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The association between the serological PCV2 status of sow herds and reproductive disorders

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Introduction

Porcine Circovirus Type 2 PCV2 is a non-enveloped, small, single-stranded DNA virus. It belongs to the family *Circoviridae*, Genus *Circovirus*. It has only 1768 nucleotides in its circular DNA *(Hamel et al., 1998)*. PCV2 DNA has 3 important Open Reading Frames, (ORF's). ORF1 encodes for two replicases: Rep and Rep' *(Mankertz et al., 1998)*. Open Reading Frame 2 encodes for capsid protein (Cap) *(Nawagitgul et al., 2000)*, while ORF 3 encodes for a protein that is probably important for apoptotic activity *(Liu et al., 2006)*.

Phagocytosis of PCV2 by macrophages is essential for the pathogenesis. Macrophages phagocyte viral material in the lungs and then transport the virus to lymph nodes and other organs. Macrophages can contain viral DNA and antigens for a prolonged period, and thereby make viral survival and viral transmission more feasible *(Gilpin et al., 2003; Vincent et al., 2003; Pérez-Martín et al., 2007)*.

PCV2 is associated with Post-weaning Multisystemic Wasting Syndrome (PMWS), Porcine Dermatitis Nefritis Syndrome (PDNS), Porcine Respiratory Disease Complex and Reproductive Disorders (PCV-RD). These symptoms together are called PCVAD, Porcine Circovirus Associated Disease *(Opriessnig et al., 2007; Segalés et al., 2005a)*.

PCV2 is endemic on swine farms in many European countries and, although most infections are subclinical, causes significant financial losses *(Armstrong and Bishop., 2004*; *Segalés et al., 2005a)*. Most research on PCV2 is done on PMWS. Whether or not a pig develops clinical PMWS signs depends on the ability of the pig to produce enough neutralizing antibodies during the infection, being mainly IgG and IgM in a lesser degree. Pigs clinically suffering from PMWS are immunocompromised and are unable to induce sufficient immune response to clear the body from the virulent virus *(Meerts et al., 2006; Fort et al., 2007)*. Lymph nodes are infiltrated by a large number of macrophages, more than physiologically normal, which suppresses normal functioning of the lymph nodes and thymus, through depletion of T- and B-lymphocytes and an altered cytokine expression *(Chianini et al., 2003; Darwich et al., 2002; Nielsen et al., 2003)*. Depletion of T- and B-lymphocytes can be due to the possible apoptotic effect of PCV2 on B-lymphocytes and due to an absence of follicles and lymphoid tissue atrophy caused by the virus *(Shibahara et al., 2000; Rosell et al., 1999; Sarli et al., 2001)*. Lymphoid tissue atrophy was shown by in vitro research by Darwich et al. (2003) where periferal blood mononuclear cells (PBMC) were impaired in their ability to respond to mitogens *(Darwich et al., 2003a)*.

Pigs suffering from PMWS develop significantly lower seroconversion rates than sub-clinically infected pigs, with lower neutralizing antibody and virus specific antibody titers *(Ladekjaer-Mikkelsen et al., 2002*; *Rovira et al., 2002; Okuda et al., 2003; Hasslung et al., 2005; Meerts et al., 2006; Fort et al., 2007)*.

Pigs that survive a PCV2 infection develop immunity *(Allan and Ellis, 2000; Krakowka et al., 2002; Ladekjaer-Mikkelsen et al., 2002).* Colostrum derived maternal antibodies can probably delay the clinical manifestations of the virus in piglets *(Allan et al., 2002; McKewon et al., 2005; Ostanello et al., 2005),* because clinical manifestation usually coincides with the decline of maternal antibodies *(Rodriguez-Arrioja et al., 2002; Rose et al., 2003; Sibila et al., 2004).*

Reproductive problems are characterized by late-term abortion, mummified piglets, stillborn near-term piglets, premature piglets and less viable piglets *(West et al., 1999; Josephson and Charbonneau, 2001; Ladekjaer-Mikkelsen et al., 2001; O'Connor et al., 2001; Kim et al., 2004).* For the reproductive problems caused by PCV2, the actual incidence and pathophysiology are unknown. Diseased piglets and less viable piglets show pulmonary edema, hepatomegaly, ascites and dilated cardiomyopathy *(West et al., 1999; Sanchez et al., 2001).* This tropism for cardiac myocytes seems to diminish as gestation advances *(Sanchez et al., 2003).*

