finde viræmiske polte i studiet. Tid fra vaccination til blodprøver blev taget kunne estimeres til 18 uger. Studier, som undersøger længden af viræmi efter vaccination med MLV finder i gennemsnit at dyrene er viræmiske i 4 uger. Sammenlignes disse 18 uger med de 4 uger, er det ikke overraskede at der ikke blev fundet nogen viræmiske dyr.

Der var 6 (8.7%) besætninger med ELISA-negative polte. Alle 6 besætninger vaccinerede med en MLV, og dette fund var derfor overraskende. Naive polte mht. PRRS i en PRRS-positiv besætning har risiko for at blive inficeret med PRRS, få viræmi og udskille virus i besætningen.

Studiet viste at polte ikke er viræmiske ved første løbning, men der er en lille del som ikke er immuniseret mht. PRRS ved første løbning. Studiet førte ikke til ændringer i anbefalingerne vedr. introduktion af polte.

Nøgleord: PRRS, MLV, viræmi, karantæne, polte, polterekruttering.

Abbreviations

dPI: Days post infection
dPV: Days post vaccination
CI: Confidence interval
DTU: Technical University of Denmark
EAV: Equine arteritis virus
ELISA: Enzyme-linked immunosorbent assay
HRP: Horseradish peroxidase
IFA: Indirect fluorescent antibody test
IFN: Interferon
IPMA: Immunoperoxidase monolayer assay
IPT: Immunoperoxidase test
LCH: Load – Close – Homogenize
LDV: Lactase dehydrogenase-elevating virus
MLV: Modified-live vaccine
NVI: National Veterinary Institute
OD: Optical density
OIE: World Organisation for Animal Health
ORFs: Open reading frames
PAMs: Porcine alveolar macrophages
PCR: Polymerase chain reaction
PRRS: Porcine reproductive and respiratory syndrome
PRRSV: Porcine reproductive and respiratory syndrome virus
qPCR: Quantitative PCR or real time PCR
RT-PCR: Reverse transcriptase PCR
RT-qPCR: Reverse transcriptase real time (or quantitative) PCR
SD: Standard deviation
SEM: Standard error of the mean
SHFV: Simian hemorrhagic fever virus
SNV: Serum neutralization test
SPF: Specific pathogen-free
$TCID_{50}$: 50% tissue culture infective dose
TNF: Tumor necrosis factor

Content

Introduction

1.1 History and theory of PRRS

Porcine Reproductive and Respiratory Syndrome (PRRS) was first described in the USA in 1987. The syndrome had many names before the name Porcine Reproductive and Respiratory Syndrome was agreed upon in 1992 at an international symposium about the disease (Christianson & Joo, 1994). Among the other titles were names including 'blue ear disease', 'mystery swine disease (MSD)' and 'swine infertility and respiratory syndrome (SIRS)'. A complete list of the various names can be found in a review by Goyal, 1993.
The aetiological agent of the disease was isolated in the city of Lelystad in the Netherlands in 1991, and designated 'Lelystad virus' (LV) (Wensvoort *et al.*, 1991). Shortly afterwards, a virus producing similar symptoms was isolated in the USA. This strain was designated American Type Culture Collection VR-2332 (VR-2332) (Collins *et al.*, 1992). 'Lelystad virus' is now the reference strain of genotype 1, and 'VR-2332' of genotype 2, respectively (Wernike *et al.*, 2012). PRRS virus (PRRSV) type 1 was diagnosed in Denmark (Als) for the first time in March 1992 (Bøtner *et al.*, 1994). In Denmark, PRRSV type 1 and type 2 corresponds to the EU-type and the US/Vac-type, respectively.
The virus is enzootic at global level (Shi *et al.*, 2010), and the estimated prevalence of PRRS-positive sow herds in Denmark – conventional and SPF – is 35% (Kristensen *et al.*, 2014).

1.1.1 Introduction of PRRSV type 2 in Denmark

In 1996, the prevalence of PRRS-positive herds in Denmark was found to be 33% (type 1). With the purpose of trying to keep PRRSV from further spread, a voluntary control programme was initiated (Bøtner *et al.*, 1997). One mission was to investigate the serological status of all swine herds. Positive herds were vaccinated with Ingelvac PRRS® modified-live virus (MLV) (Boehringer Ingelheim), which is based on the American strain 'VR-2332' (SPC for Ingelvac PRRS® MLV). The first boars were vaccinated in October 1995, with special permission from the authorities, since the vaccine was not authorised by the Danish Health authorities until July 1996. Before the approval of the vaccine, a serological screening (Sørensen *et al.*, 1997) was performed with blood samples from 2159 sows. The screening did not show evidence of occurrence of type 2 PRRSV strains in Denmark. Soon after approval of the vaccine was given, it was used in a good number of PRRS-positive herds. From October 1996 until May 1997, PRRS-positive herds on which the Ingelvac PRRS® MLV had been used experienced acute symptoms of PRRS, and samples in the form of foetuses, dead piglets, pleural fluids and long tissue were sent to a Danish laboratory. Test samples were grown in cells (MARC-145), and results showed isolation of type 2 PRRSV. Since the test could differentiate between type 1 and 2, the findings therefore strongly indicated a spread of vaccine virus to non-vaccinated animals, and the introduction of PRRS type 2 in Denmark was established (Bøtner *et al.*, 1997).

1.2 Characteristics of PRRSV

1.2.1 Taxonomy

The aetiological agent of Porcine Reproductive and Respiratory Syndrome virus (PRRSV) is a virus belonging to the order *Nidovirales*, family *Arteriviridae* (formerly *Togaviridae*), genus *Arterivirus.* It is related to lactase dehydrogenase-elevating virus (LDV), equine arteritis virus (EAV), and simian hemorrhagic fever virus (SHFV), all of which belong to *Arteriviridae* (Christianson & Joo, 1994; Quinn *et al.*, 2011).

1.2.2 Characteristics of the genome

PRRSV is medium-sized (50-72 nm in diameter), enveloped, and contains an icosahedral capsid, containing a single-stranded positive-sense RNA genome of approximately 15 kb (Quinn *et al.*, 2011).

The genome consists of at least nine open reading frames (ORFs). ORF1a and ORF1b represent about 75% of the viral genome, and encode non-structural proteins such as enzymes needed for replication. ORF2a, ORF2b, ORF3 and ORF4 are minor structural proteins, and encode membrane-associated glycoproteins (GP2, GP3 and GP4). ORF5, ORF 6 and ORF7 encode major structural proteins. ORF5 encodes envelope glycoprotein (GP5), ORF6 encodes membrane (M) protein, and ORF 7 encodes nucleocapsid (N) protein (Dea *et al.*, 2000; Shi *et al.*, 2010). ORF5 has a high variability, and genomic analysis is often based on this ORF sequence and ORF7 (Shi *et al.*, 2010; Stadejek *et al.*, 2008). It has been suggested that another ORF exists – ORF5a (Johnson *et al.*, 2011). The function of ORF5a is not known for sure (Johnson *et al.*, 2011).

1.2.3 Genomic diversity

PRRSV has, like other RNA-viruses, a high mutation rate, due to a lack of proofreading by the RNA replication enzyme (Dea *et al.*, 2000; Rossow, 1998).

PRRSV consists of two genotypes: type 1 (European type, DK/EU), and type 2 (American type, US/Vac) (Labarque *et al.*, 2004). Type 1 and type 2 have about 60% nucleotide identity (Allende *et al.*, 1999).

Based on ORF5 and ORF7 sequences, Stadejek *et al.* (2008) divided PRRSV type 1 into three subtypes: a pan-European subtype 1, and East European subtypes 2 and 3. PRRSV type 1 subtype 1 is widespread in Western Europe. Subtypes 2 and 3 have only been reported from Eastern Europe (Stadejek *et al.*, 2008). In Denmark there are two major clusters within PRRSV 1 subtype 1 circulating. One is Lelystad-like, and one is an almost unique Danish cluster (Kvisgaard *et al.*, 2013). The diversity of PRRSV type 1 exceeds that of type 2 (Stadejek *et al.*, 2008).

The genomic difference between the strains affects the extent of cross-protection, which is poor (van Woensel *et al.*, 1998). Animals vaccinated with a PRRS MLV are protected against a challenge with a homologues strain, but not necessarily against a heterological strain (Labarque *et al.*, 2004).

A thorough phylogenetic tree can be found in Wernike *et al.* (2012).

1.3 Epidemiology

There are many possible routes – direct and indirect – of transmission with PRRSV. Direct contact between pigs (nose-to-nose contact) is the most common means of transmission (Rossow, 1998).

Airborne transmission has been demonstrated by Kristensen *et al.* (2004). Good conditions for an airborne spread are high humidity, low wind speed, low temperature, and when ultra-violet light exposure is low (Albina, 1997; Goyal, 1993). The distance between farms is very relevant when it comes to airborne spread. It is shown that PRRSV can spread over distances up to 20km, but the risk is highest within 500m to 1km (Albina, 1997). Whether people can act as mechanical vectors was studied by Amass et al., 2000. This study found that – under experimental circumstances – people could not transmit PRRSV from clinically ill pigs to healthy, PRRSV-seronegative pigs. However, they did find a limited proportion of people that were contaminated with PRRSV after contact with clinically ill PRRSV-positive pigs; but they did not succeed in shedding the virus to the sentinels. Other studies succeeded in showing that people and fomites – such as coveralls and boots – could transmit PPRSV, but that the transmission of virus could be controlled by the use of sanitation protocols (Otake *et al.*, 2002). Vehicles are also proven to be a source of transmission of PRRSV (Dee *et al.*, 2004). Spread through semen has been proved in a study with 2 boars, which were inoculated with PRRSV intranasally. Semen was collected 6 days after inoculation, and two PRRS-negative gilts were in-

seminated. Both gilts seroconverted (Yaeger, 1993). In Denmark it is possible to buy semen from PRRS-vaccinated boars and from PRRS-negative boars (Hatting, 2015). Wills *et al.*, (1997b) showed that PRRSV can spread though serum, urine, saliva and tracheal rinse. The study did not succeed in finding the virus in conjunctival swabs or faeces. However, Yoon *et al.* (1993) managed to show that PRRSV is excreted in faeces, and it can thereby be concluded that faeces can potentially transmit PRRSV. Vertical transmission from viremic sows transplacentally to the foetuses might result in foetal death, or birth of virus-positive piglets that might appear weak (Bøtner *et al.*, 1994).
Infection with PRRSV can be persistent (Allende *et al.*, 2000; Wills *et al.*, 1997a). One study found that virus could persist in tissue for up to 157 days in weaners (Wills *et al*., 1997a). A persistent infection is defined as "the continued presence of a pathogen in a host beyond the acute symptomatic phase of infection" (Batista *et al.*, 2004). Allende *et al.* (2000) provided evidence that the persistence of PRRSV involve continuous viral replication (replicates at low levels over time), and therefore it cannot be classified as a latent persistent infection. Based on this article, a persistent infection with PRRSV should rather be called a "chronic infection".
It is shown that sows persistently infected with PRRSV (86 days post intranasal inoculation) can transmit the virus to PRRSV-negative sows, even though the infected sows were not found to be viremic (Bierk *et al.*, 2001).
The introduction of PRRS type 2 in Denmark proved that pigs vaccinated with an MLV could transmit vaccine virus to non-vaccinated seronegative pigs (Bøtner *et al.*, 1997). This means that MLV is a potential source of infection with PRRS in a farm.

1.3.1 Stability of virus

All arteriviruses are relatively labile. PRRSV can survive for 6 days at 20°C and 1 month at 4°C, but only 10-24 hours at 37°C. The virus is stable between pH 5 and 7, but a pH value of less than 5 or greater than 7 will inactivate the virus (Christianson & Joo, 1994). Lipid solvents, detergent treatments, drying out, UV irradiation and many different disinfectants will inactivate the virus (Benfield *et al.*, 1992).

1.4 Pathogenesis and immune response

Infection occurs via the respiratory tract in most cases (Quinn *et al.*, 2011). PRRSV infects mononuclear cells, especially porcine alveolar macrophages (PAMs), and replicates there (Duan *et al.*, 1997). Replicated new virus particles are transported to regional lymph nodes, where replication also occurs. From here the virus particles can be transmitted to the lymph and blood, resulting in viremia (Allende *et al.*, 2000; Goyal, 1993).

1.4.1 Duration of viremia

The length of viremia after vaccination with PRRS MLV and after intranasal inoculation with PRRSV has been investigated in several studies. Table 1 shows a collection of studies which tested length of viremia after intranasal inoculation. Table 2 is similar, but instead of being intranasally inoculated, the pigs were vaccinated with an MLV.
From Table 1 and 2 is it calculated that length of viremia post inoculation and post vaccination with MLV is in average 29 days for both cases. Different methods to investigate the presence of virus in serum have been used in the studies. In general newer studies utilized PCR e.g. Wills *et al.* (2003), Bierk *et al.* (2001) and Park *et al.* (2015), and older studied utilized virus isolation e.g. Yoon *et al.* (1993), Duan *et al.* (1997) and

Halbur *et al.* (1995). Some studies used both PCR and virus isolation (Martelli *et al.*, 2009; Mengeling *et al.*, 2003; Wills *et al.*, 1997a, 2003).

The day when bled is shown in the tables to give an impression of the interval between sampling. In general the studies sampled once a week. Blood sampling every day would have given more precise knowledge about the length of viremia.

Table 1: Results from different studies investigating length of viremia after intranasal inoculation.

Reference	*n*	Viremia, dPI*	Bled day?	Isolate / strain
Batista *et al.* (2002)	120	14	0, 3, 7, 14, 30, 60, 90, 120, 150, 180	MN30100
Batista *et al.* (2004)	80	30	0, 3, 7, 21, 30, 50, 70, 90, 100, 110, 120, 135	MN30100
Bierk *et al.* (2001)	12	14	1, 3, 5, 7, 10, 14, 21, 28, 35, 42	Not known
Díaz *et al.* (2012)	8 8	28 56	7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84	Strain 3262 (type 1) Strain 3267 (type 1)
Duan *et al.* (1997)	16	28	3, 14, 21, 28, 35, 42, 82	Lelystad virus
Halbur *et al.* (1995)	25 25 25	28 28 28	1, 2, 3, 5, 7, 10, 15, 21, 28	ATCC VR2385 ATCC VR2431 Lelystad-virus
Wills *et al.* (1997a)	4	23	0, 2, 4, 7, 9, 11, 14, 16, 18, 21, 23, 25, 28, 30, 32, 35, 37, 39, 42 and every 14th day until day 220	ATCC VR-2402
Wills *et al.* (1997b)	12	21	7, 14, 21, 28, 35, 42	ATCC VR-2402
Wills *et al.* (2003)	28	56, 251[a]	0, 7, 14, 28, 56, 84, 119, 147, 168, 196, 225, 251	16244B
Yoon *et al.* (1993)	4	35	0, '2- to 7- days intervals', 56	PRRS MN-1b
Yoon *et al.* (1995)	8	15	0, 3, 5, 7, 9, 11, 13, 15, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 91, 98, 105	ATCC VR-2402

**dPI, days post inoculation. [a]All animals was negative between 84 and 196 dPI. In the study they discuss whether the finding of one viremic animal at 251 dPI might be a false positive.*

Table 2: Results from different studies investigating length of viremia after vaccination with MLV against PRRSV.

Reference	*n*	Viremia, dPV*	Bled day?	Vaccine
Díaz *et al.* (2006)	5	42	0, 7, 14, 21, 28, 42, 63, 70, 91	Porcilis® PRRS
Duengelhoef *et al.* (2014)	28	15	0, 2, 4, 8, 15	Porcilis® PRRS
Foss *et al.* (2002)	5	21	0, 7, 14, 21, 28, 35, 42	Ingelvac PRRS® MLV
Labarque *et al.* (2004)	49	35	5, 9, 15, 20, 28, 35, 42	Porcilis® PRRS
Martelli *et al.* (2007)	18	28	0, 7, 14, 21, 28, 35	Porcilis® PRRS
Martelli *et al.* (2009)	30	14	0, 7, 14, 28, 45	Porcilis® PRRS
Martínez-Lobo *et al.* (2013)	125	21	0, 3, 6, 7, 9, 12, 14, 15, 18, 21	Ingelvac PRRS® MLV Amervac PRRS® Pyrsvac-183® Porcilis® PRRS
Mengeling *et al.* (2003)	8	42	0, 7, 13, 21, 28, 35, 42	Ingelvac PRRS® MLV
Nielsen *et al.* (1997)	5	21	0, 4, 7, 14, 21, 28, 35, 42	Ingelvac PRRS® MLV
Pawlowski & Carlsen (2015)[a]	66	56[b]	-1, 1, 2, 5, 6, 14, 21, 28, 35, 42, 49, 56, 62	Porcilis® PRRS Ingelvac PRRS® MLV
Pileri *et al.* (2015)	40	35	0, 7, 14, 30, 35	Porcilis® PRRS
Sipos *et al.* (2003)	5	22	2, 7, 14, 22, 30, 44, 80	Porcilis® PRRS
Stadejek *et al.* (2005)	12	42	2, 21, 42, 68, 92	Porcilis® PRRS
Park *et al.* (2015)	60	10	0, 3, 5, 7, 10, 14, 21, 35	Fostera® PRRS

**dPV, days post vaccination. [a]Unpublished. [b]Three groups of 18 pigs were vaccinated with Porcilis® PRRS, Ingelvac® PRRS MLV and Porcilis® PRRS plus Ingelvac® PRRS MLV, respectively. All had same length of viremia of 56 days.*

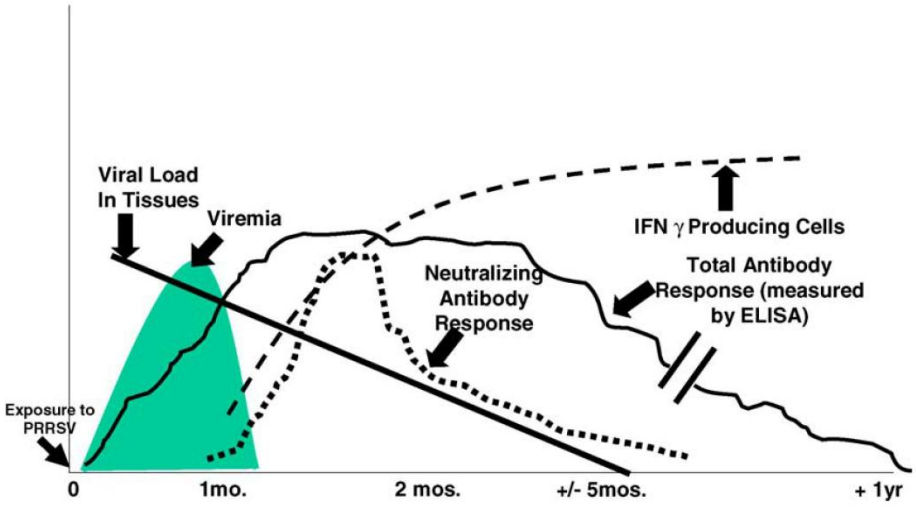
1.4.2 Immune response

Infection with PRRSV elicits an innate immune response as the first thing. Infected porcine alveolar macrophages (PAMs) generates an antiviral response in the cytoplasm, but the innate response is weak – characterized by a low production of inflammatory cytokines, a low TNF (tumor necrosis factor) expression, and a weak recruitment of natural killer cells (NK cells). The production of type 1 interferons (IFN α and β) is down-regulated, and this implies a reduction in antiviral activity, and thus might allow PRRSV replication in the cell (Murtaugh *et al.*, 2002).