In experiments, fetuses were infected intra-uterine *(Sanchez et al., 2003)* and transplacentary *(Park et al., 2005),* and it was suggested that clinical signs can be linked to the route of transmission of the virus. Intra-uterine infections were shown to cause viral invasion of cardiac myocytes *(Sanchez et al., 2003)* while transplacentary infections led to invasion of the macrophages *(Park et al., 2005).*
Sanchez et al. (2001), Kim et al. (2004), and Park et al. (2005) strongly suggest that PCV2 can be transferred transplacentary at any stage of gestation *(Sanchez et al., 2001; Kim et al., 2004,* and *Park et al., 2005).* Not only was PCV2 detected in still-born piglets at any time of gestation *(Kim et al., 2004),* PCV2 was also able to replicate in fetuses after intra-fetal inoculation at any time during gestation *(Sanchez et al., 2001).*

Pigs have a diffuse epitheliochorial placenta and it is believed that this type of placenta does not support diffusion of large molecules from mother to fetus *(MacDonald, Bosma., 1985).* However, research has proven that antibodies against PCV2 are able to cross the placental barrier. The titer of transplacentary transferred antibodies shows a linear relation with the maternal antibody titer *(Saha et al., 2014).* When infection takes place after approximately 70 days post insemination, fetuses are able to produce antibodies themselves *(Saha et al., 2010).* The presence of PCV2 antibodies in neonates on its own is therefore not a confirmation of a peri gestational infection.
Since sow PCV2 antibody titers are positively correlated with fetal PCV2 antibody titers *(Saha et al., 2014),* together with the finding that antibodies have a diminishing effect on clinical signs in piglets *(Allan et al., 2002; McKewon et al., 2005; Ostanello et al., 2005; Fort et al., 2007; Fort et al., 2009),* sow serological PCV2 profiles seem to be a good predictor for the incidence of clinical manifestations of PCV2-Reproductive Disorders in sow populations. Gilt serological PCV2 statuses and PCV2 vaccination may have an effect on the sows' serological PCV2 statuses, therefore gilt serological PCV2 statuses and PCV2 vaccination statuses are also reflected in this thesis *(Opriessnig et al., 2008).* If gilts have high PCV2 antibody titers, possibly because of a vaccination, as sow they are more likely to have high titers too. Porcine reproductive and respiratory syndrome virus (PRRSV) is known to cause similar reproductive disorders as PCV, so the possible role of PRRSV as a confounder was also tested in this thesis *(Goyal., 1993; Rossow et al., 1995).*

The aim of this study was to determine the association between the PCV2 serological status of sows (at farrowing) or gilts (prior to insemination) and reproductive disorders at farm level.
The hypothesis that the prevalence of reproductive disorders in sow and gilt herds is lower when sows and gilts are seropositive was tested in this thesis by analyzing data from the MSD Respig program.

Accepting the hypothesis will support farmers and veterinarians in their decision to ensure that all inseminated sows and gilts have a positive serological PCV2 status either by natural infection or vaccination to prevent reproductive disorders.

Materials and Methods
MSD Animal Health has set up the RESPIG program, that focusses on the Porcine Respiratory Disease Complex (PRDC). The aim of MSD is to determine and visualize factors that contribute to the PRDC on Dutch farms. In the RESPIG program collection of blood samples of pigs in every stage of production and an audit on risk factors for PRDC such as biosecurity, climate and management, are combined to determine which pathogens can possibly contribute to PRDC at individual farms. The samples are taken by a consulting veterinarian in one farm visit, making it a cross-sectional study. Approximately 10% of the Dutch piggeries co-operates in the RESPIG program. Sow- and gilt data from this study is also suitable for other than respiratory disorders, for instance reproductive disorders, because some of the pathogens, PCV2 and PRRSV, can not only cause respiratory, but also reproductive disorders.

The farms were subdivided on basis of the region, type, PRRSV serological status and PCV2 vaccination status. The PCV2 serological status of each farm was categorized in 6 groups based on the number of positive samples. For gilts, blood samples were taken prior to insemination and for sows, blood samples were taken one or two weeks post farrowing. In order to assess the serological antibody status of the sows and gilts blood samples were subjected to an in house Alfalise test. This is a quantitative test based on the ORF2-encoded capsid protein. This test determines the concentration of antibodies aimed at the ORF2-encoded capsid protein. Concentrations of 4log2 and higher were considered positive. The data collected at the farms was screened prior to statistical analysis using multiple inclusion- and exclusion criteria.