The humoral immune response executes the production of antibodies. The first detectable antibodies are seen by day 7-14 after exposure to PRRSV (Bøtner, 1997), and can be detected by ELISA, IFA or IPMA (Christopher-Hennings *et al.*, 2002). They target the N and M protein, encoded by ORF 7 and 6 respectively, but especially the N-protein. These first antibodies are not neutralizing. Neutralizing antibodies appear 3-4 weeks after exposure - Yoon *et al.* (1995) demonstrated neutralizing antibodies 9 days after experimental infection with PRRSV - and are directed against GP4, GP5 and the M-protein. GP5 is the major neutralizing antibodies determinant, and seems to play a big role in immune response to PRRSV (Murtaugh *et al.*, 2002). The antibody response against GP5 is delayed, and this might be due to a so-called 'decoy epitope' distracting the immune system from the neutralizing epitope, the 'epitope B' (Lopez & Osorio, 2004).

Whether neutralizing antibodies can prevent viremia or not is not clear (Lopez & Osorio, 2004).

Figure 1: Reactions by the immune system when a pig is infected with PRRSV (Lopez & Osorio, 2004).



A cell-mediated immune response against PRRSV can be measured by the level of IFN-γ. This will reflect the T cell response, since IFN-γ is secreted by T cells. An IFN-γ response will appear about 4 weeks after infection (Murtaugh *et al.*, 2002), see Figure 1.

PRRSV is most likely eliminated by means of neutralizing antibodies and cell-mediated immunity (Murtaugh *et al.*, 2002). PRRSV probably has an immunosuppressive effect, triggering secondary bacterial infections. Maternal antibodies transferred through colostrum against PRRSV (passive immunity) can be found in piglets for up to 8 weeks of age (Murtaugh *et al.*, 2002). Stadejek *et al.* (2005) found maternal antibodies in a piglet 43 days old.

The duration of protective immunity was studied by Lager *et al.* (1997), who found it to last for at least 604 days post experimental exposure to PRRSV (homologues challenge). Other studies found specific antibodies measured by serum neutralization test (SNT) lasting for 105 days (Yoon *et al.*, 1995), and 213 days (Wills *et al.*, 1997a).

1.5 Clinical signs

Clinical signs are described in a review by Rossow (1998), which encompasses both field and experimental observations. Clinical signs of infection are mainly reproductive failure in sows, and pneumonia in pigs of all ages. Infection with PRRSV may be subclinical (Rossow, 1998).

Pneumonia due to infection with PRRSV is commonly seen in nursery and finishing pigs. Pneumonia will often be complicated by concurrent respiratory bacterial infections. In weaned pigs, fever, lethargy and death can also be seen (Rossow, 1998).

Reproductive failure because of infection with PRRSV can be seen through abortions (ranging from sporadic abortions to abortion storms), premature or late farrowings, and late return to estrus after weaning. Infection with PRRSV in the third trimester will result in late-term abortion or premature farrowing, or stillborn and mummified piglets. Sows may show signs of infection in the shape of anorexia, pyrexia, agalactia and lethargy (Rossow, 1998). It has been shown that sows having viremia shortly after insemination might give birth to viremic piglets due to the transplacental transmission of PRRSV (Han *et al.*, 2012).

Litters from affected sows will have a higher prevalence of stillborn and mummified piglets. Furthermore, these litters will often be unthrifty and have higher morbidity and mortality than litters from healthy sows. The piglets may show clinical signs such as dyspnea, tachypnea, periocular edema, conjunctivitis, inappetence, cutaneuos erythema and anorexia (Done *et al.*, 1996; Rossow, 1998). It is described how the infection can give blue (cyanosed) skin, especially ears and vulva (Done *et al.*, 1996; Rossow, 1998).

PRRSV in finishing pigs, boars, gilts and sows is seen mainly by a transient fever and inappetance (Rossow, 1998).

Type 2 PRRSV is more virulent than type 1 PRRSV, according to a study by Halbur *et al.*, (1995), which demonstrated that type 2 PRRSV caused more severe clinical signs than type 1 PRRSV.

1.5.1 Lesions at necropsy

At necropsy, no or minimal signs of infection with PRRS will show macroscopically. Interstitial pneumonia and consolidation of the lungs can be seen, but affection of the lungs is often caused by secondary bacterial infections, which PRRS predisposes for (Rossow, 1998; Wensvoort *et al.*, 1991). Markedly enlarged lymph nodes might be seen, especially in young pigs (Rossow, 1998).

At histology, interstitial pneumonia with septal thickening by mononuclear cells, necrotic debris in alveoli, and type 2 pneumocyte hypertrophy and hyperplasia can be seen. Furthermore, lymphadenopathy, characterized by follicular hypertrophy, hyperplasia and necrosis, may show (Halbur *et al.*, 1995).

1.6 Diagnostics

A diagnosis of PRRS can be confirmed by the use of clinical signs and diagnostic tests. Serological conversion can be shown by the use of ELISA, IPMA, IFA or SNT (Christopher-Hennings *et al.*, 2002). Virus can be shown by the use of PCR (infective and non-infective virus) and/or cultivation in cells (infective virus). The virus can be cultivated in vitro in non-porcine cell clones derived from MA104 monkey kidney cells (MARC-145 and CL2621) (Bautista *et al.*, 1993; Kim *et al.*, 1993).

1.6.1 Serological tests

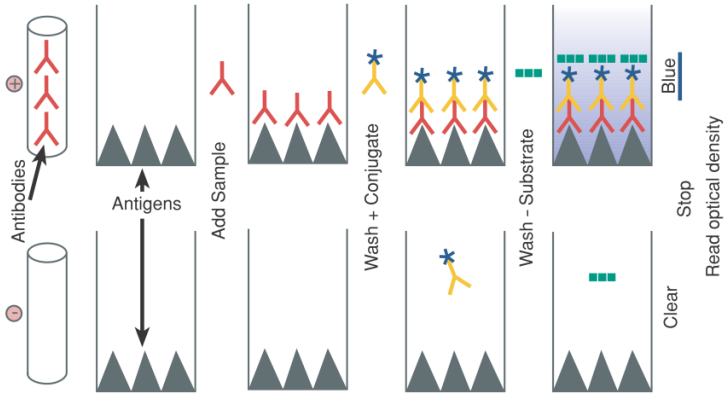
Serological tests are used for herd diagnostics, and are not suitable for individual testing (Bøtner, 1997). Nevertheless, the tests are used for testing individual animals e.g. boars at semen collection centers and with animals for export (personal communication; Klara Tølbøll Lauritsen, NVI, DTU).

Serological tests to diagnose PRRS include (Christopher-Hennings *et al.*, 2002):

- ELISA (enzyme-linked immunosorbent assay)
- IPMA (immunoperoxidase monolayer assay)
- IFA (indirect fluorescent antibody test)
- SNT (serum neutralization test)

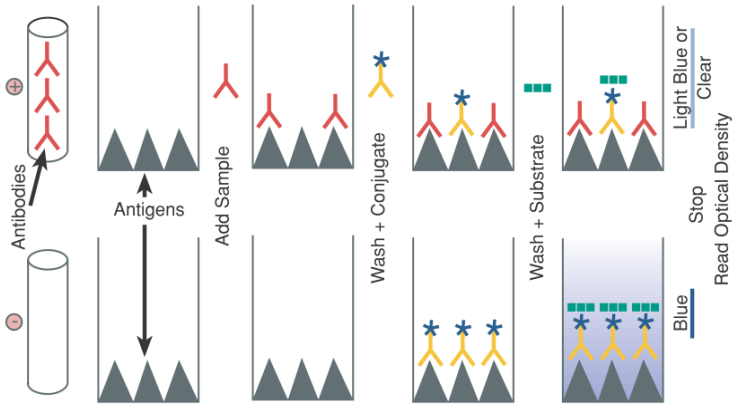
ELISA (enzyme-linked immunosorbent assay) is a diagnostic method used to detect antibodies or antigens in a sample. There exist different kinds of ELISA, but all utilize the same principle, which is binding between antigens and antibodies in a well. Through the use of conjugated antibodies and a substrate, colour changes can be observed, and can be read off by the use of a plate spectrophotometer and an optical density (OD) value is given (Christopher-Hennings *et al.*, 2002).
'Herdchek PRRS ELISA' is a commercial ELISA, which utilizes an indirect ELISA method (Ferrin *et al.*, 2004), see Figure 2. The kit includes both type 1 and type 2 PRRSV antigens. In this ELISA, a sample-to-positive (S:P) ratio ≥ 0.4 is interpreted as positive, meaning there are antibodies against PRRSV in the sample (Christopher-Hennings *et al.*, 2002). The sensitivity and specificity are described as 97.4% and 99.6%, respectively (Ferrin *et al.*, 2004).

Figure 2: Steps in an indirect ELISA. The figure shows a positive sample on the top and a negative sample in the bottom. Antibodies from the positive sample react with the conjugated antibodies, and by the use of a substrate a colour change can be read off by a spectrophotometer ('ELISA Technical Guide', https://www.idexx.com/pdf/en_us/livestock-poultry/elisa-technical-guide.pdf).



The principle in a blocking ELISA is almost the same as in an indirect ELISA. The difference between these two tests is that in the blocking ELISA the conjugated antibodies bind to the antigens in the well, instead of potential antibodies in the sample. Positive samples will be colourless in this type of ELISA – compared to other types of ELISA, where colour indicates a positive result.

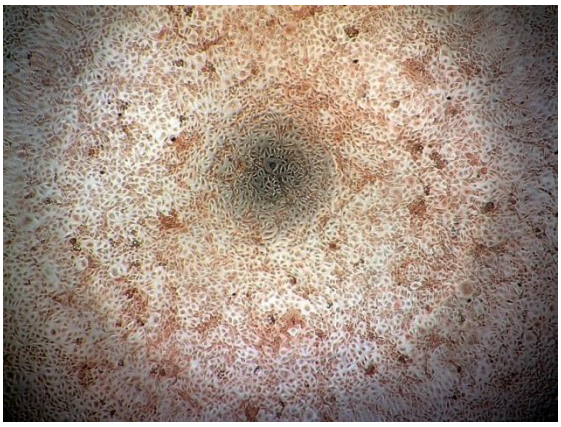
Figure 3: Steps in a blocking ELISA. The figure shows a positive sample on the top and a negative sample in the bottom. In the positive sample, antibodies will bind to the antigens in the well. Conjugated antibodies will bind to the antigens in the well, and by the use of a substrate a colour change can be read off by the use of a spectrophotometer (ELISA Technical Guide', https://www.idexx.com/pdf/en_us/livestock-poultry/elisa-technical-guide.pdf).



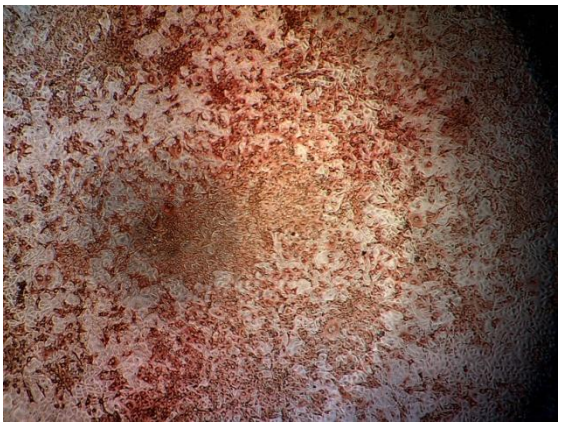
In Denmark there are two options when it comes to ELISA testing. In Kjellerup, at "Laboratorium for Svinesygdomme", the commercial HerdChek PRRS ELISA is used. At NVI, DTU, a blocking ELISA is performed. The difference between the two ELISA types is that HerdChek PRRS ELISA can't differentiate between type 1 and 2 PRRSV. HerdChek PRRS ELISA will show whether there are antibodies against PRRSV in the sample or not. The blocking ELISA performed at NVI has the ability to distinguish between type 1 and type 2 antibodies.
The advantages of using ELISA is that it is a rapid and simple method, which can be used on a large scale (Christianson & Joo, 1994). It is shown that pigs will seroconvert in ELISA 7-14 days after infection (A. Bøtner, 1997).

IPMA (immunoperoxidase monolayer assay, Danish: IPT, immunoperoxidase test) is not routinely used in North America, but is widely used in Europe including Denmark (Christianson & Joo, 1994). The principle of the test is that a serum sample is added to a microtiter plate with a fixated monolayer of PRRSV-infected cells (type 1 or type 2, one type for each plate). Different dilutions are used to determine a titre value. If there are antibodies present in the serum sample, they will bind to the antigens in the wells. Peroxidise-labelled IgG antibodies are added, and will bind to potential antibodies in the sample. An addition of ethylcarbazole induces a colour change in the positive samples, and the results can be read off through the use of a microscope (Bøtner *et al.*, 1994). If a sample is positive, the cells will be stained dark red. The highest positive titre value constitutes the result. The interpretation of the results has to be carried out by a trained person, because the test has a subjective endpoint (see Picture 1 and 2). It is a semiquantitative method due to the different dilutions. The advantages of IPMA is that it is highly specific, but the disadvantage is that is has a subjective endpoint (Christianson & Joo, 1994). A high IPMA-value is an indication of an early stage of infection with PRRSV or recent vaccination (Bøtner, 1997). Detection of antibodies against PRRSV is possible 7-14 days after infection (Bøtner, 1997).

Picture 1: IPMA, PRRSV type 2, negative, titre value: 0.



Picture 2: IPMA, PRRSV type 2, positive, titre value: 250.



Pictures kindly provided by Klara Tølbøll Lauritsen, NVI, DTU.

IFA (indirect fluorescent antibody test) is a test similar to IPMA, with the difference that the conjugated antibodies used are fluoroscein-labelled anti-porcine antibodies and not peroxidise-labelled. IFA is used extensively in the USA (Christianson & Joo, 1994; Christopher-Hennings *et al.*, 2002). The advantages and disadvantages of IFA are the same as in IPMA (Christianson & Joo, 1994).

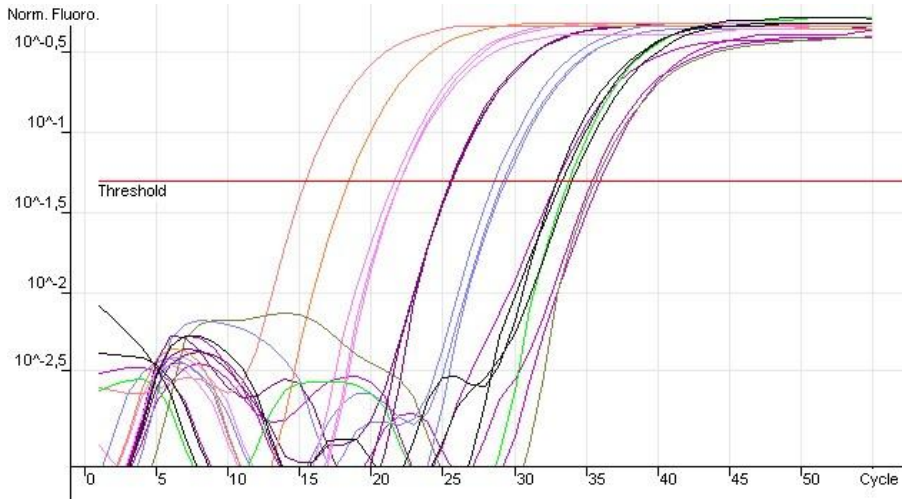
SNT (serum neutralization test) is a test based on a cell-culture with a known level of virus. Serum from a sample is diluted into various dilutions and added to the assay, and after incubation it can be determined whether the antibodies in the sample were capable of neutralizing virus. The result is read off in a microscope, where cytopathic changes are indicative of neutralized virus (Christopher-Hennings *et al.*, 2002).
This test is less sensitive than IFA and ELISA, because it is measuring neutralizing antibodies, and they appear later than non-neutralizing antibodies (Christopher-Hennings *et al.*, 2002; Lopez & Osorio, 2004). The advantages of the SNT test are that it can show whether the antibodies in the serum sample are neutralizing or not. The disadvantages are that it is expensive and time-consuming. In general this test should mainly be used as a research tool (Christopher-Hennings *et al.*, 2002).

It should be kept in mind that a serological test can't differentiate between vaccination-derived antibodies and wild type antibodies (Christopher-Hennings *et al.*, 2002). Furthermore, it does not tell whether the animals are viremic or not.

1.6.2 PCR

The principle in PCR is to amplify a specific genetic target to a detectable level. This is carried out through the use of primers, dNTPs (deoxyribonucleotides) and a Taq-polymerase that is a heat-resistant enzyme. The reaction is performed by temperature cycling. High temperatures are required for the separation of DNA strands, then the temperature is lowered to let primers anneal. Finally, the temperature is raised again (to 72°C) to let polymerase extend the primers by incorporating the dNTPs (Kubista *et al.*, 2006).
The genetic target can be detected in different ways. In conventional PCR, this is carried out through the use of agarose gel electrophoresis. In real-time PCR (qPCR) it is carried out through the use of fluorescent probes or dyes. The amount of fluorescent signal is related to the amount of PCR product in the sample, and the amount of fluorescent signal is measured in each cycle. Results are illustrated by a graph drawn by the use of computer software - see Figure 4.

Figure 4: An example of a graph carried out by Rotor-Gene Q Series Software (QIAGEN). One curve represents one sample. The cycle threshold, C_t, is defined as the number of cycles required for the fluorescent signal to cross a defined threshold (horizontal line). The figure is kindly provided by senior adviser Charlotte Kristiane Hjulsager, NVI, DTU.



In a conventional PCR, the result provided is either 'positive' or 'negative', and is thus qualitative. qPCR provides quantitative information about the amplification process as it progresses, and results can be seen in *real time* through the use of computer software.

The real time detection used in qPCR is performed using non-specific DNA binding dyes or sequence-specific probes. An example of a DNA binding dye is SYBR® Green I, which detects all double-stranded DNA in a sample. The most commonly used method is the probe-based detection, where dual-labelled target-specific probes are used. The probe consists of oligonucleotides which are complementary to a sequence within the target template, and it has a quencher and a fluorophor attached. The probe anneals to the target strand, and as the Taq-polymerase elongates the strand, the probe is hydrolysed by exonuclease activity. The result is that the fluorophor is no longer quenched, and a signal is released and can be read by computer software, which plots the results into a graph (see Figure 4) (Kubista *et al.*, 2006).

A cycle threshold (C_t) is defined as the number of cycles required for the fluorescent signal to cross a defined threshold (to exceed background noise). This is shown on the graph drawn by the computer software - see Figure 4. The more viral copies in a sample, the higher the fluorescent signal will be, and the lower the C_t-value. In other words, a low C_t-value indicates a strong positive result, and a high C_t-value indicates a weak positive result. No C_t-value means that there was no signal crossing the threshold, and the sample is therefore interpreted as negative. C_t-value corresponds to a number of viral copies which can be quantified by interpolation of a standard curve (not shown). Standard curves are generated by diluting the template control (with a known concentration of virus) into tenfold dilution series, and plotting the C_t-values against the log copy number (Pestana *et al.*, 2010).

When PCR has to be performed on an RNA template, such as viral RNA from PRRSV, the RNA has to be extracted from the sample matrix and converted to DNA before running the PCR-process. The conversion from RNA to complementary DNA (cDNA) is catalyzed by the enzyme reverse transcriptase (RT) (Christopher-Hennings *et al.*, 2002). A real time PCR process preceded by the reverse transcriptase step is called RT-qPCR, or real time RT-PCR.

Most RT-qPCR assays for detection of PRRSV targets ORF6 and/or ORF7 (Christopher-Hennings *et al.*, 2002; Wernike *et al.*, 2012). The reason for this is that ORF6 and ORF7 are considered the most conserved parts of the PRRSV genome (Christopher-Hennings *et al.*, 2002). When sequencing to type PRRSV, the ge-

nome of PRRSV ORF5 is typically used, due to the high variability of this ORF (Christopher-Hennings *et al.*, 2002; Shi *et al.*, 2010). It is possible to perform a multiplex RT-qPCR (Wernike *et al.*, 2012), where two or more targets are amplified by the use of multiple primers in a single reaction (Pestana *et al.*, 2010). This is beneficial for PRRSV, due to the high genomic variability and the existence of different types of PRRSV (Wernike *et al.*, 2012), which can be detected in a single reaction with a multiplex RT-qPCR.
Various different types of specimens are possible in testing for PRRSV. What is important to know is where virus exists in the body. Examples of what is usually used for RT-qPCR, with regard to PRRSV, are semen, serum (Christopher-Hennings *et al.*, 2002), and oral fluid samples (Heiselberg *et al.*, 2012).
Due to the high costs of individual sampling, and to save time, pooling of samples for RT-qPCR is widely used. Whether pooling of samples for PRRSV detection decreases sensitivity was investigated by Rovira *et al.*, (2007). The study found a decrease in sensitivity in serum pools of 3 and 5 samples compared to individual samples. When pooling samples, the potential decrease in sensitivity has to be taken into account, as well as potential interference between components in the sample material, which might also lead to a lower sensitivity. The assay therefore has to be validated.
Advantages of the use of RT-qPCR are that it is a rapid method, and it has a high sensitivity and specificity. The risk of contamination is reduced in RT-qPCR compared to conventional PCR, because it takes places in a 'closed system'. Furthermore, it is possible to quantify the amount of virus in the sample. Disadvantages of the use of RT-qPCR are that the equipment is expensive and advanced. When using an assay with a high sensitivity, it has to be taken into account that any contamination might show as a weak positive result. Primers have to be very specific, since viral genome might not be detected if the genomic variance is too large between primers and the viral RNA in the sample. This implies that PCR assays often have to be revalidated, especially when it comes to PRRSV due to the high mutation rate and high genomic variability (Christopher-Hennings *et al.*, 2002).

1.7 Treatment, control and eradication

Since PRRSV is a virus, there is no direct treatment. However, PRRSV predisposes for bacterial infections, which can be treated with antibiotics. Focus must lie on prevention of introducing the disease into the herd. This can be achieved by following certain regulations and recommendations set up by SPF SUS, Denmark. Examples of guidelines could be having an 'access room', and when purchasing new animals (e.g. replacement gilts) they should always have the same or higher status (SPF-Sundhedsstyringen, 2015).
When wishing to eradicate or control PRRS in a sow herd, it is crucial to know whether the herd is stable. A stable breeding herd is defined as a herd without evidence of sow-to-sow or sow-to-piglet transmission of PRRSV, or in short a herd with a lack of virus transmission (Dee, 2003). This can be evaluated by looking at the status of the weaned or older piglets which is desired to be PRRSV negative. A way to achieve stability in a breeding herd can be through management of the replacement gilt pool (Dee *et al.*, 1995), and by following 'MCREBEL' (Management Changes to Reduce Exposure to Bacteria to Eliminate Losses) (McCaw, 2003) to limit transmission of virus. Points in MCREBEL are (copied from Dee *et al.*, 1996):

- Cross-foster only during the first 24 hours of life
- Do not move sows or piglets between rooms
- Humanely destroy piglets that become sick and are unlikely to recover
- Minimize handling of piglets, especially administration of routine antibiotics or extra iron injections
- Do not transfer undersized pigs back to rooms containing younger litters
- Move nursery pigs according to strict all in / all out principles, allowing for 2–3 days between groups for cleaning and disinfecting

Eradication of PRRS from a herd can be achieved in several ways (Baker, 2009); there are however two main ways which can be modified: complete depopulation, and partial depopulation (Kristensen *et al.*, 2014).
In a complete depopulation all animals are culled, and the farm is washed and disinfected. New PRRS-negative animals are inserted after 3 weeks (Kristensen *et al.*, 2014). In a partial depopulation, only sections with transmission of virus are emptied, washed and disinfected. To gain knowledge of virus transmission and whether the herd is stable or not, a serological profile of the herd can be made (Kristensen *et al.*, 2014; Rossow, 1998). Partial depopulation is used in the case the herd is not stable. Transmission of virus will often be seen among the weaned piglets for which reason this section is often depopulated in an eradication strategy. This is called nursery depopulation (Dee, 2003).
An example of another known eradication strategy is the 'Load – Close – Homogenize' (also known as LCH) strategy. In this method the farm is loaded with replacement gilts for 200 days' use, and then the farm is closed for new animals. The pigs on the farm are homogenized by the use of MLV corresponding to the type of PRRS existing on the farm. All adult pigs are vaccinated twice, with a month between. Piglets are vaccinated once. The principle in LCH is that there is no transmission of virus after 200 days, and thus no option of transmission to the new PRRS-seronegative animals introduced (Baker, 2009; Charlotte Sonne Kristensen & Glenting, 2013).
Before starting an eradication process, a few things have to be considered. Among these are the odds of reinfection, whether the workers are motivated and what the sales market looks like – is it possible to sell the PRRS-negative pigs (Kristensen *et al.*, 2014)? The odds of reinfection by transmission of virus through the air can be calculated by studying a geographic information system (GIS) map (Mortensen, 2001).
Biosecurity is crucial when it comes to PRRS strategies. In Lambert *et al.*, (2012) a definition of biosecurity is given as "procedures, efforts and programs established to reduce the risk of new disease introduction (external biosecurity) or to slow down the transmission of endemic pathogens into populations (internal biosecurity)". In a PRRS-positive a good internal biosecurity can be achieved by following MCREBEL. When maintaining a stable status regarding PRRS it has to be emphasized that all adult animals have the same immunological status with regards to PRRS (Kristensen *et al.*, 2014). Improper management of replacement gilts can have consequences affecting the PRRS stability in the herd. Introduction of seronegative replacement gilts in a breeding herd might be a reason for persistent transmission of virus (Dee *et al.*, 1995).
Additional risk factors for the introduction of PRRSV to a farm can be herd size (the larger the herd, the greater the risk), absence of quarantine facilities, and introduction of new animals into the herd (Albina, 1997).

1.7.1 Vaccines available and safety of MLV

A list of vaccines available in Denmark is seen in Table 3. The protection of inactivated vaccines compared to MLV against challenge with PRRSV was studied by Zuckermann *et al.*, (2007). The study showed that the inactivated vaccine (Progressis®, Merial) applied did not protect against a homologues strain of PRRSV when challenged. The inactivated vaccine is proposed by the company (Merial Norden A/S) to be used as a booster after vaccination with MLV type 1 (personal communication; Michael Albin, Merial Norden A/S). Several studies have shown an efficiency of MLV against homologues challenge (Labarque *et al.*, 2004; Martelli *et al.*, 2007; van Woensel *et al.*, 1998). As mentioned, there is poor cross-protection between the different strains (Labarque *et al.*, 2004; van Woensel *et al.*, 1998), meaning that vaccination must target the relevant type of PRRSV existing in the herd. Newer studies has demonstrated a partial cross-protection of a new PRRSV type 2 MLV (Fostera® PRRS, Zoetis) against challenge with PRRSV type 1 (Park *et al.*, 2015).
Regarding the safety of MLV, it has to be emphasized that vaccination of sows (lactating and pregnant) with MLV is off-label use (medicintildyr.dk) in Denmark, but is approved in the rest of Europe. This is due to a risk of vertical transmission of virus to the foetuses (Bøtner *et al.*, 1997) potentially resulting in stillborn, mummi-

fied and weak piglets (Rossow, 1998). The risk of potential replication of vaccine virus in the herd also has to be taken into account when using MLV (Martínez-Lobo *et al.*, 2013). An example of the spread of vaccine virus is the introduction of PRRS type 2 in Denmark (Bøtner *et al.*, 1997). Lymph node enlargement and lung lesions might be seen after vaccination with MLV (Martínez-Lobo *et al.*, 2013; Mengeling *et al.*, 2003), but in general the MLV's are considered clinically safe.

Table 3: A list of selected vaccines available in Denmark (medicintildyr.dk and personal communication with Boehringer Ingelheim).

Modified-live vaccine (MLV)	Inactivated vaccine
Ingelvac PRRS® MLV	**Progressis®**
Porcilis® PRRS	**Suivac® PRRS-IN**

1.8 Gilt recruitment in PRRS-positive sow herds

When introducing new animals to a farm, it is important that the new animals match the existing animals on the farm in terms of immunity. For pig farms there are two ways to recruit replacement gilts. Either they buy the gilts from another farm, or they produce the gilts themselves. The most secure way, when it comes to spread of disease, is to produce the gilts on the farm. The disadvantage of this might be a loss of genetic progress, but that subject is beyond the scope of this paper.

New replacement gilts – bought or produced on the farm – have to be immunized against the relevant diseases existing on the farm. When introducing replacement gilts into a PRRS-positive farm, they have to be immunized (Kristensen *et al.*, 2014), and this can be carried out in different ways (Mengeling, 2005). It has to be emphasized that animals vaccinated with a PRRS MLV are protected against a challenge with a homologues strain, but not against heterological strain (Labarque *et al.*, 2004; van Woensel *et al.*, 1998).

The most common methods in Denmark are vaccination with PRRS MLV, or natural immunization. In natural immunization, the idea is to mix PRRSV-positive animals with PRRSV-naïve replacement gilts. The disadvantage of this method is that it can't be known for sure whether the PRRS-positive animals are actually shedding virus, and thus immunizing the replacement gilts (Mengeling, 2005).

Replacement gilts that are vaccinated with a PRRS MLV can be vaccinated at the supplier's farm, or on the farm itself. If the replacement gilts have to be vaccinated on the farm, it is recommended to utilize a quarantine for at least 12 weeks after vaccination to avoid the spread of virus (Kristensen *et al.*, 2014). An example of a way to handle this is to have two quarantines ('double twelve'), which are filled with replacement gilts every 6th week, thus making it possible to take PRRSV stable replacement gilts into the sow herd every sixth week (Kristensen *et al.*, 2014).

When introducing seronegative gilts to a PRRS-positive sow herd with an ongoing virus transmission, there is a risk of transmission of virus to the naïve gilts, which results in viremia and shedding of PRRSV (Kristensen *et al.*, 2014). Sows having viremia shortly after insemination might give birth to viremic piglets due to the transplacental transmission of PRRSV (Han *et al.*, 2012).

1.9 Economic impact of PRRS

The consequences of a PRRS outbreak in a herd can be economically devastating due to multiple factors. Stillborn piglets and abortions cause reduced litter size, which is further reduced due to deaths caused by respiratory problems among weaners, owing to PRRSV. Daily weight gain and feed efficiency is also affected negatively. To this is added increased costs for medicine and an increased amount of work. The economic losses are mainly due to the reduced number of sold piglets / gilts. A Dutch study from 2012 found that the

average loss during an outbreak was 1.7 pigs per sow, with a mean loss per sow of €125 (Nieuwenhuis *et al.*, 2012). An American study from 2005 found a loss per litter of $74 (Neumann *et al.*, 2005). A Danish study showed a loss during a PRRS outbreak of between 30-1059 DKK per sow, with a mean average of 329 DKK per sow, and no difference in number of weaned piglets per sow per year compared to the Dutch study (Kristensen *et al.*, 2013).
The quotation for a 30kg pig in Denmark is higher (20 DKK, January 2016) if the pig is PRRS-negative (Smågrisenotering, 2016). This encourages pig producers to be/remain PRRS-negative.

1.10 PRRS surveillance in Denmark

PRRS in Denmark is mainly monitored by the SPF-system, and approximately 78% of all sow herds are a member of this system. Of them, 70% are PRRS-negative (SPF Sundhedsstyringen, 2015). A qualified estimate of the prevalence of PRRS-positive herds – including conventional herds – in Denmark is 35% (Kristensen *et al.*, 2014).
SPF is an abbreviation for 'specific pathogen free'. The aim of the SPF-system in Denmark is to monitor health status in swine herds in Denmark. The health status is taken into account when trading and moving pigs. In the system there is a list of 7 diseases which need to be declared in all member farms. The list can be found at spfsus.dk. PRRS (type 1 and type 2) is one among the seven SPF-diseases. To stay in the SPF-system, it is required to send in blood samples from a number of animals for surveillance once a year or once a month (depending on status). The blood samples are tested by ELISA and IPMA, to prove that there are no antibodies against PRRSV in the herd.
PRRS is an OIE-listed disease, thus a reportable disease (OIE, 2015). According to Danish law, *"BEK nr. 54 af 26/01/2011: Bekendtgørelse om lister over smitsomme sygdomme til lov om hold af dyr"*, PRRS is a list 2 disease, and when diagnosed it has to be reported to the public authorities according to *"LBK nr. 466 af 15/05/2014: Bekendtgørelse af lov om hold af dyr"*.
Certain countries (Argentina, China and Ukraine) won't accept imported pork from PRRSV-positive herds, meaning that virus must not have been detected within the last year. If a herd is investigated for PRRS virus, the slaughterhouse therefore has to be advised, and the consequence is no export from the herd concerned (Petersen, 2014).
For research matters it is possible to detect PRRS virus by PCR, as long as the samples are blinded.

2. The Gilt Project

One way to control Porcine reproductive and respiratory syndrome (PRRS) is by having a Porcine reproductive and respiratory syndrome virus (PRRSV)-stable sow herd, where breeding animals have antibodies against PRRSV and there is no circulation of PRRSV. A challenge to the PRRSV stable sow herd is the introduction of replacement gilts, which may be obtained by vaccination followed by a quarantine period. Quarantine is needed due to the viremia, which develops after vaccination with MLV (Martínez-Lobo *et al.*, 2013) or natural infection with PRRSV. Recommendations in Denmark for farms with PRRS are to introduce new breeding animals through a quarantine of 12 weeks. The animals are supposed to be vaccinated with a PRRS MLV or exposed to a wildtype virus, and then wait for 12 weeks before being taken into the sow herd (Kristensen *et al.*, 2014). In some herds however it is not practically possible to manage an all-in all-out quarantine for 12 weeks. Therefore there is a need for alternative ways of introducing replacement gilts which ensure that the sow herd remains PRRSV-stable. Hopefully this study will lead to optimised guidelines in how to introduce replacement gilts to a PRRS-positive sow herd.

2.1 Objectives

The objective of this study was to compare the use of PRRS MLV and quarantine facilities with the PRRSV status of replacement gilts at first insemination. Furthermore, the study aimed at looking at antibody levels compared to time from vaccination with PRRS MLV and age of the animals when vaccinated.

2.2 Hypotheses

The main hypothesis was that – at herd level – there is no correlation between the use of PRRS MLV, quarantine facilities, and PRRSV status at first insemination. PRRSV status was defined by RT-qPCR status (positive / negative) and ELISA status (positive / negative).