Criteria for the division of farms, independent variables:
Region:
- Mid: Zuid-Holland, Utrecht, Overijssel, Gelderland, Flevoland
- South: Noord-Brabant, Limburg, Zeeland

Types of farm:
- Farrow – to – finish
- Farrowing only
- Breeding

Farm sow- and gilt status:

On each farm 10 blood samples were taken, 5 sow samples and 5 gilt samples. The outcome at each farm falls in one of 6 classes representing 0 to 5 positive sows and 0 to 5 positive gilts.

Farm PCV2 vaccination status:

- PCV2 vaccinated *(Porcilis® PCV, Ingelvac CircoFLEX® or Circovac®)*
- PCV2 unvaccinated

Farm serological PRRSV status

- PRRSV positive (1 or more positive samples)
- PRRSV free (0 positive samples.*)

*Based on 5 samples, a PRRSV free status can not be guaranteed. In this thesis herds are rather unsuspected than free.

Anamnesis, dependent variable:

The veterinarians provided data about the clinical symptoms seen on the farm around the time of the consultation. If a farm had endured one or more types of reproductive disorders in at least one sow or gilt, it was allocated to ‘’Reproductive disorders’’
The types of reproductive disorders were:

- Abortion
- Premature birth
- Weak piglets
- Infertility
- Repeat breeders

Farms with clinical signs other than reproductive disorders and farms with no clinical signs have been allocated to ‘’No reproductive disorders’’.

Inclusion- and exclusion criteria:

The inclusion and exclusion of farms was done in four steps.

Step 1: Data from RESPIG 2013 was taken and all fattening farms were taken out. Some of them did have information about PCV2 vaccination- and PRRSV serological status. However, fatting farms could not provide an answer to the question about the incidence of reproductive disorders.

Step 2: All farms with unknown PCV2 vaccination status were removed. In this way the possible relation between PCV2 vaccination status and the farms’ sow PCV2 status could be tested (table 8).

Step 3A: Farms with unclear anamnesis, anamnesis that gave no clear information about whether or not any of the reproductive disorders were experienced, were removed. The data that was then left gave the opportunity to calculate the possible relation between the farms PCV2 vaccination status and the incidence of reproductive disorders (table 5).

Step 3B: Here farms with unclear anamnesis were not removed. Here farms with a positive PCV2 vaccination status were removed. With this data the possible relation between farms’ PRRSV status and the farms’ sow PCV2 status ánd the possible relation between the farms’ gilt PCV2 status and the farms’ sow PCV2 status could be calculated (table 9 and 7).

Step 4: Step 4 combines step 3A and step 3B. Now all farms with unclear anamnesis and with a positive PCV2 vaccination status were removed. The farms that were not excluded during either of the steps were used to calculate the effect of the

region, the type of farm, the farms' PRRSV status, the farms' sow PCV2 status and the farms' gilt PCV2 status on the incidence of reproductive disorders (table 1,2,3,4 and 6).

Statistical approach

To calculate the association between the individual factors and the incidence of reproductive disorders cross tables were made. The serological statuses were calculated on farm level. Cross tables were designed for respectively the effect of region, type of farm, farm status sow, farm status gilt, farm vaccination status and farm PRRSV status on the incidence of reproductive disorders. To test the association between the farm vaccination status, the farm status gilt, or the farm PRRSV status and the farm status sow a separate set of cross tables was made.

The independent variables were:

- Region — categorical: 1 (mid), 2 (south))
- Type of farm — categorical: 1 (farrow-to-finish), 2 (farrowing), 3 (breeding)
- Farm status sow — categorical: 0 (0 positive sows), 1 (1 positive sow), 2 (2 positive sows), 3 (3 positive sows), 4 (4 positive sows), 5 (5 positive sows)
- Farm status gilt — categorical: 0 (0 positive gilts), 1 (1 positive gilt), 2 (2 positive gilts), 3 (3 positive gilts), 4 (4 positive gilts), 5 (5 positive gilts)
- Farm vaccination status — categorical: 0 (PCV2 unvaccinated), 1 (PCV2 vaccinated)
- Farm PRRSV status — categorical: 0 (PRRSV free), 1 (PRRSV positive)

The dependent variables were:

- Reproductive disorders — binomial: 0 (no reproductive disorders), 1 (reproductive disorders)
- Farm status sow — categorical: 0 (0 positive sows), 1 (1 positive sow), 2 (2 positive sows), 3 (3 positive sows), 4 (4 positive sows), 5 (5 positive sows)

IBM SPSS Statistics 23 was used as statistical analysis software. The dependent variable, reproductive disorders, was binomial and the independent variables, region, type-of-farm, farm PRRSV status, farm-status-sow-and farm-status-gilt were categorical, therefore the multivariable logistic regression model was the appropriate statistical model to be used in this study. In the multivariable logistic regression model all mentioned factors were tested simultaneously. As a reminder, in the logistic regression test only farms without PCV2 vaccination were included. Significant associations were assumed if the 95% C.I. for the Odds Ratios did not include 1.

To test if there was an association between the farm vaccination status and farm-status-sow, the Fisher's exact statistics was used. To test if there was an association between farm-status-gilt and farm-status-sow, the Fisher's exact test and the Cohen's kappa test were done. A significant association was assumed if p- values were below 0.05. A moderate, substantial, or good measure of agreement was assumed if the kappa coefficient was between 0.41-0.60, 0.61-0.80 and >0.80, respectively *(Petrie and Watson, 2006).*

Results

Descriptive statistics

For the first analysis cross tables were made. The cross-tables provide percentages on the incidence of reproductive disorders in relation to the various factors. The factors region, type-of-farm, farm-status-sow, farm-status-gilt, farm-vaccination-status and farm-PRRSV-status are depicted in tables one to six.

Table 1: *Counts and percentages for the presence of reproductive disorders on farms in different regions*

	Reproductive Disorders			
	Yes		No	
Region	count	%	count	%
Mid	26	22.2	91	77.8
South	23	29.9	54	70.1
Total	49	25.3	145	74.7

In the mid region 22.2% of the sampled farms had reported reproductive disorders (26/117), with 29.9% of the sampled farms in the south region (23/77). On average 25.3% of the sampled farms encountered reproductive disorders (49/194).

Table 2: *Counts and percentages for the presence of reproductive disorders on different types of farms*

	Reproductive Disorders			
	Yes		No	
Type of farm	Count	%	count	%
Farrow - to - finish	20	26.3	56	73.7
Farrowing	24	25.3	71	74.7
Breeding	5	21.7	18	78.3
Total	49	25.3	145	74.7

The prevalence of reproductive disorders on the different types of farms was 26.3% for the farrow – to – finish farms (20/76), 25.3% for the farrowing farms (24/95) and 21.7% for the breeding farms (5/23). On average 25.3% of the farms reported reproductive problems (49/194).

Table 3: *Counts and percentages for the presence of reproductive disorders on farms with different serological sow status*

Farm status sow no. of seropositive sows	Reproductive Disorders					
	Yes		No		Total	
	count	%	count	%	count	%
0	7	31.8	15	68.2	22	11
1	11	28.9	27	71.1	38	20
2	7	17.5	33	82.5	40	21
3	11	25.6	32	74.4	43	22
4	11	32.4	23	67.6	33	17
5	2	12.5	14	87.5	16	8
Total	49	25.4	144	74.6	193	100

On the sampled farms, the majority of the sow herds did not encounter reproductive disorders (RD). Over twenty-five percent of the farms did encounter problems, against 74.6% which did not encounter RD (49 and 144 farms respectively). Prevalence ranged from 12.5% on farms with 5 seropositive sows to 32.4% on farms with 4 seropositive sows (2/16 and 11/34 respectively). Most farms had one, two, or three seropositive sows.

Table 4: *Counts and percentages for the presence of reproductive disorders on farms with different serological gilt status*

Farm status gilt no. of seropositive gilts	Reproductive Disorders					
	Yes		No		Total	
	count	%	count	%	count	%
0	10	35.7	18	64.3	28	15
1	8	30.8	18	69.2	26	13
2	6	19.4	25	80.6	31	16
3	7	29.2	17	70.8	24	12
4	5	19.2	21	80.8	26	13
5	13	22.4	45	77.6	58	30
Total	49	25.4	144	74.6	193	100

Prevalence of gilt herds experiencing RD ranged from 19.2% (5/26) to 35.7% (10/28) in herds with 4 and 0 positive gilts respectively. Herds with 5 positive gilts encountered RD in 22.4% (13/58). On average, 25.4% (49/193) herds encountered RD. Most herds had 0, 1, 2, 3, or 4 seropositive gilts.