To investigate this hypothesis, other hypotheses needed to be investigated:

- **1a.** H0: There is no relation between PRRSV status of replacement gilts at first insemination and the use of quarantine
- **1b.** H0: There is no relation between PRRSV status of replacement gilts at first insemination and the duration of quarantine
- **1c.** H0: There is no relation between PRRSV status of replacement gilts at first insemination and the gilt recruitment strategy
- **1d.** H0: For farms that buy gilts, there is no relation between PRRSV status of replacement gilts at first insemination and number of suppliers
- **1e.** H0: For farms that buy gilts, there is no relation between PRRSV status of replacement gilts at first insemination and number of deliveries of replacement gilts

Other hypotheses regarding antibodies levels were tested:

- **2a.** H0: There is no relation between the level of antibodies and age when vaccinated
- **2b.** H0: There is no relation between the level of antibodies and time from vaccination to blood samples is taken

3. Materials and methods

3.1 Study design

The present study was a retrospective cross-sectional study performed in 75 Danish sow herds, of which 69 were included. The criteria for the farms to be included in the study were that they were known to have antibodies against PRRSV; type 1, 2 or both. Furthermore they should have an immunisation strategy for replacement gilts.
The study aimed at being a representative sample for PRRS-positive sow herds in Denmark

3.1.1 Sample size

It was assumed that 20% of the herds using quarantine and 60% not using quarantine would have unstable replacement gilts at first insemination. Being stable was defined as being negative by PCR and positive by ELISA, and the opposite applied for being unstable. With a power of 0.8 and an alpha value of 0.05, it required 23 herds in each group to prove this difference, meaning a total of 46 herds (Houe *et al.*, 2004).

3.1.2 Sampling

Five blood samples were taken at each farm from replacement gilts close to first insemination (a few days before, or on the day of, insemination). The farmer pointed out the gilts which were close to insemination. Blood samples were taken from the jugular vein with a needle (18G), serum tubes and a vacutainer. The gilts were restrained by the use of a nose snare.
The first 24 herds were visited in spring 2014, in connection with another project performed by SEGES Danish Pig Research Centre. Veterinarians from Team Health took the blood samples. The remaining herds (25 to 75) were visited from spring 2015 to September 2015, mainly by the author, but also with help from veterinarian practitioners.
The blood samples were sent to “Laboratorium for Svinesygdomme” in Kjellerup, Denmark. Samples were blinded here, and then send to the National Veterinary Institute (NVI), Technical University of Denmark, for analysis. The blood samples were tested for PRRSV by RT-qPCR, and for antibodies against PRRSV by ELISA. If a sample was positive in ELISA, it was tested by IPMA.
A stable group of gilts was defined as ‘all animals in the group having antibodies against PRRSV shown by ELISA’ and ‘all animals in the group not having viremia shown by RT-qPCR’. A group of gilts corresponds to the 5 blood samples taken in each herd.

3.1.3 Questionnaire

In each farm a questionnaire regarding handling of PRRS vaccination, quarantine facilities and replacement gilts was filled out (see Appendix A / B), by asking the farmer or their veterinarian for the answers. One question from the questionnaire was removed due to ambiguity (Danish: “Køres poltestalden efter AI/AU”; English: “Is the quarantine managed all-in all-out”) - see Appendix A / B.
Each farm was visited once. For the first 24 herds processed in spring 2014 by SEGES Danish Pig Research Centre, questionnaires were filled out by using an iPad. For the remaining herds a paper questionnaire was filled out by hand, and later on typed into a computer.
From the questionnaire it could be decided whether the quarantine facilities in the herd were optimal or not. Optimal quarantine facilities were defined on the basis of 3 questions regarding quarantine:

- Does the quarantine share air with the other sections? Optimal answer: ‘no’
- Is there a separate entrance to the quarantine? Optimal answer: ‘yes’
- Is the quarantine empty of animals before new animals are introduced? Optimal answer: ‘yes’

Non-optimal quarantine facilities included every other farm that was not included in ‘optimal’. The purpose of these questions was to investigate if the quarantine really was a quarantine. It can be discussed whether a quarantine that shares air with other sections, is missing a separate entrance, or is not empty before the introduction of new animals, actually functions as a quarantine.
Contact with the farms was made with help from their veterinarian, which means that farms were not chosen randomly.

3.2 Laboratory analysis

The laboratory tests were all performed at the National Veterinary Institute (NVI), Technical University of Denmark. The level of PRRSV was assessed by testing serum samples by real time reverse transcriptase polymerase chain reaction (RT-qPCR). Initially, five samples from one herd were tested in a pool, and, if positive, they were analysed individually. The serum samples were also tested for antibodies against PRRSV using the serological tests ELISA and IPMA. IPMA was only run when the samples were positive in ELISA.

3.2.1 Real time reverse transcriptase polymerase chain reaction (RT-qPCR)

The RT-qPCR assay used in this study for detection of PRRSV RNA was the Kleiboeker modified 1 assay (Wernike *et al.*, 2012), modified by using HEX instead of TEX in the NA-probe. It is a multiplex RT-qPCR assay, which uses multiple primers and probes to simultaneously detect and type PRRSV - see Table 4.
Five serum samples (all from the same farm) were pooled into one sample due to cost and time constraints. RNA from a 140 µL pooled serum sample was extracted using QIAamp Viral RNA Mini Kit (QIAGEN, Copenhagen, Denmark), automated on a QIAcube (QIAGEN) extraction robot according to instructions from the supplier. RNA was kept at -80°C until the RT-qPCR was run.
Each PCR reaction contained: 10 µL RNase-free water, 5 µL Qiagen OneStep RT-PCR buffer, 1 µL dNTP mix 10 mM, 0.75 µL of each primer (see Table 4) 10 µM, 0.5 µL of each probe (see Table 4) 10 µM, 1 µL Qiagen OneStep RT-PCR Enzyme Mix (QIAGEN), and 2 µL purified RNA, in a total volume of 25 µL.
The reactions were run on Rotor-Gene Q (QIAGEN). The thermal cycler program consisted of 50°C for 1800 sec, 95°C for 900 sec, and 45 cycles at 94°C for 15 sec, 60°C for 60 sec, and 72°C for 10 sec. Fluorescent signal was acquired at the 60°C step in the green and yellow channels, detecting PRRSV type 1 and type 2 respectively. The data was analyzed with Rotor-Gene Q Series Software (QIAGEN).

Table 4: Specific designed primers used (Wernike *et al.*, 2012).

Forward primer	Reverse primer	Probe	Genomic target
EU-1: GCACCAC CTCACCCRRAC	EU-1: CAGTTCCTG CRCCYTGAT	EU-1: 6-FAM-CCTCTGYYTG CAATCGATCCAGAC-BHQ1	ORF 6, ORF 7
EU-2: CAGATGCAGAY TGTGTTGCCT	EU-2: TGGAGDCC TGCAGCACTTTC	EU-2: 6-FAM-ATACATTCTGG CCCCTGCCCAYCACGT-BHQ1	ORF 6, ORF 7
NA: ATRATGRG CTGGCATTC	NA: ACACGGTC GCCCTAATTG	NA: *TEX-TGTGGTGAATGGCA CTGATTGACA-BHQ2	ORF 7, 3'UTR

**In this study HEX fluorophore was used instead of TEX.*

3.2.2 Serological tests: Blocking ELISA

The ELISA used was an 'in-house' blocking ELISA conducted at NVI, DTU. The laboratory facilities and methods are accredited by The Danish Accreditation Fund, ISO/IEC17025:2005 (DANAK, 2015). The ELISA is a serological analysis which detects PRRSV antibodies in serum samples. The analysis can discriminate between type 1 and type 2 PRRSV. The method is described by Sørensen *et al.* (1997).

ELISA microplates were coated with type 1 and type 2 PRRSV, one type per plate. Serum samples were added to the plates and incubated overnight. Type 1 plates were incubated at room temperature, type 2 plates at 15 degrees. If there were any antibodies present in the sample, they would bind to the antigens in the well. Next day the plates were washed, and biotinylated antibodies (secondary antibodies) were added, specific to each type of PRRSV. These conjugated polyclonal antibodies bind to antigens in the well - if the antigens are not occupied by antibodies from the sample (if the test samples are positive). After incubation, avidin horseradish peroxidase (HRP) was added, binding to the biotinylated antibodies present in cases where the sample was negative. Another incubation step later, tetra-methyl-benzidine (TMB) was added, reacting with the HRP present in the negative sample. Finally, sulphuric acid, H_2SO_4, was added to stop the reaction. The potential colour change seen in negative samples was read using a spectrophotometer and an OD-value (optical density) was given. OD-values above 44 were considered negative, and OD-values of 44 or below were considered positive (Sørensen *et al.*, 1997).

Type 1 / type 2 ratio was determined (Sørensen, 1998) by dividing the type 1 OD% values with the type 2 OD% values:

$$R = OD\%\ type1\ /\ OD\%\ type2$$

A ratio of less than 1.3 indicates type 1 PRRSV antibodies, and a ratio of above 1.9 indicates type 2 PRRSV antibodies.

Blocking ELISA was performed on all blood samples in the study.

3.2.3 Serological tests: IPMA

The technique used was described by Bøtner *et al.* (1994). IPMA microtiter plates with a fixated monolayer consisting of a cell culture of PRRSV infected cells in wells were prepared at Lindholm, NVI. PRRSV type 1 on one plate, and type 2 on another. Serum samples were diluted 1:50, 1:250, 1:1250 and 1:6250. Each serum sample was tested separately both on PRRSV type 1 and type 2 microtiter plates. The plates were incubated and washed, and peroxidase conjugated goat anti-swine IgG was added. After incubation and wash, ethylcarbazole and hydrogen peroxide (H_20_2) was added. These two reagents react with the peroxidase, and a red colour staining of the cells can be seen if there are antibodies against PRRSV present in the serum

sample. Results were read off through a microscope, and the highest positive titre value constituted the result. The interpretation of the results was carried out by a trained technician. IPMA was only performed when ELISA was positive, meaning that IPMA has not been performed on every blood sample in the study. IPMA was used as a confirmatory test for ELISA.

IPMA categorization

A categorization of IPMA-values (high or low) was needed in order to fulfill the work on the hypotheses in this area. The categorization was carried out on the basis of the claim stated by Bøtner (1997), which is that a high IPMA-value is an indication of an early stage of infection with PRRSV or recent vaccination. In other words: a high IPMA-value could be an indication of a recent viremia. Low IPMA-values were categorized as serum concentration titre values of 1:0, 1:50 and 1:250, and high titre values were categorized as being 1:1250 and 1:6250.

To categorize the results, it was necessary to interpret the IPMA results from each farm and decide which category the farm belonged to. These questions had to be answered in order to categorize the farms:

- Does the farm have type 1 or type 2 PRRSV seen from the ELISA ratio, and do the IPMA results confirm this? A majority of samples pointing at a certain type of PRRSV is concluded to have that type of PRRSV. For example, a majority of OD-ratios below 1.3 indicates PRRSV type 1, and the IPMA results should then primarily have positive titre-values for PRRSV type 1.
- Is the interpretation of the ELISA and IPMA results consistent with the vaccine used at the farm? If yes, the farm is included.
- Which titre category does the farm belong to, low or high?

3.3 Statistical analysis

When relevant it was evaluated whether data was following a normal distribution or not. This was carried out by making histograms and QQ-plots. Normally distributed data is described with minimum and maximum values, mean, standard deviation and standard error of the mean (SEM). SEM was calculated to give an estimate of the true mean in the population of Danish gilts. Mean is shown with a 95% confidence interval. Data that are not distributed normally are described using minimum and maximum values, and quartiles including the median. Level of significance used was 5%. The statistical analyses were performed using R, 'The R Project for Statistical Computing' (www.r-project.org).

For the first five hypotheses, stability of the gilts ('stable' or 'not stable') was the dichotomous outcome, and the independent variables tested were:

1a. Use of quarantine: 'yes' or 'no'
1b. Duration of quarantine (categorized): 'short' or 'long'
1c. Gilt recruitment strategy: 'own' or 'bought'
1d. Number of suppliers (categorized): '1' or '>1'
1e. Number of deliveries (categorized): '<=5' or '>5'

For the remaining two hypotheses, level of antibodies was categorized into 'low' or 'high' which was the outcome. The independent variables tested were:

2a. Age when vaccinated (categorized): 'young' or 'old'
2b. Time from vaccination (categorized): 'short' or 'long'

All variables are categorical. To evaluate the association between two qualitative variables, Fisher's exact test was used. Level of significance used was 5%.

4. Results

4.1 Farms excluded from the study

Six farms were removed from the project for different reasons. One farm was taken out due to discrepancy in the questionnaire. In another farm, the wrong blood sample tubes were used, and the necessary blood sample analyses could not be completed, so the farm had to be excluded. Three farms were in the middle of a sanitation process and thus didn't have a routine strategy for handling PRRS and gilts, and therefore they had to be excluded. In one farm there had been a vaccination failure, and the gilts that were blood sampled were thus not following the regular PRRS vaccination strategy on the farm. The gilts in this particular farm had been vaccinated 2 weeks before sampling. Before this was known, RT-qPCR, ELISA and IPMA were run on the samples from this particular farm. It was decided to include these results to verify the RT-qPCR - see section 4.3.

4.2 Data from questionnaires

The description of the data from the questionnaires is shown in the following section.

4.2.1 Status in the herds

The distribution of the type of PRRS on the farm is shown in Figure 5. The majority of the farms have PRRS type 1, one-third has type 2 and the remaining farms have both types. The distribution of PRRS type 1 and 2 in Danish sow herds are not known, since the SPF-system does not divide status in sow herds from status in other herds, e.g. slaughter herds (SPF Sundhedsstyringen, 2015). As a result it can't be determined whether this graph represents sow herds in Denmark.

The distribution of SPF sow herds and conventional sow herds in the Gilt Project and in Denmark is shown in Figure 6. The Gilt Project and the distribution in Denmark are shown together to give an impression of whether the Gilt Project could represent Denmark when it comes to SPF status or conventional status.

To investigate this, a Fisher's exact test was carried out. The null hypothesis tested was that there was no difference between the Gilt Project and Denmark, in terms of status. The *p*-value was 0.01, and the hypothesis was accepted, meaning that there was a difference between the Gilt Project and Denmark in terms of status, and thus the Gilt Project could not represent Denmark.

Figure 5: Distribution of PRRS status in the herds in the dataset (*n*=69).

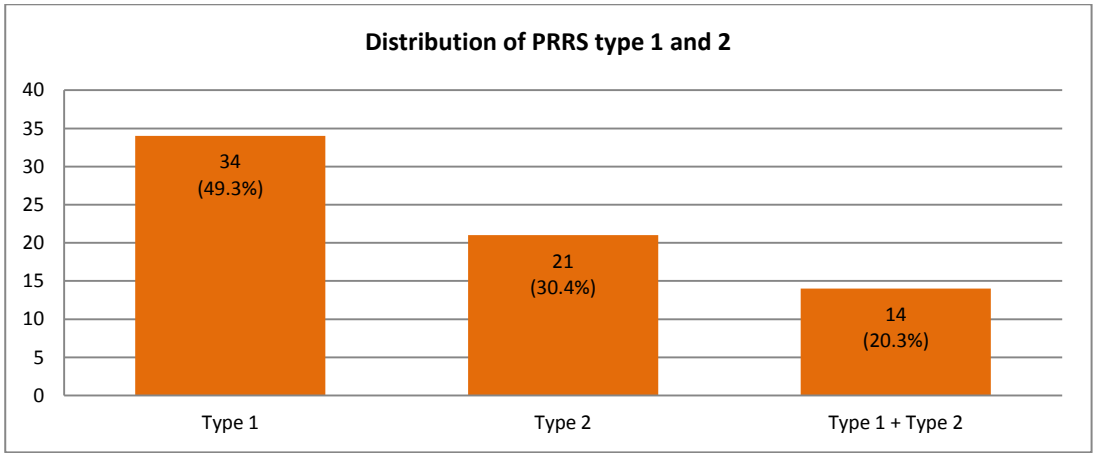
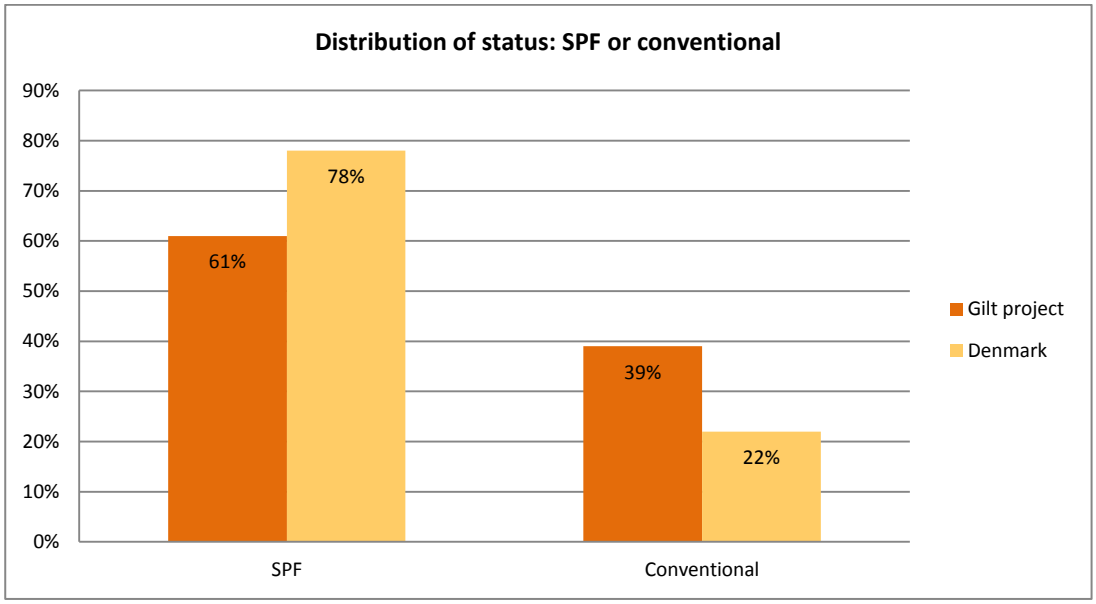


Figure 6: Distribution of SPF sow herds and conventional sow herds in Gilt Project and in Denmark (SPF Sundhedsstyringen, 2015).



The production of PRRSV-negative weaners is an indication of PRRSV is under control in the herd i.e. the herd is stable. Whether the farmer presumed that the piglets were PRRSV negative at weaning was investigated. In Table 5 it can be seen that a majority of farmers presume that the piglets are PRRSV negative at weaning. Please note that these answers represent the farmer's own perception, and may not reflect the truth in the herd.

Table 5: Distribution of answers to the question: 'Do you suppose that the pigs are PRRSV free at weaning?' (*n*=69).

Do you suppose that the pigs are PRRSV free at weaning?		
Yes	**No**	**Don't know**
51 (73.9%)	16 (23.2%)	2 (2.9%)

4.2.2 Gilt recruitment strategy

It was asked which gilt recruitment strategy was used on the farm. The distribution of farms that buy gilts, farms that produce their own gilts, and farms that do both, are shown in Figure 7. The majority of the farms in the study buys gilts (*n*=40), and a small proportion has a mixed gilt recruitment strategy (*n*=7). Out of the 7 farms that mix, 4 farms primarily (more than 50%) buy gilts, and 3 farms primarily produce their own gilts - see Figure 8.