Table 5: *Counts and percentages for the presence of reproductive disorders on farms with different PCV2 vaccination status*

	Reproductive Disorders			
	Yes		No	
Farm vaccination status	count	%	count	%
Positive	9	37.5	15	62.5
Negative	48	24.7	146	75.3
Total	57	26.1	161	73.9

The prevalence of sow- and gilt herds to experience RD was 37.5% (9/24) for vaccinated herds and 24.7% (48/194) for non-vaccinated herds.

Table 6: *Counts and percentages for the presence of reproductive disorders on farms with different PRRSV status*

	Reproductive Disorders			
	Yes		No	
Farm PRRSV status	count	%	count	%
PRRSV positive	37	27.8	96	72.2
PRRSV free	12	20.0	48	80.0
Total	49	25.4	144	74.6

PRRSV positive farms had a higher odds of reproductive disorders than farm that were PRRSV free, 27.8% prevalence and 20.0% prevalence respectively.

Since the gilts did not undergo gestation and parturition yet, the incidence of possible reproductive disorders for gilts was unknown. However, since gilts are sows after first parturition, gilt information represents sow data. For this reason, a cross-table was made in table 7 for the association between gilt serological status and sow serological status.

Table 7: *Relation between farms' gilt serological status and farms' sow serological status*

Farm status gilts, number of seropositive gilts	Farm status sow, number of seropositive sows					
	0	1	2	3	4	5
0	**4**	**9**	9	3	4	0
1	**7**	**8**	**6**	8	3	1
2	8	**9**	**8**	**4**	1	4
3	2	5	**2**	**3**	**10**	2
4	2	1	9	**7**	**6**	**3**
5	2	7	11	24	**26**	**6**

The cells in the highlighted diagonal row describe the relation between the farm status gilt and the farm status sow. Sixteen percent (16.4%) was on the diagonal (tainted cells). Fifty-five percent (55.1%) was on the diagonal or 1 cell left or right off the diagonal. This shows a weak relation between the farms' sow –and –gilt status. The SPSS 23 Fisher's Exact test gives a p-value of 0.000. The Kappa test gives Kappa

coefficient of 0.015 with an approximate significance of 0.628, suggesting no correlation.

Cross-table 8 shows the efficacy of PCV sow vaccination.

Table 8: *Counts and percentages for the number of farms with status 0, 1, 2, 3, 4, 5 with respect to their PCV2 vaccination status*

Farm vaccination status	Farm status sow, number of seropositive sows											
	0		1		2		3		4		5	
	count	%	count	%	count	%	count	%	count	%	count	%
Positive	3	11.1	5	18.5	3	11.1	1	3.7	3	11.1	12	44.4
Negative	25	11.6	39	18.1	46	21.3	50	23.1	40	18.5	16	7.4
Total	28	11.5	44	18.1	49	20.2	51	21.0	43	17.7	28	11.5

On the non-vaccinated farms most of the sow herds (23.1%) had 3 out of 5 positive sows, while only 7.4% had 5 out of 5 positive sows. On the vaccinated farms however, 44.4% had 5 out of 5 positive sows, with only 3.7% having 3 out of 5 positive sows. Fisher's Exact test gives p=0.000, assuming a significant difference in sow status between vaccinated and non-vaccinated farms.

Furthermore, a final cross-table was made for the possible relation between the farms' PRRSV status and the farms' PCV2 status, as PRRSV was a possible confounder (Table 9).

Table 9: *Counts and percentages for the number of farms with status 0, 1, 2, 3, 4, 5 with respect to their PRRSV status*

Farm PRRSV status	Farm status sow, no. of seropositive sows											
	0		1		2		3		4		5	
	count	%	count	%	count	%	count	%	count	%	count	%
Positive	15	10.0	27	18.0	38	25.3	35	23.3	24	16.0	11	7.3
Free	9	13.8	12	18.5	8	12.3	15	23.1	16	24.6	5	7.7
Total	24	11.2	39	18.1	46	21.4	50	23.3	40	18.6	16	7.4

The Pearson chi-square test gave p = 0.277, indicating no significant correlation between the farms' PRRSV status and their PCV2 status.