To give an overview of the seven farms which have a mixed gilt recruitment strategy, a short description of each farm is given:

- No 1 produces 20% gilts and buys 80%. All gilts go into quarantine
- No 2 produces 60% gilts and buys 40%. All gilts go into quarantine
- No 3 produces 5% gilts themselves, and buys 95%. All gilts go into quarantine
- No 4 produces 35% gilts and buys 65%. All gilts go into quarantine
- No 5 produces 50% gilts and buys 50%. All gilts go into quarantine
- No 6 produces 95% gilts and buys 5%. Only bought gilts go into quarantine
- No 7 produces 75% gilts and buys 25%. No gilts go into quarantine

Figure 7: Gilt recruitment (*n*=69).

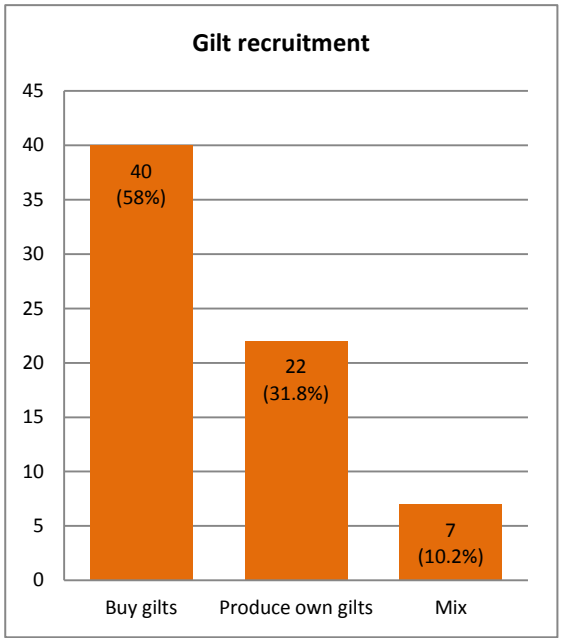
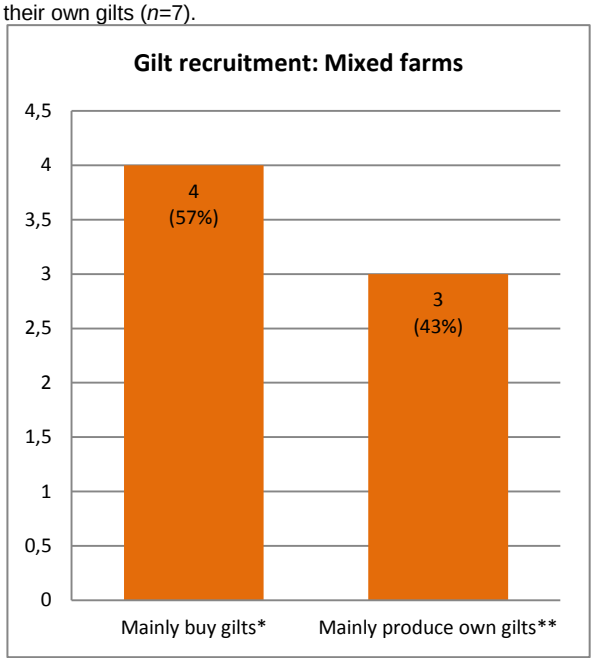


Figure 8: Distribution of farms that both buy gilts and produce their own gilts (*n*=7).



**Over 50% of the gilts are bought*

***Over 50% of the gilts are produced on the farm*

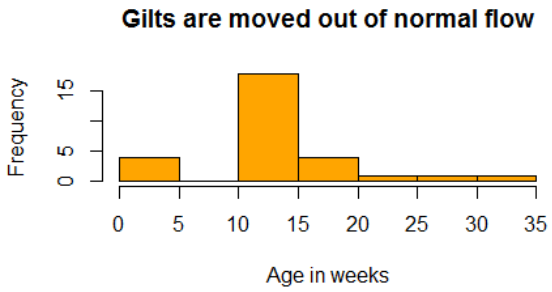
4.2.3 Production of own gilts

There was one question regarding the management of gilts for farms that produce the gilts themselves (see Table 6 and Appendix A/B for the questionnaire). The purpose of the question was to gain knowledge of how the gilts are handled compared to normal slaughter pigs in the herd. The majority of the farms (*n*=15, 52%) move the gilts out of the normal flow at 12 weeks. One farm did not move the gilts out of the normal flow until insemination. Data does not follow a normal distribution, as seen by the histogram in Figure 9.

Table 6: Data from farms that produce own gilts (*n*=22), including farms that have a mixed gilt recruitment strategy (*n*=7). Total *n*=29.

Own production of gilts	Minimum	25% quantile	Median	75% quantile	Maximum
At what age are the breeding animals moved out of the normal flow [weeks]?	4	12	12	15	33

Figure 9: Data are not normally distributed.



4.2.4 Purchase of gilts

There were several different questions regarding the purchase of gilts (see Appendix A/B for the questionnaire).

Number of bought gilts per year is shown in Table 7. The median is 340 gilts per year, ranging from 50 to 2000, as seen in the boxplot in Figure 11. From the histogram in Figure 10 it can be seen that data is not normally distributed.

Number of suppliers over the last two years is also shown in Table 7. The majority of the farms only had one supplier over the last two years (72.5%), 9 farms had two suppliers (22.5%), and 2 farms had three suppliers over the last two years (5%). The median is 1. From the histogram in Figure 12, it can be seen that data is not normally distributed.

Table 7: Data from farms that purchase gilts (*n*=40). Data for 'Number of purchased animals each year ' and 'Number of suppliers over the last two years' does not follow a normal distribution as seen in Figure 10, 11 and 12.

Purchase of gilts	Minimum	25% quantile	Median	75% quantile	Maximum
Number of bought gilts pr year	50	257.5	340	525	2000
Number of suppliers the last two years	1	1	1	2	3

Figure 10: Number of purchased gilts per year, data is not normally distributed.

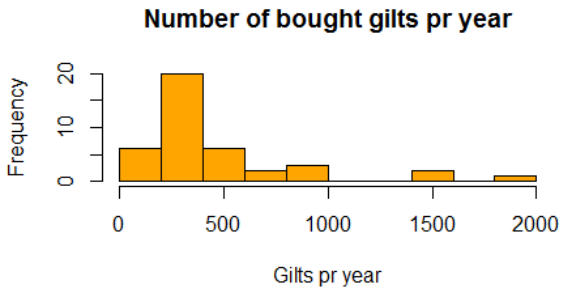


Figure 11: Boxplot showing number of bought gilts per year. Outliers are 1000, 1500, 1600 and 2000.

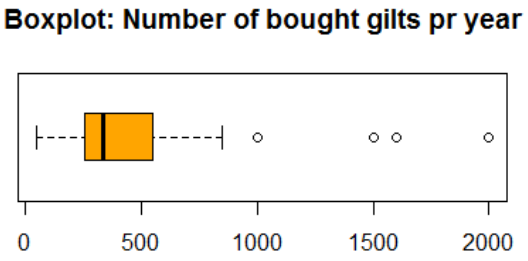
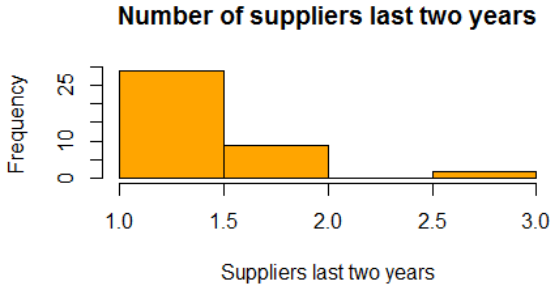


Figure 12: Number of suppliers last two years, data is not normally distributed.



Number of deliveries in the last year is shown in Table 8. Data ranges from 1 to 17 deliveries in the last year. From the histogram in Figure 13, it can be seen that the value '17' is an outlier, meaning this number stands out from the rest. When this value is removed, and a new histogram is drawn (see Figure 14), it can be seen that the data follows a normal distribution. This is confirmed by looking at the QQ-plot in Figure 15, where it

can be seen that the dots lie approximately on a straight line. Had there been more observations, the value of 17 might not have been interpreted as an outlier.
Mean when outlier is removed is 5.9±0.74 deliveries in the last year. Standard deviation is 2.3, and standard error of the mean is 0.38.

Table 8: Table showing 'number of deliveries in the last year'. Number of observations was not 40 as expected due to missing answers in two farms and due to removal of an outlier (*n*=37).

	Minimum	Mean±95%CI	SD	SEM	Maximum
Number of deliveries in the last year					
- Without outlier (*n*=37)	1	5.9±0.74	2.3	0.38	12

Figure 13: Number of deliveries in the last year, not normally distributed.

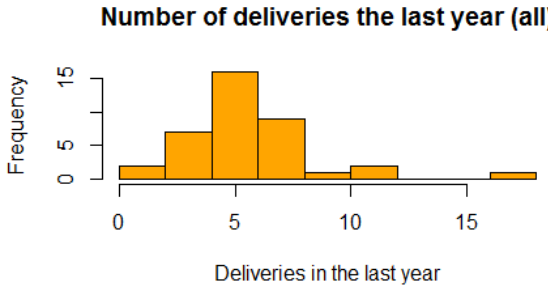


Figure 14: Number of deliveries in the last year without outlier (value=17), the data follows a normal distribution.

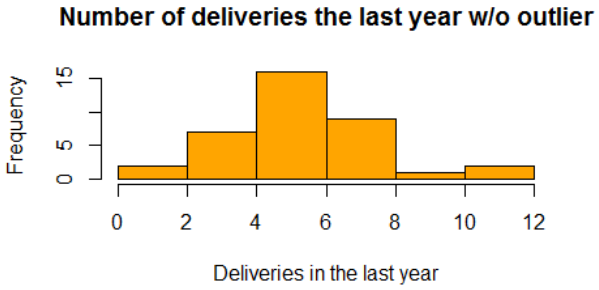
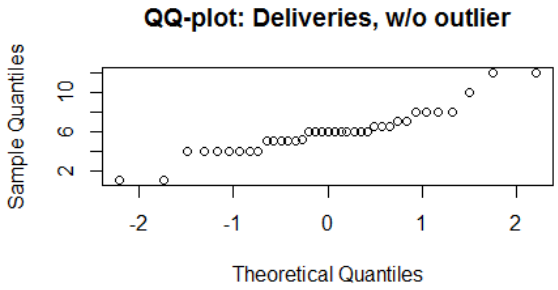


Figure 15: QQ-plot over number of deliveries in the last year without outlier (=17); the data follows a normal distribution.



Age of the purchased gilts at arrival is described with an interval, meaning that answers on the questionnaires ranged from minimum age to maximum age (see Appendix A/B). From the histograms in Figure 16 and 17 it can be seen that data is not normally distributed. In Table 9, data is summarized. An average gilt in the present study is between 15-22 weeks when bought. The youngest gilts are 6 weeks and the oldest are 31 weeks.

Table 9: Data from farms (*n*=39). One farm did not answer these questions.

Age of purchased animals at arrival	Minimum	25% quantile	Median	75% quantile	Maximum
From minimum [weeks]	6	12	15	17.5	28
To maximum [weeks]	13	20	22	24	31

Figure 16: Histogram showing age at arrival, minimum. Data is not normally distributed.

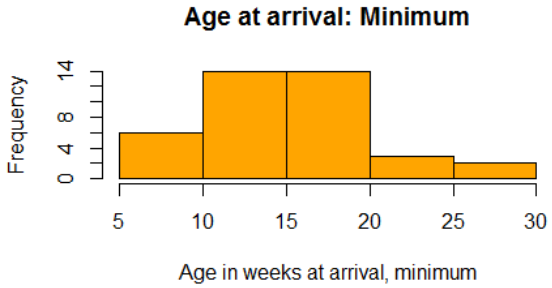
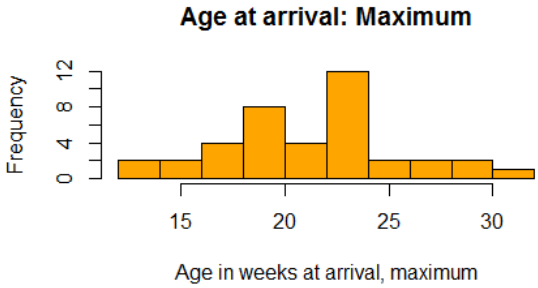


Figure 17: Histogram showing age at arrival, maximum. Data is not normally distributed.



PRRS-status of the bought gilts and whether they are vaccinated or not before arrival is shown in Table 10. Regarding the PRRS-status of the bought gilts, 9 farms buy gilts that are PRRS type 1 positive. Among these 9 farms, 8 farms buy gilts vaccinated with PRRS type 1 before arrival. One farm buys PRRS type 1 positive gilts that are not vaccinated before arrival. Distribution of the PRRS status of the 9 farms, is that 5 farms has PRRS type 1 and 4 farms has both PRRS type 1 and 2.
Another farm buys PRRS-negative gilts, but they are vaccinated against type 1 PRRS before arrival. It could be discussed whether these gilts are PRRS-negative, when they are vaccinated. No farms in the study buy gilts that are PRRS type 2 positive.

Table 10: Data from farms (*n*=40).

Status of purchased animals	'Yes'	'No'	Missing answer
PRRS type 1	9 (22.5%)	31 (77.5%)	0
PRRS type 2	0	39 (97.5%)	1 (2.5%)
Are purchased animals vaccinated before arrival*	9 (22,5%)	31 (77,5%)	0

**They might be vaccinated again in the farm; that's why these numbers are not consistent with the numbers in Table 15.*

4.2.5 Quarantine and quarantine facilities

In Table 11 and in Figure 18 the distribution of farms that utilize quarantine for the gilts, own and bought, are shown. For farms that buy gilts the majority use quarantine (72.5%). For farms that produce their own gilts, half of the farms use quarantine. In total the majority of gilts in the present study go to quarantine.

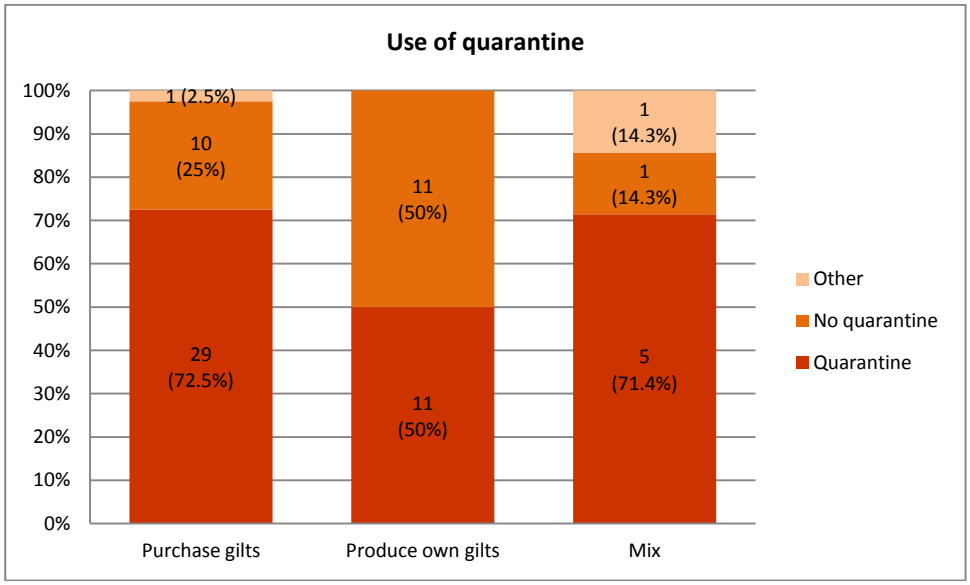
Table 11: Proportion of farms which utilizes quarantine (*n*=69).

	Purchase gilts (*n*=40)	Produce own gilts (*n*=22)	Mix* (*n*=7)	Total (*n*=69)
Quarantine	29 (72.5%)	11 (50%)	5 (71.4%)	45 (65.2%)
No quarantine	10 (25%)	11 (50%)	1 (14.3%)	22 (31.9%)
Other	1 (2.5%)[a]	-	1 (14,3%)[b]	2 (2.9%)

**Mix means farms that have a mixed gilt recruitment strategy (both buying gilts and producing their own gilts).*

[a]Missing answer, [b]One farm that has a mixed gilt recruitment strategy used quarantine for the bought gilts, but not for the gilts that are produced on the farm, see also section 4.2.2.

Figure 18: Use of quarantine (*n*=67).



The quarantine facilities are investigated with three different questions seen in Table 12. The purpose of these questions was to see whether the quarantine was 'optimal' or 'not optimal'. An optimal quarantine was defined on the basis of the 3 questions mentioned before:

- Does the quarantine share air with the other sections? Optimal answer: 'no'
- Is there a separate entrance to the quarantine? Optimal answer: 'yes'
- Is the quarantine empty of animals before new animals are introduced? Optimal answer: 'yes'

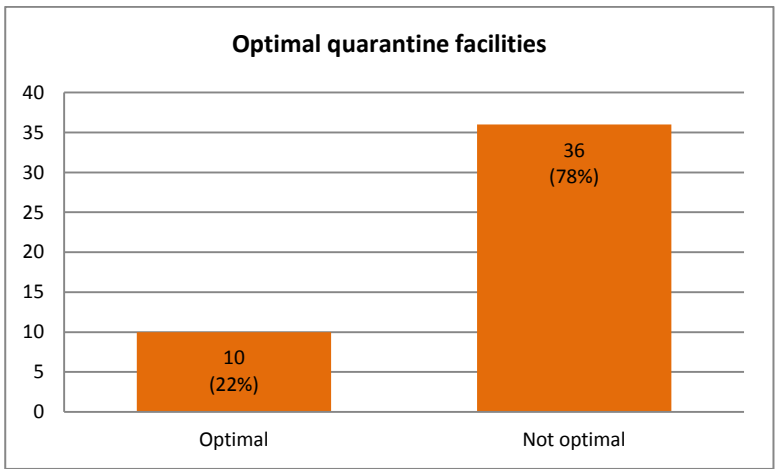
Non-optimal quarantine facilities include every other farm that is not included in 'optimal'.
Results are shown in Figure 19, where it can be seen that the majority of the farms claiming to use quarantine do not have 'optimal' quarantine facilities (78%). It can be discussed whether the quarantines used are actually quarantines.
The 10 farms with optimal quarantine facilities all had a quarantine length between 8 and 16 weeks (not shown).

Table 12: Data from farms with quarantine concerning quarantine facilities (*n*=46). Expected *n*=45, but this table included the one farm had a mixed gilt recruitment strategy and utilized quarantine for the bought gilts, but not for the gilts produced on the farm.

Quarantine facilities	Yes	No	Total
Does the quarantine share air with the other sections?	25 (54.3%)	21 (45.7%)	46 (100%)
Is there a separate entrance to the quarantine?	30 (66.6%)	15 (33.3%)	45[a] (100%)
Is the quarantine empty before new animals are introduced?	27 (58.7%)	19 (41.3%)	46 (100%)

[a]*One missing answer*

Figure 19: Distribution of farms claiming to use quarantine, divided into farms which have optimal quarantine facilities and farms which do not have optimal quarantine facilities. The reason why the number of farms is 46 and not 45 (see Table 11) in this graph is that there is one farm with a mixed gilt recruitment strategy (bought gilts go to quarantine, but own gilts don't), and this was not included in the 45 in Table 11. *N*=46.



Length of quarantine is shown in Table 13. From the histogram in Figure 20 and the QQ-plot in Figure 21 it can be seen that data roughly follows a normal distribution. It is decided to show all possible data in Table 13, because data roughly follows a normal distribution.
It can be seen that the median is 8 weeks, and the mean is 9.4±0.93 weeks. Interval of quarantine length ranges from 2 weeks to 18 weeks. One-third of the observations were 8 weeks (not shown). 7 farms were following recommendations of 12 weeks quarantine. One-third of the farms had a quarantine length between 12 and 18 weeks.

Table 13: Length of quarantine (*n*=48). From Table 11 it can be seen that 45 farms utilize quarantine. The reason why the number of farms is 48 and not 45 in this table is that the one farm with a mixed gilt recruitment strategy was not included in the 45 in Table 11, even though the bought gilts go to quarantine. In addition there is one farm, that doesn't answer whether the gilts go to quarantine or not, but the answers the question regarding length of quarantine. It can be assumed they use a quarantine. Another farm answers that they don't use a quarantine, but gives an answer as to how long the gilts are in quarantine.

	Minimum	25% quantile	Median	Mean±95%CI	SD	SEM	75% quantile	Maximum
How long are the animals in quarantines? [weeks]	2	8	8	9.4±0.93	3.3	0.48	12	18

Figure 20: Histogram showing length of quarantine; data does roughly follow a normal distribution.

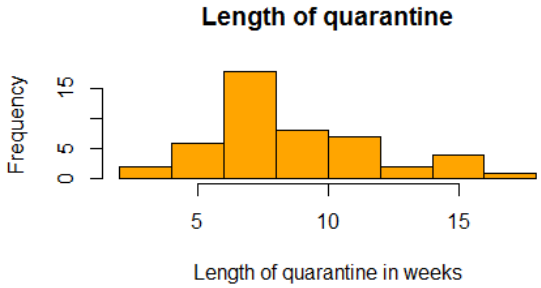
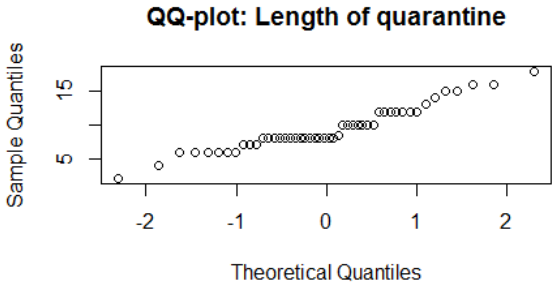


Figure 21: QQ-plot showing that data on length of quarantine does roughly follow a normal distribution.



4.2.6 Blood samples before introduction to the sow herd

Whether the gilts are blood-sampled to investigate them for PRRSV before introduction to the sow herd is shown in Table 14. The absolute majority of the farms never blood-sample the gilts before introduction to the sow herd. 2 farms always blood-sample the gilts, and 5 farms do it occassionally.

Table 14: Blood samples before introduction to the sow herd (*n*=49). 46 farms used quarantine, but for some reason 49 farms answer the question regarding blood samples. Of the 49 farms, 2 farms claim that the gilts don't go to quarantine, and 1 farm does not answer whether the gilts go to quarantine or not.

Blood samples before introduction to sow herd	Always	Sometimes	Never
Are the animals investigated for PRRSV before being introduced to the sow herd?	2 (4%)	5 (10%)	42 (86%)

4.2.7 Vaccination and vaccines

Almost every farm the in the study vaccinates the gilts against PRRSV (91%). This can be seen in Table 15. There are 4 farms that do not vaccinate the gilts on the farm, but the gilts are vaccinated at the supplier's place and the vaccine used is known.

There is missing information regarding vaccines in two farms. One farm, which buys PRRSV type 1 positive gilts, does not give information about the age when the gilts are vaccinated, so it can't be decided if the gilts are vaccinated on farm. Maybe the farmer doesn't know, or maybe the gilts are simply not vaccinated against PRRSV. The other farm with missing data produces their own gilts and doesn't give any information on vaccines.

Table 15: Data on vaccination on the farm (*n*=69). 4 of the 4 farms in the 'no' category buy gilts that are already vaccinated upon arrival.

Vaccination	Yes	No	Missing data
Are animals vaccinated against PRRS on the farm?	63 (91%)	4 (6%)	2 (3%)

Vaccine used in the farm is compared to the PRRS-status of the farm (Figure 5) in Table 16. It can be concluded that the chosen vaccine is consistent with the type of PRRS in the farm.

Table 16: Distribution of vaccines compared to PRRS status (type 1, type 2, type 1+ type 2) in the herd (see Figure 5) (*n*=69).

Vaccine	Type 1 (*n*=34)	Type 2 (*n*=21)	Type 1 + type 2 (*n*=14)	Total (*n*=69)
Porcilis® PRRS	33 (97%)	-	3 (21.5%)	36
Ingelvac® PRRS MLV	-	20 (95%)	3 (21.5%)	23
Both	-	-	7 (50%)	7
Other [a]	-	-	1 (7%)	1
No info of vaccination[b]	1 (3%)	1 (5%)	-	2

[a] *Other vaccine is Ingelvac® PRRS MLV and Suivac® PRRS-IN (inactivated vaccine), which was a combination used only in one farm.*

[b] *Gilts are already vaccinated when they arrive, name of vaccine is not known.*

Age of the gilts when they are vaccinated is shown in Table 17. As seen in Figure 23 data just roughly follows a normal distribution, so all data possible is shown. The youngest gilts are 6 weeks old when vaccinated and the oldest is 33. Mean is 15.9±1.26 weeks, and the median is 15 weeks. From the histogram in Figure 22 it can be seen that the majority of the gilts are vaccinated between 10 weeks old and 20 weeks old.

Table 17: Age when vaccinated (*n*=67).

	Minimum	25% quantile	Median	Mean± 95%CI	SD	SEM	75% quantile	Maximum
Age when vaccinated [weeks]	6	12	15	15.9±1.26	5.27	0.64	18	33

Figure 22: Histogram showing distribution of age when vaccinated. Data roughly follows a normal distribution.

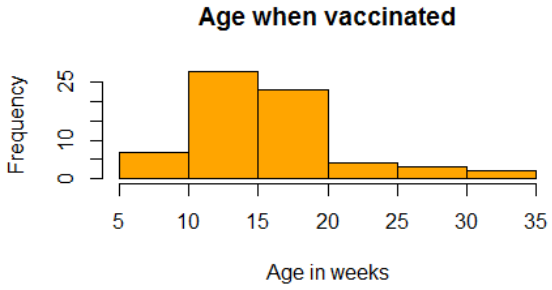
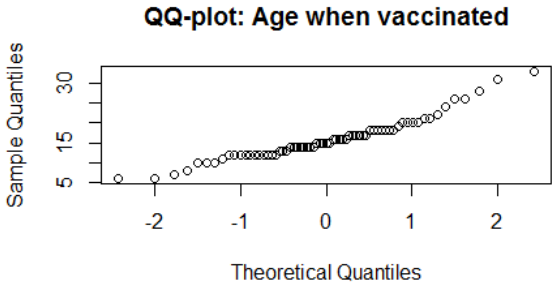


Figure 23: QQ-plot showing age when vaccinated. Data roughly follows a normal distribution.



4.2.8 Results outside the questionnaire

Age of the gilts when blood samples were taken was not a part of the questionnaire, and because of that, this data was not collected on every farm. Some farms did not know the age of the gilts, but the primary reason for the missing data is that it was not requested from the farmer. Age of the gilts when blood sampled was known in 11 cases. The majority of the observations were 32 weeks (*n*=6), and the mean was 32.8±1.08 - see Table 18. Whether data follows a normal distribution or not can't be determined, due to the small number of observations.

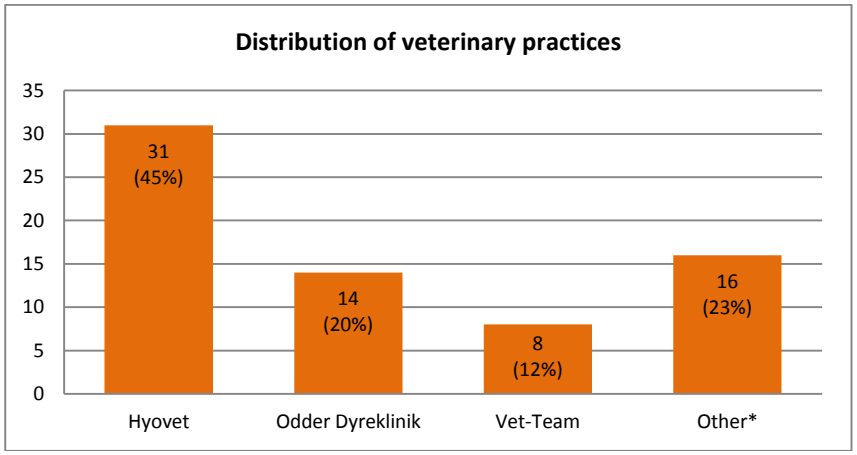
Since blood samples were taken from gilts that were about to be inseminated (same week), the age when blood sampled can be interpreted as the age when inseminated.

Table 18: Age when blood samples were taken (*n*=11).

	Minimum	Median	Mean±95%CI	SD	Maximum
Age when blood sampled [weeks]	30	32	32.8±1.08	1.8	36

The distribution of veterinary practises engaged in each farm is shown in Figure 24. The majority of the farms belong to a certain veterinarian practice (Hyovet). This data was found by looking up the CHR-numbers on the internet (http://chr.fvst.dk).

Figure 24: Distribution of veterinary practices (*n*=69).



**Other veterinary practices.*

4.2.9 The average gilt in the study

Based on all the prior sections in this chapter it can be summarized what an average gilt in the present study looks like. The average gilt is bought (58%) and not produced on the farm. It comes from the same supplier every time, and is delivered to the farm approximately 6 times a year. The age of the gilt when bought is between 15-22 weeks, and the gilt is most likely negative for PRRS type 1, and negative for type 2 PRRS. Most likely the average gilt goes into quarantine, since more than half of the gilts in the study do so.
In cases where the gilt is produced on the farm, it will follow the normal flow until an age of around 12 weeks. It is 50/50 whether the homemade gilts go into quarantine.
The majority of the gilts are vaccinated on the farm, and the age when vaccinated is 15 weeks old.

4.3 RT-qPCR results

RT-qPCR results are shown in Table 19. There were no positive samples among the 69 farms included. Before exclusion of the 6 farms (see section 4.1 for description of farms that were excluded) out the 75 farms that were included at the beginning of the project, all analyses including RT-qPCR was carried out. This means that RT-qPCR results for the excluded farms were available. Among these 6 excluded farms, one farm turned out positive in the PCR test. This is shown in Table 19. The reason for excluding this particular farm was that there had been a failure regarding the vaccine procedure (see section 4.1). The gilts had been vaccinated two weeks before samples were taken, and the farm was therefore not following their vaccine strategy. When a pooled sample was positive in RT-qPCR, it was decided to run the sample again separately. As seen in Table 20, 4 out of 5 serum samples turned out positive for PRRSV type 2. This finding confirms that the RT-qPCR conducted had a high enough sensitivity to find virus in the samples, and that's why this result is included in this report.

Table 19: RT-qPCR results (*n*=69).

PCR positive	PCR negative
0 (0%)	69 (100%)

Table 20: RT-qPCR results for the one positive farm (excluded from the study) run individually. Note: This farm was excluded from the study.

Sample	Ct-value	Type PRRSV
Pooled 55	37.74	Type 2
55A	-	-
55B	36.34	Type 2
55C	33.03	Type 2
55D	38.58	Type 2
55E	37.6	Type 2

4.4 ELISA data

To classify a group as positive, all five gilts had to have a positive result in ELISA. To classify a group as negative, at least one gilt in a group of 5 had to be negative. See Appendix C and D for an example of a group classified as positive in ELISA and a group classified as negative in ELISA, respectively.
In Table 21 it can be seen that the majority of the sampled gilts were ELISA-positive, thus having antibodies against PRRSV. To summarize the 6 farms which were classified as ELISA-negative, and later on as 'unstable', a short description is given:

- 3 farms had one negative gilt, and 4 positive – corresponding to 3 out of 69 farms (4.3%)
- 2 farms where all gilts were negative – corresponding to 2 out of 69 farms (2.9%)
- 1 farm had 2 negative and 3 positive – corresponding to 1 out of 69 farms (1.5%)

All 6 farms had PRRS type 1 and were vaccinating against type 1, expect one farm that had both types and was vaccinating against both types. It can be questioned whether the gilts at the two farms where all gilts were negative were vaccinated at all. When looking into data (not shown) it can be seen that both farms claim to vaccinate with Porcilis® PRRS.

Table 21: ELISA results (*n*=69).

ELISA-positive	ELISA-negative
63 (91.3%)	6 (8.7%)

4.5 IPMA data

IPMA was only performed when samples were positive in ELISA. Results from IPMA were categorized into two groups: Low IPMA-values and high IPMA-values, see section 3.2.3. Not all farms were suitable for this categorization, e.g. farms that were vaccinating against both types of PRRSV or were classified as having both types on the farm, and farms having ELISA-negative gilts. Farms with inconclusive results regarding type of PRRSV were also excluded. An example of an inconclusive IPMA results can be seen in Appendix E.
After the classification principle was conducted, 45 farms were left. Out of the 45 farms, 2 farms did not give information on vaccination (age and vaccine), so they were excluded as well, leaving 43 farms included in the IPMA analysis in Table 22. The farms that were excluded after the principle was conducted were not analyzed further.

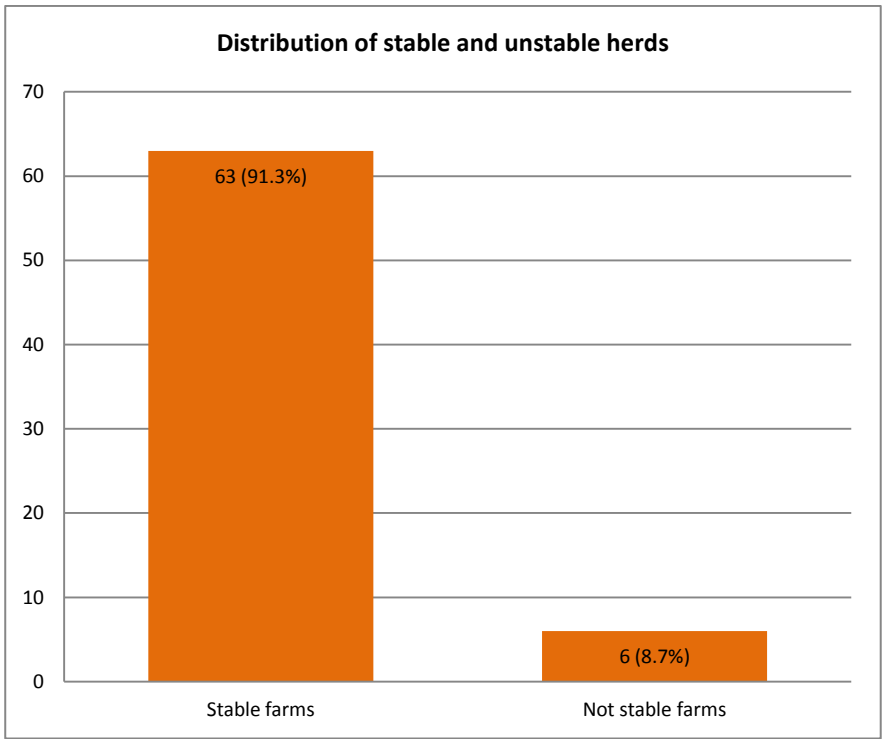
Table 22: IPMA results (*n*=43). Low IPMA-values were classified as serum concentrations of 1:0, 1:50 and 1:250 and high IPMA-values as 1:1250 and 1:6250.

	Type 1 PRRS (*n*=25)	Type 2 PRRS (*n*=18)	Total (*n*=43)
Low IPMA-values	7	9	16
High IPMA-values	18	9	27

4.6 Distribution of stable and unstable farms

When concluding on RT-qPCR results and ELISA results, it can be determined how many stable and unstable farms there are in the study. See section 3.1.2 for definition of a stable farm. Since no included farms were positive by RT-qPCR, the distribution is made solely on ELISA results. There were 6 farms that were negative in ELISA. See Figure 25.

Figure 25: Distribution of stable and unstable herds in the study (*n*=69).



4.7 Analysis of hypotheses

The main hypothesis was that - at the herd level - there is no correlation between the use of PRRS MLV, quarantine facilities and PRRSV status at first insemination. To investigate this hypothesis, other hypotheses needed to be studied. They are studied one by one in the following pages.

4.7.1 Hypotheses regarding stability of the gilts

1a. H0: There is no relation between PRRSV status of replacement gilts at first insemination and the use of quarantine

It is recommended to use a quarantine, and it is seen as a risk factor for having unstable gilts if no quarantine is used.