Statistical analysis

The Logistic Regression model was used to determine the p values and the odds ratios (C.I. 95% included) for the five variables that were tested. These five variables are region, type of farm, farm status sow, farm status gilt and farm PRRSV status. None of the factors were proven to have a significant effect on the presence of reproductive disorders in the tested sow herds and gilt herds. The type of farm the herds were housed was found to have a far from significant effect. P-value was 0.923 and the C.I. 95% ranged from 0.374 to 4.169. The PCV2 antibody status of gilt herds

was found to have a p-value of 0.790. C.I. 95% ranged from 0.275 to 5.672. The PCV2 antibody status of sow herds had a larger effect on the presence of reproductive disorders. P-value was 0.621 and therefore not significant either. C.I. 95% ranged from 0.234 to 17.637. The next factor in line was the region in which the herds lived. The p-value was 0.334 and the C.I. 95% ranged from 0.354 to 1.423. The PRRSV status of the sow herds and gilt herds had a p-value of 0.203 and a C.I. 95% from 0.277 to 1.313.

Two-hundred fifty-two farms out of the 285 farms were seropositive. This means that at least one out of the five tested sows on those farms was seropositive. Therefore, the PCV2 sero-prevalence on the tested Dutch farms was 88% (252/285). Seropositivity was probably caused by natural infection, vaccination or a combination of both.

Discussion

This study focussed on sow- and gilt herd PCV2 serological status and the prevalence of reproductive disorders in those herds. It was hypothesized that a positive PCV2 serological status would be related to a low prevalence of reproductive disorders, and visa versa.

No relations were found between farm's PCV2 serological sow- and gilt status and between farm's PVC2 serological status and farm's PRRSV serological status. There was however, a significant relation between PCV2 vaccination and PCV2 serological status. The hypothesis on the other side, could not be confirmed. No significant relations were found between the factors region, type of farm, farm status sow, farm status gilt and farm PRRSV status and the prevalence of reproductive disorders on the farms.

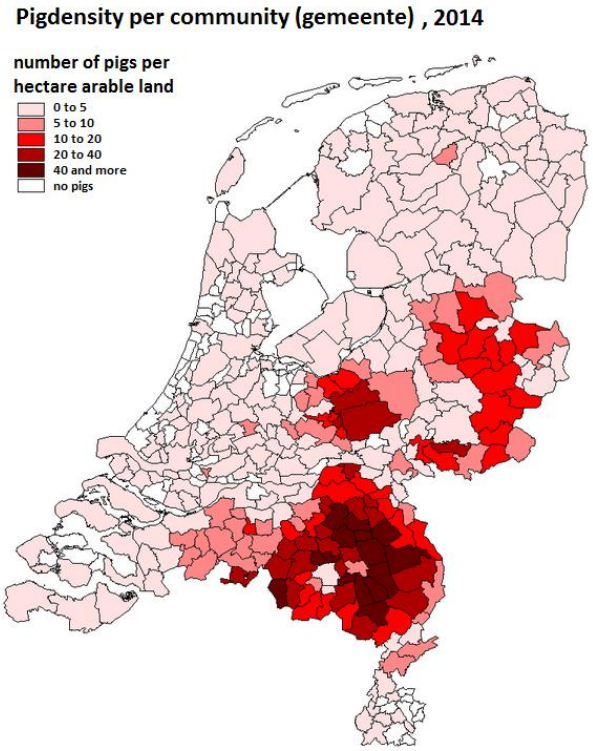
The cooperating farms have a contract with MSD-AH Intervet Nederland BV. By signing the contract, information about swine health and swine health problems are mapped out in the RESPIG program. The program is used for both diagnostic and monitoring purposes.

One could think that farms with considerable health issues were more willing to apply the monitoring program than farms with splendid health status, ór that those farms are less keen to apply the monitoring program to mask their swine health issues. In either way the data could be biased. It is possible that there were many reproductive disorders because there was low immunity in the herds. On the other hand, it is also possible that there were many reproductive disorders caused by other, unidentified, factors, while PCV2 immunity was relatively high. Imagine a farm with good PCV2 vaccination protocols but with other factors strongly stimulating the presence of reproductive disorders. Such farm would be keen to enrol in the study. As a result, PCV2 antibody titres could be associated with reproductive disorders, while this in general may not be true.