Table 23: 2x2 table showing the outcome: 'stable' or 'not stable' and the independent variable: quarantine: 'yes' or 'no'. *p*-value = 0.08, (*n*=67). Two farms with either missing data or with a mixed quarantine strategy, see Table 11 for explanation.

	Quarantine – yes	Quarantine – no	Total
Stable	43	18	61
Not stable	2	4	6
Total	45	22	67

The *p*-value was 0.08 and thus the null hypothesis could not be rejected, meaning there was no relation between the PRRSV status of replacement gilts at first insemination and the use of quarantine.
If a lower level of significance was applied, e.g. 0.10, the null hypothesis could have been rejected, meaning that there was a relation between the use of quarantine and whether the gilts are stable or not - in the sense that the use of quarantine results in stable gilts. Overall it could be concluded that there was a strong tendency towards a relation between the use of quarantine and the PRRSV status of replacement gilts at first insemination, meaning that farms that were using quarantine had PRRSV-stable gilts.

Since the first analysis included all answers on 'yes' to using a quarantine (*n*=45), without taking into account whether the quarantine was an optimal quarantine, another analysis was carried out. In this analysis, only farms actually using an optimal quarantine were categorized as using a quarantine (see Figure 19). The rest were categorized as not using quarantine.

Table 24: 2x2 table showing the outcome: 'stable' or 'not stable', and the independent variable: optimal quarantine: 'yes' or 'no'. *p*-value = 1, (*n*=67).

	Optimal quarantine – yes	Quarantine – no	Total
Stable	9	52	61
Not stable	1	5	6
Total	10	57	67

The *p*-value was 1, and thus the null hypothesis could not be rejected, meaning there was no relation between the PRRSV status of replacement gilts at first insemination and the use of an optimal quarantine.

1b. H0: There is no relation between PRRSV status of replacement gilts at first insemination and the duration of quarantine

Duration of quarantine in the study ranged from 2 to 18 weeks, with a mean of 9.4±0.93 - see Table 13. Since about one-third of the observations were 8 weeks, this was chosen as a dividing value. Observations on length of quarantine were categorized into two groups:

- Short quarantine period did include farms with quarantine periods of less than 8 weeks
- Long quarantine period did include farms with quarantine period of 8 weeks or more

Note that this hypothesis only included farms with quarantine. This left 2 farms with unstable gilts, as seen in Table 25.

Table 25: 2x2 table showing the outcome: 'stable' or 'not stable' and the independent variable: quarantine: 'short' or 'long', *p*-value = 1, (*n*=48).

	Short <8 weeks	Long ≥8weeks	Total
Stable	11	35	46
Not stable	0	2	2
Total	11	37	48

The *p*-value was 1, and thus the null hypothesis could not be rejected, meaning there was no relation between the PRRSV status of replacement gilts at first insemination and the duration of quarantine.

1c. H0: There is no relation between PRRSV status of replacement gilts at first insemination and the gilt recruitment strategy

It is considered safer to produce own gilts, because then the herd can remain 'closed', meaning that no new animals are introduced.

Table 26: 2x2 table showing the outcome: 'stable' or 'not stable' and the independent variable: gilt recruitment strategy: 'own' or 'bought', *p*-value = 0.65, (*n*=62).

	Own gilts	Bought gilts	Total
Stable	21	36	57
Not stable	1	4	5
Total	22	40	62

Note, farms that both bought and produced own gilts (*n*=7) were not included in this analysis. The *p*-value was 0.65, and thus the null hypothesis could not be rejected, meaning there was no relation between the PRRSV status of replacement gilts at first insemination and the gilt recruitment strategy.

1d. H0: For farms that buy gilts, there is no relation between PRRSV status of replacement gilts at first insemination and number of suppliers

A high number of suppliers are thought to constitute a risk for having unstable gilts.

Table 27: 2x2 table showing the outcome: 'stable' or 'not stable' and the independent variable: suppliers: '1' or '>1', *p*-value = 1, (*n*=47).

	Suppliers = 1	Suppliers > 1	Total
'Stable'	30	12	42
'Not stable'	4	1	5
Total	34	13	47

Note, farms that both bought and produced own gilts (*n*=7) were included in this analysis. The *p*-value was 1, and thus the null hypothesis could not be rejected, meaning there was no relation between the PRRSV status of replacement gilts at first insemination and number of suppliers.

1e. H0: For farms that buy gilts, there is no relation between PRRSV status of replacement gilts at first insemination and number of deliveries of replacement gilts

A high number of deliveries, and thus a high number of new animal introductions to the herd or quarantine, is thought to constitute a risk for having unstable gilts.

Table 28: 2x2 table showing the outcome: 'stable' or 'not stable' and the independent variable: deliveries: '<=5' or '>5', *p*-value = 1, (*n*=45).

	Deliveries <= 5	Deliveries > 5	Total
'Stable'	17	23	40
'Not stable'	2	3	5
Total	19	26	45

5 or fewer than 5 deliveries was one group, and more than 5 deliveries was another. The *p*-value was 1, and thus the null hypothesis can't be rejected, meaning there was no relation between the PRRSV status of replacement gilts at first insemination and number of deliveries of replacement gilts.

4.7.2 Hypotheses regarding antibodies

2a. H0: There is no relation between the level of antibodies and age when vaccinated

This analysis does not differentiate between the types of PRRS. The IPMA-values were categorized according to the method described in section 3.2.3. 'Age when vaccinated' was categorized into:

- Younger: Gilts that are 12 weeks or younger when vaccinated
- Older: Gilts older than 12 weeks when vaccinated

The rationale for this categorization is that optimal time of vaccination is when maternal antibodies against PRRSV are gone, and they can be detected up to 8 weeks of age (Murtaugh *et al.*, 2002). 12 weeks was chosen, because this is a normal time to move or sell the pigs.
A large portion of the gilts was vaccinated at this time, see Figure 22.

Table 29: 2x2 table showing the outcome: 'high' or 'low' IPMA-value, and the independent variable: age when vaccinated: 'younger' or 'older', *p*-value = 0.75 (*n*=43).

	Age when vaccinated, younger ≤12 weeks	Age when vaccinated, older >12 weeks	Total
Low IPMA-value	5	11	16
High IPMA-value	10	17	27
Total	15	28	43

The *p*-value was 0.75, and thus the null hypothesis could not be rejected, meaning there was no relation between 'age when vaccinated' and the level of antibodies.

2b. H0: There is no relation between the level of antibodies and time from vaccination to blood samples is taken

To calculate 'Time from vaccination to blood samples is taken' it was necessary to know the time of vaccination and the age when blood sampled. Since data on age when blood sampled were only available for 11 farms (see Table 18), this hypothesis could not be tested.

If the age when blood sampled was known for all farms, the interval (in weeks) between vaccination and blood sampling would have been categorized like this:

- Short: Interval between vaccination and blood sampling is 4 weeks or less
- Long: Interval between vaccination and blood sampling is more than 4 weeks

This categorization is based on an article suggesting that IPMA-values will remain high approximately 2-4 weeks after infection (Bøtner, 1997).

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5. Discussion

Recommendations in Denmark for PRRS-positive farms are to introduce replacement gilts through a quarantine of 12 weeks (Kristensen *et al.*, 2014) due to the length of viremia after vaccination with MLV or natural infection. In some herds it is practical impossible to manage a quarantine for 12 weeks. Therefore there is a need for alternative ways to introduce replacement gilts which secure that the sow herd remains PRRS-

stable. This study was investigating whether there exist alternative ways – e.g. shorter duration of quarantine – to introduce replacement gilts and still maintain a stable sow herd.
The present study was conducted in 69 PRRS-positive farms. By using a questionnaire and blood samples, the use of PRRS MLV and quarantine facilities was compared with the PRRSV status of replacement gilts at first insemination. Antibody levels compared to time from vaccination with PRRS MLV and age of the animals when vaccinated was also investigated. Five blood samples were taken at each farm, and analyzed by RT-qPCR, ELISA and IPMA.
PRRSV status was divided into 'stable' and 'unstable', stable being defined as being negative by RT-qPCR and positive by ELISA. The distribution of stable and unstable replacement gilts was 6 (8.7%) and 63 (91.3%) respectively. Among the farms included, no positive in RT-qPCR was found. Six farms had seronegative gilts in ELISA and thus constituted all the unstable farms.
The study found no significant relation between the use of PRRS MLV and quarantine facilities, compared to the PRRSV status of replacement gilts at first insemination. Nor did the study find a significant relation between antibody levels and time from vaccination with PRRS MLV, and age of the animals when vaccinated. This was – to the best of the author's knowledge – the first study conducted investigating PRRSV status of replacement gilts.
From this study it can be concluded, that quarantine, duration of quarantine and gilt recruitment strategy was not related to the status of the replacement gilts at first breeding. There was a tendency towards the use of quarantine and having stable gilts at first insemination, but this was not significant. This study demonstrated that there is a high probability of having PRRSV PCR-negative gilts at first insemination, and there is a small probability of the gilts being naïve regarding PRRSV at first insemination.
Below this section the results of the study are discussed in details.

5.1 Discussion on results

Seronegative replacement gilts
The basis for the five hypotheses described in section 4.7.1 was that there were two groups: a 'stable' group and an 'unstable' group. When making these hypotheses it was expected that some gilts were PCR-positive for PRRSV and thus belonged to the 'unstable' group. A large portion of ELISA-negative gilts were not predicted, since the gilts had been vaccinated either on the farm or at the supplier's farm. The study did show that 6 farms had ELISA-unstable gilts, and, among these 6, 2 farms were completely negative.
According to the questionnaire, the gilts had been vaccinated (and according to vetstat.dk, the farms had bought PRRS MLV vaccines – Porcilis® PRRS), leaving two scenarios. Either the gilts *were* vaccinated, and there had been a vaccine failure somehow; or, the gilts had simply not been vaccinated. Suggestions to causes of vaccine failure could be injection technique, temperature and storage of the vaccine, not used before the expiration date (Duengelhoef *et al.*, 2014) and the health status of the gilts when vaccinated. Animals have to be healthy when being vaccinated (Agger *et al.*, 2011). A likely scenario of vaccine failure in a farm is the use of a formerly opened and already mixed vaccine. According to the manufacturers of Porcilis® PRRS it has to be used within 3 hours after mixing (SPC for Porcilis® PRRS). For Ingelvac® PRRS MLV is has to be used directly after mixing (SPC for Ingelvac PRRS® MLV). Another explanation to negative ELISA results is failure in the execution of the ELISA-test, but since the ELISA test includes positive and negative controls, this seems unlikely. In these two particular farms it might be an idea for the veterinarian to make sure that vaccination is performed in a correct way.
4 farms (5.8%) had a mixture of ELISA-positive and ELISA-negative gilts. This raises the question whether it can be expected that in about 5% of farms vaccinating gilts against PRRS, there will a small proportion of

PRRS naïve gilts. In several studies the proportion of seroconverted animals after vaccination with MLV is measured by ELISA, and all studies finds 100% of the animals seroconverting e.g. Martínez-Lobo *et al.* (2013), Nielsen *et al.* (1997) and Pileri *et al.* (2015). Based on this it can be concluded that – if used properly – the vaccines makes the animals seroconvert. This leaves poor management and human errors as the most likely reason for the lack of detectable antibodies.
Consequences of PRRSV seronegative gilts being introduced to a PRRS-positive sow herd are depending on whether the herd is considered stable or not. If the herd is stable (no transmission of virus), the gilts will remain seronegative (function as sentinels) and might contribute to a spontaneous eradication of PRRS over time (Freese, 1994). If the herd is considered unstable (with ongoing transmission of virus) the seronegative gilts will – with great certainty – become viremic at some time. This is not desirable since this will lead to a continued ongoing transmission of virus in the herd (Dee *et al.*, 1995), and might show clinically in the form of abortions, early farrowings and weak piglets (Rossow, 1998).
When introducing seronegative gilts to a PRRS-positive sow herd, the role of persistently infected animals has to be taken into account. It has been shown that a sow infected/vaccinated with an MLV, can infect another sow, even though she is not viremic at the time of infection of the other sow (Bierk *et al.*, 2001). Thus it is very important that all gilts and sows in a herd, is immunized against PRRS. It is concerning, that 8.7% of replacement gilts was not immunized at time of insemination.

Viremic replacement gilts

It was expected that more farms would have viremic gilts at insemination, due to the length of viremia after vaccination with MLV or natural infection, than turned out to be the case (e.g. Pawlowski & Carlsen, 2015; Pileri *et al.*, 2015; Wills *et al.*, 1997b). The time from vaccination to when blood samples were taken was not known due to a lack of data regarding age of the gilts when blood samples were taken, but with the data on age when vaccinated (mean 15.9±1.26 weeks) and a knowledge of the recommended time of first breeding (second heat and about an age of 33-36 weeks according to the Gilt Manual (2012) from SEGES Danish Pig Research Centre), the time from vaccination to when blood samples was taken could be estimated to about 18 weeks on average.
Several studies (Díaz *et al.*, 2006; Duengelhoef *et al.*, 2014; Foss *et al.*, 2002; Labarque *et al.*, 2004; Martelli *et al.*, 2007, 2009; Martínez-Lobo *et al.*, 2013; Mengeling *et al.*, 2003; Nielsen *et al.*, 1997; Park *et al.*, 2015; Pawlowski & Carlsen, 2015; Pileri *et al.*, 2015; Sipos *et al.*, 2003; Stadejek *et al.*, 2005) investigated the length of viremia after vaccination with MLV. The average of the duration of viremia based on these studies is 4 weeks, but is shown to last as long as 8 weeks (Pawlowski & Carlsen, 2015) and for up to 251 days in experimentally inoculated animals (Wills *et al.*, 2003). In the study by Wills *et al.* (2003) viremic animals was found until day 56 after inoculation, and again at day 225 and 251. The reasons for the second occurrence of viremia were not known, but the finding emphasizes the importance of all animals in a herd being immunized against PRRS based on the conviction that viremic animals potentially sheds virus.
Comparing the time from vaccination to blood samples were taken (estimated 18 weeks) to the 4 weeks, it is thus not surprising that no farms included in the study had positive samples in the RT-qPCR.
In the present study, five serum samples were pooled into one sample, which was analysed by RT-qPCR. This was due to cost and time. A study by Rovira *et al.* (2007) showed that the sensitivity on pooled serum samples was reduced compared to individual serum samples. The decrease in sensitivity is likely when thinking of the potential dilution of viral RNA in a pooled sample. Among the pooled serum samples included in the study there were no samples positive in RT-qPCR. However, one farm excluded from the study, came up positive in the pool. When tested individually, 4 out of 5 of the samples from the pool came up positive, showing a good agreement between pooled samples and individually tested samples. The farm was excluded because they did not follow their usual vaccination routine for gilts (the sampled gilts had been vacci-

nated two weeks prior to sampling). In an interlaboratory ring trial to evaluate RT-qPCR detection methods by Wernike *et al.* (2012), the assay that revealed the best results regarding analytical sensitivity was the assay Kleiboeker modified 1 used in this study. Since this assay has been modified from Kleiboeker modified 1 described by Wernike *et al.* (2012), it is likewise expected to have a high sensitivity.

Use of quarantine

There was a strong tendency towards the use of quarantine and having stable gilts at first insemination, but this was not significant. The objective of having a quarantine is to ensure that new gilts doesn't transmit new pathogens to the existing sow herd (Hvolgaard, 2013). With regards to PRRS the quarantine is used to make sure the new animals are no longer viremic i.e. shedding virus when introduced to the sow herd.

IPMA data

To analyze the IPMA-data, a classification principle was conducted. The farms that were excluded due to this principle was not analysed further, and this was due to limitations of this project. It could have been interesting to see how the IPMA-values were distributed on farms with both types of PRRS circulating. The specific diagnosis of double infected herds by serology can be challenging because the second infection with another type of PRRS may boost the antibody response against the initial virus. This is a mechanism termed antigenic sin (Morens *et al.*, 2010). Sampling of several age groups of pigs may therefore be necessary.

Gilt recruitment

A total of 40 (58%) farms bought gilts, 22 (31.8%) produced their own gilts and 7 (10.2%) had a mixed gilt recruitment strategy. For the farms that produced their own gilts, the majority of gilts were moved out of the normal flow at 12 weeks. When the remaining pigs go to slaughter stable, the forthcoming gilts are probably moved to a gilt stable. The earliest the new gilts were moved was 4 weeks old, corresponding to weaning. The latest the new gilts were moved from normal flow was 33 weeks old, corresponding to when they are about to be inseminated. It is not recommended to feed slaughter pigs and coming gilts in the same way due to the longevity of the replacement gilts (Bruun & Sørensen, 2014).

In Denmark there are circulating two major clusters of PRRSV type 1 both belonging to subtype 1 (Kvisgaard *et al.*, 2013). A cluster including Lelystad-like viruses and the Porcilis® PRRS MLV, and an 'unique' Danish cluster (Kvisgaard *et al.*, 2013). The diversity between these clusters is 7-15%. A total of 9 farms bought PRRS type 1 positive gilts. Even though all 9 farms were PRRS type 1 positive, there is a potential risk of introducing another cluster of PRRSV type 1 when buying PRRS type 1 positive gilts. Unpublished data demonstrated that the genetic diversity within PRRSV type 1 affects the efficiency of Porcilis® PRRS (Pawlowski & Carlsen, 2015). The study proved evidence, that a group vaccinated with Porcilis® PRRS, contracted viremia when challenged another cluster. The finding was consistent to other studies (Labarque *et al.*, 2004). Based on this, PRRS-positive farms should always buy PRRS-negative replacement gilts to make sure not to introduce new strains of PRRSV type 1 in the herd.

Quarantine and quarantine facilities

45 (65.2%) farms utilize quarantine (excluding one farm with a mixed gilt recruitment strategy, which utilizes quarantine for the bought gilts, but not for the homemade gilts). For farms that are buying gilts, three quarters utilizes quarantine, and for farms that produce their own gilts, half of them do. For the farms that utilize quarantine, the quarantine facilities were investigated. It was found that only 10 out of 46 farms had optimal

quarantine facilities, and 5 out of the 10 were following recommendations regarding length of quarantine (12 weeks or more). In total that means that only 5 farms out of the total 69 farms (7%) had both optimal quarantine facilities and an optimal length of quarantine. This was surprising and might indicate that farmers need advice regarding quarantine.
In the present study 14 farms were following the recommendations regarding length of quarantine, and had a quarantine length between 12 and 18 weeks. Less than 25% of the farms had less than 8 weeks of quarantine, so in general it can be stated that length of quarantine is reasonable for the majority of farms.
Blood sampling to investigate the gilts for PRRS before introduction to the sow herd is almost never done. In cases where duration of quarantine is 4 weeks or less (seen in two farms) it should be strongly recommended to sample the gilts for PRRSV by PCR. The 4 weeks are based on the collection of studies showing viremia after vaccination with MLV e.g. Martelli *et al.* (2007), Nielsen *et al.* (1997) and Pawlowski & Carlsen (2015). Due to the fact that PRRS is a reportable disease in Denmark, no veterinarians wish to send in blood samples for PCR. If PRRS was not a reportable disease there would maybe be an increase in submissions to the laboratories, but still the economic aspect would have to be taken into account. From this present study, it can't be concluded if it is necessary to blood sample the gilts before introduction to the sow herd, since PRRSV-status of the gilts when introduced is not known.

A large part (45%) of the farms belongs to a certain veterinarian practice (Hyovet). The distribution of veterinary practices belonging to the farms in the study might have had some bias on the results of the study. Each veterinary practice has its own ideological notions about how to handle PRRS in a herd. It might be that the veterinary practices represented in this study are the best examples when it comes to strategies around the handling of PRRS.

The present study does not provide new data that should lead to a change in the existing Danish guidelines for introduction of replacement gilts in PRRS-positive farms. Recommendations for introduction of replacement gilts is thus still to vaccinate the gilts with a PRRS MLV or expose them to a wildtype virus, and let them be introduced through a quarantine of 12 weeks (Kristensen *et al.*, 2014).

5.2 Discussion on methods and study design

Time of blood sampling
The blood samples were taken from replacement gilts around first insemination, and not by the time of introduction to the sow herd. It can't be proven or shown that the gilts were stable when they were introduced to the sow herd. Blood samples could have been taken by the time of introduction to the sow herd to really say something about the PRRSV status of the replacement gilts when introduced.
The proportion of viremic gilts might be bigger when introduced to the sow herd compared to time of first insemination, since time from vaccination is shorter. A group of gilts that are viremic at first insemination could potentially be newly infected from the sow herd and going through their second viremia – or even their first viremia if vaccination hasn't been successful. Gilts being viremic when introduced to a sow herd are potentially shedding virus. If there exist seronegative sows in the herd, they might get infected with PRRSV eliciting clinical signs.
Age of the gilts when introduced to the sow herd was not known. If the age when introduced to the sow herd was consistent with the age of the gilts when blood samples were taken, the blood samples could say something about status when introduced to the sow herd. It would be interesting to do the same study again, with

only difference to take the blood samples when the gilts are introduced to the sow herd to see if the proportion of viremic gilts would be higher.

Representation

Whether the Gilt Project could represent Denmark was decided based on status (SPF or conventional) in the herds compared to the distribution in Denmark. It was concluded that the Gilt Project could not represent Denmark. It was not possible to include other variables to determine this, since other variables for Denmark such as gilt recruitment strategies are unknown. The farms in the study should have been randomly selected to achieve representation.

Method

The chosen cross-sectional study design has its limitations e.g. the distribution of the usage of quarantine and duration of quarantine was not known before the end of the study period. If a case-control method had been used, two groups could have been defined from the start: a group with short quarantine e.g. 4 weeks and group following the recommendations of 12 weeks quarantine. The benefit of this would have been to more precisely investigate the effect of length of quarantine, and maybe come with suggestions to new recommendations.

Sample size

Number of herds to be included in the project was calculated to be 46, based on the assumption that 20% of the herds using quarantine and 60% not using quarantine would have unstable replacement gilts at first insemination. The study did not meet these assumptions. In the study 5% of the herds using quarantine had unstable gilts, and 20% of the herds not using quarantine has unstable gilts. With a power of 0.8 and an alpha value of 0.05, it would have required 75 herds in each group, meaning a total of 150 herds (Houe *et al.*, 2004). The sample size of 69 was thus not adequate statistically speaking.

Number of blood samples was 5 in each herd. The purpose of the blood samples was to deduce the PRRSV status of the team of gilts. If the sow herds sampled consisted of 1000 sows in average, they would have to inseminate about 10 replacement gilts pr week (corresponding to having 20% gilts in a team). With an expected prevalence of PRRS-unstable gilts of 20%, this would require a sample size of 7 (Houe *et al.*, 2004). The larger the herd, the larger the sample size has to be. A sample size of 5 was corresponding to expecting a proportion of PRRS-unstable gilts of 0.4 in a group of gilts of 10. Based on this, the sample size of 5 seems acceptable.

6. Conclusion

Based on the present study it can be concluded, that the use of quarantine, duration of quarantine and gilt recruitment strategy was not related to the status of the replacement gilts at first breeding. There was a tendency towards the use of quarantine and having stable gilts at first insemination, but this was not significant. This study demonstrated that there is a high probability of having PCR negative replacement gilts at first insemination, and there is a small probability of not having immunized replacement gilts at first insemination.

This study does not change the existing guidelines in how to introduce replacement gilts to a PRRS-positive sow herd.

7. Perspectivation

To investigate future recommendations for introduction of replacement gilts a case-control study could be conducted with the purpose of comparing two different lengths of quarantine (e.g. 6 weeks and 12 weeks) to the PRRSV status of the gilts when introduced to the sow herd. Another option is to repeat the present study with the only difference of taking the blood samples when the gilts are introduced to the sow herd. This would provide information to the effects of quarantine. If any PCR-positive serum samples were found, they could be sequenced to provide interesting knowledge about clusters circulating in Danish replacement gilts.

The optimal vaccine against PRRS should protect against all genotypes and subtypes, not make the animals viremic, protect against PRRSV for a life-time, and should be easy to handle for the farmers. If such a vaccine was developed, it would change the guidelines for handling of PRRS and replacement gilts. Another benefit of such vaccine would be an increased degree of animal welfare in PRRS-positive herds, since no PRRS-vaccinated animals would be sick due to PRRSV.

Is it somehow possible to manipulate with the length of viremia? In the case that the duration of the second viremia is shorter than the first viremia, it might be possible to decrease length of quarantine by vaccinating the piglets e.g. at weaning, and then again e.g. at 12 weeks. This could be an interesting study.

The guidelines in how to introduce replacement gilts to a PRRS-positive sow herd is maintained, meaning replacement gilts should be vaccinated with a PRRS MLV or exposed to a wildtype virus, and then wait for 12 weeks before being taken into the sow herd (Kristensen *et al.*, 2014).

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Appendix

A) Questionnaire, Danish:

One question from the questionnaire was taken out due to ambiguity ("Køres poltestalden efter AI/AU", English: "Is the quarantine managed all-in all-out").

Spørgeskema

CHR:

Udfyldt af:

			Sæt X / udfyld:		
Besætningen har PRRS DK			Ja	Nej	Ved ikke
Besætningen har PRRS VAC			Ja	Nej	Ved ikke
Status er deklareret af SPF-SUS			Ja	Nej	
Anses soholdet for at være stabilt (uden cirkulation af PRRS virus)			Ja	Nej	
Polterekruttering		Egen avl	%	Indkøb	%
Opstaldning af polte fra egen avl					
Indtil hvilken alder følger poltene det normale flow i besætningen				uger	
Kommer polte af egen avl i karantæne			Ja	Nej	
Polte indkøb					
Hvor mange polte indkøbes per år				polte	
Antal leverandører indenfor de sidste 2 år				leverandører	
Antal gange der er indkøbt polte det seneste år				gange	
Hvilken PRRS-status har indkøbte polte	PRRS-dk		Ja	Nej	Ved ikke
	PRRS-vac		Ja	Nej	Ved ikke
Er poltene vaccineret mod PRRS før ankomst			Ja	Nej	Ved ikke
Hvor gamle er polte ved indkøb		Fra	uger	til	uger
Kommer indkøbte polte i karantæne			Ja	Nej	
Polte karantæne (gælder både polte af egen avl og indkøbte polte)					
Deler karantænen luftrum med øvrige stalde			Ja	Nej	
Er der separat indgang			Ja	Nej	
Vaccineres poltene mod PRRS			Ja	Nej	
Hvis Ja	hvilken alder		uger		
	hvilken vaccine				
Hvor længe er poltene i karantæne			uger		
Køres poltestalden efter AIAU			Ja	Nej	
Er karantænen helt tom for dyr før nye dyr indsættes			Ja	Nej	
Blodprøves poltene for PRRS før indsættelse i soholdet			Altid	Nogle gange	Aldrig

B) Questionnaire, English:

One question from the questionnaire was taken out due to ambiguity ("Køres poltestalden efter AI/AU", English: "Is the quarantine managed all-in all-out").

Questionnaire

CHR:

Filled in by:

Sæt X / udfyld:

Is the herd PRRSV type 1 positive?	Yes	No	Unknown
Is the herd PRRSV type 2 positive?	Yes	No	Unknown
Is the herd declared in SPF-SuS?	Yes	No	
Do you suppose that the pigs are PRRSV free at weaning?	Yes	No	
The replacement gilts comes from	Own breeding	%	Purchase %

OWN BREEDING OF YOUNG BREEDING ANIMALS

At what age are the breeding animals moved out of the normal flow?		weeks	
Are the young breeding animals in quarantine	Yes	No	

PURCHASE OF YOUNG BREEDING ANIMALS

Number of purchased animals each year		gilts		
Number of suppliers the last two years		suppliers		
Number of deliveries in the last year		times		
Status of purchased animals	PRRSV type 1 positive	Yes	No	Unknown
	PRRSV type 2 positive	Yes	No	Unknown
Are purchased animals vaccinated before arrival		Yes	No	Unknown
Age of purchased animals at arrival	from	weeks	to	weeks
Are the coming young breeding animals in quarantine		Yes	No	

ALL YOUNG BREEDING ANIMALS

Does the quarantine share air with the other sections?		Yes	No
Is there a separate entrance to the quarantine?		Yes	No
Are animals vaccinated?		Yes	No
If yes:	Age of animals	weeks	
	Vaccine		
How long are the animals in the quarantine		weeks	
Is the quarantine managed all-in all-out		Yes	No
Is the quarantine empty for animals before new animals are introduced?		Yes	No
Are the animals investigated for PRRSV before introduced to the sow herd?		Always	Sometimes	Never

C) An example of an ELISA and IPMA result interpreted as positive (PRRSV type 2):

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DTU

D.S. Projektafd.

Vinkelvej 11
Att.:Charlotte S. Kristensen
8620 Kjellerup

Sagsnr.:	15-16967
Deres ref.:	VSP7140420030
Udtaget:	Ikke oplyst
Modtaget:	09-09-2015
Analyse påbegyndt:	09-09-2015
Besvaret:	12-10-2015
Sagsansvarlig:	Lars Ole Andresen
Telefon:	+45 3588 6250
E-mail:	laoa@vet.dtu.dk

Laboratoriesvar

Dyreart	**Materiale**	**Antal**
Svin	Serum	5

Prøvenr./Mærke	**A** OD %	**B** OD %	**C** -	**D** titer	**E** titer
1 66 A	29	8	**3,6**	0	**1250**
2 66 B	31	11	**2,8**	**250**	**250**
3 66 C	33	6	**5,5**	**50**	**250**
4 66 D	26	6	**4,3**	**250**	**250**
5 66 E	34	10	**3,4**	**50**	**250**

A: PRRS virus (PRRSV) EU type Antistof-test ved ELISA, OD% >44 = negativ
Anvendt metode: V05-03-029

B: PRRS virus (PRRSV) US type Antistof-test ved ELISA, OD% >44 = negativ
Anvendt metode: V05-03-029

C: Ratio EU/US Kun relevant ved positive ELISA resultater
Anvendt metode: V05-03-029
Ratio >1,9 i hovedparten af prøverne indikerer smitte med Amrk type
Ratio <1,3 i hovedparten af prøverne indikerer smitte med EU type
Positive resultater er angivet med fed skrift

D: PRRS virus (PRRSV) EU type Antistof-test ved IPT, titer <50 = negativ
Positive resultater er angivet med fed skrift

E: PRRS virus (PRRSV) US type Antistof-test ved IPT, titer <50 = negativ
Positive resultater er angivet med fed skrift

DANAK
TEST Reg.nr. 0536

Resultaterne gælder alene for de undersøgte prøver. Undersøgelser mærket # i dette laboratoriesvar er ikke omfattet af akkrediteringen. Laboratoriesvaret må kun gengives i sin helhed med mindre skriftlig tilladelse til andet er indhentet hos Veterinærinstituttet og/eller Fødevareinstituttet.

Laboratorieundersøgelser udføres i henhold til Veterinærinstituttes/Fødevareinstituttets generelle forretningsbetingelser, herunder bestemmelser om ansvarsbegrænsning, som kan ses på www.vet.dtu.dk/www.food.dtu.dk

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www.vet.dtu.dk, vet@vet.dtu.dk

D) An example of an ELISA result interpreted as negative (one negative sample means the group is interpreted as negative), page 1/2:

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DTU

D.S. Projektafd.

Vinkelvej 11
Att.:Charlotte S. Kristensen
8620 Kjellerup

Sagsnr.:	15-1269
Deres ref.:	
Udtaget:	20-08-2014
Modtaget:	12-01-2015
Analyse påbegyndt:	12-01-2015
Besvaret:	22-01-2015
Sagsansvarlig:	Christian Gräs
Telefon:	+45 3588 6250
E-mail:	chrgra@vet.dtu.dk

Laboratoriesvar

Prøvenr.	Prøveinformation
1	17A, Svin, serum,
2	17B, Svin, serum,
3	17C, Svin, serum,
4	17D, Svin, serum,
5	17E, Svin, serum,

Sagsbemærkninger:
Besætning nr. 17

Prøvenr./Mærke	PRRSV EU	PRRSV US	PRRSV Ratio	PRRSV IPT EU
	OD %	OD %	-	titer
1 17A	38	22	**1,7**	**250**
2 17B	17	15	**1,1**	**1250**
3 17C	72	75	**-**	
4 17D	16	18	**0,9**	**250**
5 17E	11	15	**0,7**	**1250**

PRRS virus (PRRSV) EU type Antistof-test ved ELISA, OD% >44 = negativ
Anvendt metode: V05-03-029

PRRS virus (PRRSV) US type Antistof-test ved ELISA, OD% >44 = negativ
Anvendt metode: V05-03-029

Ratio EU/US Kun relevant ved positive ELISA resultater
Anvendt metode: V05-03-029
Ratio >1,9 i hovedparten af prøverne indikerer smitte med Amrk type
Ratio <1,3 i hovedparten af prøverne indikerer smitte med EU type
Positive resultater er angivet med fed skrift

PRRS virus (PRRSV) EU type Antistof-test ved IPT, titer <50 = negativ
Positive resultater er angivet med fed skrift

DANAK
TEST Reg.nr. 0536

Resultaterne gælder alene for de undersøgte prøver. Undersøgelser mærket # i dette laboratoriesvar er ikke omfattet af akkrediteringen. Laboratoriesvaret må kun gengives i sin helhed med mindre skriftlig tilladelse til andet er indhentet hos Veterinærinstituttet og/eller Fødevareinstituttet. Oplysninger om måleområde og usikkerhed m.m. kan rekvireres.

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Appendix D, page 2/2:

Prøvenr./Mærke	PRRSV IPT US titer
1 17A	**50**
2 17B	**50**
4 17D	**250**
5 17E	0

PRRS virus (PRRSV) US type Antistof-test ved IPT, titer <50 = negativ
Positive resultater er angivet med fed skrift

E) An example of an inconclusive ELISA result:

DTU Veterinærinstituttet

D.S. Projektafd.

Vinkelvej 11
Att.:Charlotte S. Kristensen
8620 Kjellerup

Sagsnr.:	15-16957
Deres ref.:	
Udtaget:	Ikke oplyst
Modtaget:	09-09-2015
Analyse påbegyndt:	09-09-2015
Besvaret:	25-09-2015
Sagsansvarlig:	Christian Gräs
Telefon:	+45 3588 6250
E-mail:	chrgra@vet.dtu.dk

Laboratoriesvar

Prøvenr.	Prøveinformation
1	56 A, Svin, serum,
2	56 B, Svin, serum,
3	56 C, Svin, serum,
4	56 D, Svin, serum,
5	56 E, Svin, serum,

Prøvenr./Mærke	PRRSV EU	PRRSV US	PRRSV Ratio	PRRSV IPT EU
	OD %	OD %	-	titer
1 56 A	13	11	**1,2**	**250**
2 56 B	13	15	**0,9**	**250**
3 56 C	19	8	**2,4**	0
4 56 D	19	8	**2,4**	0
5 56 E	12	7	**1,7**	0

PRRS virus (PRRSV) EU type Antistof-test ved ELISA, OD% >44 = negativ
Anvendt metode: V05-03-029

PRRS virus (PRRSV) US type Antistof-test ved ELISA, OD% >44 = negativ
Anvendt metode: V05-03-029

Ratio EU/US Kun relevant ved positive ELISA resultater
Anvendt metode: V05-03-029
Ratio >1,9 i hovedparten af prøverne indikerer smitte med Amrk type
Ratio <1,3 i hovedparten af prøverne indikerer smitte med EU type
Positive resultater er angivet med fed skrift

PRRS virus (PRRSV) EU type Antistof-test ved IPT, titer <50 = negativ
Positive resultater er angivet med fed skrift

DANAK
TEST Reg.nr. 0536

Resultaterne gælder alene for de undersøgte prøver. Undersøgelser mærket # i dette laboratoriesvar er ikke omfattet af akkrediteringen. Laboratoriesvaret må kun gengives i sin helhed med mindre skriftlig tilladelse til andet er indhentet hos Veterinærinstituttet og/eller Fødevareinstituttet.

Laboratorieundersøgelser udføres i henhold til Veterinærinstituttes/Fødevareinstituttets generelle forretningsbetingelser, herunder bestemmelser om ansvarsbegrænsning, som kan ses på www.vet.dtu.dk/www.food.dtu.dk

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Appendix E, page 2:

Prøvenr./Mærke	PRRSV IPT US titer
1 56 A	**50**
2 56 B	**250**
3 56 C	0
4 56 D	0
5 56 E	0

PRRS virus (PRRSV) US type Antistof-test ved IPT, titer <50 = negativ
Positive resultater er angivet med fed skrift