The blood samples were analysed by an in house Alfalise test. In order to determine if a sow or gilt was positive or negative a cut-off value of 4.3 log2 was used. Research suggests levels of 6 log2 or higher give a decrease in virus load. Possibly, a cut-off value of 6 log2 would have been more suitable. In the current case, values between 4.3 log2 and 6 log2 may have been ascribed as positive serological status wrongly. Farms with 4.3 log2, thus with possible reproductive disorders since 4.3 log2 may not be protective, are related to positive serological status, suggesting the opposite of the hypothesis.

Anamneses have been taken by several veterinarians. One veterinarian will ask slightly different anamnesis questions than the other. This will lead to different reproductive disorders administration. Prevalence of reproductive may be over- or underestimated leading to either a deviation in the statistical analysis outcome. This deviation can be towards conformation of the hypothesis, or away from it.
Further, like veterinarians, farmers also differ. One farmer might think that one abortion is worthy to be mentioned, while another farmer might think only 5 or more abortions matter. Aberrations of the normal farm specific pattern are listed. There are no fixed numbers that can serve as a threshold. In the anamnesis quotes as "to many" abortions, repeat breeders, early farrowing etcetera are listed. These are subjective observations of the veterinarian or the farmer. It is not clear what the actual prevalence of abortions or repeat breeders was on the farms. As a result, the hypothesis that the prevalence of reproductive disorders is lower when sows and gilts are seropositive could be made more or less plausible. Some of the cooperating farms have been sampled twice. Minimal inter-sampling time is six months. For this reason, one could claim that these data was not independent. But as the porcine reproduction cycle is five months, the sampling took place in a completely new cycle. However, the average replacement rate on Dutch sow farms is 40-45%, so sow herds did not completely change after six months. Therefore, the independence of the data can still be questioned. If farms with for example 5/5 positive sows with no RD were sampled twice that could make the hypothesis that the prevalence of RD is lower when sows and gilts are seropositive more plausible. If farms with 3/5 positive sows with no RD are sampled twice that could make this hypothesis less plausible.

Figure 1:



The first variable tested was Region. Logistic Regression gives a significance of 0.753 which means that the region in which the tested farms were situated does not significantly influence the incidence of reproductive disorders. In the current study two distinct regions were made. It may have been better to have made three regions, according to the pig density in the Netherlands in 2014. Figure 1 shows the map of the Netherlands. There are three pig density hotspots, possibly suitable to be the ''regions''.
Figure 1 from Centraal Bureau voor de Statistiek NL

During the Avian Influenza (AI) outbreak in 2014 the Dutch government divided the Netherlands into 4 regions, based on the poultry density. The poultry density is somewhat similar to the pig density, therefore using the Dutch government's division model could also be a useful model for the current study.

The avian influenza model's regions B, C and D are approximately the same regions as the three hotspots in the pig density model. A combination of those models may be more appropriate on veterinary behalf than current model. If those three regions appear to differ in their contribution to the presence of reproductive disorders, it would be interesting to investigate which exact factors underlie that difference, for example the contact structures. *Figure 2 from Rijksoverheid NL*

Figure 2:Four regions according to Dutch government during 2014 AI outbreak

The second variable tested was the type of farm the sows and gilts were housed. The type of farm does, like the region, is not significantly associated with the incidence of reproductive problems on the tested farms. The confidence intervals show clear overlap.

The third variable is the farm status sow. The outcome of this variable roughly provides the answer to the scientific question of the research. Table 3 provides the cross-table and percentages for this variable. When looking at the significance value ($p = 0.541$) the conclusion can be drawn that there is no significant relation between sow serological antibody status and the presence of reproductive problems. A reason for finding no significant relation is that there is no clear control group for RD. If there was a group of farms that were sure to have no reproductive problems, a better comparison could be made.

The fourth variable that may influence the prevalence of reproductive disorders on the tested Dutch piggeries is the PCV2 antibody status of gilts on a farm. The tested gilts have been examined prior to their first insemination, therefore before farrowing. However, information about gilt reproductive problems is available, paradoxically. After a gilt has farrowed, a gilt is a sow. Since we have information about sow reproductive disorders, we do have information about gilt reproductive disorders. The Logistic Regression model gave $p = 0.955$, a value far from significant. Explanation for this could be that the reproductive disorders administration was not representable for gilt parturition but rather for higher parity. Another explanation could be that sows and gilts are exposed to different factors that can influence reproduction success.

In the present study, vaccination has a positive association with serological antibody status in the tested sows. Eleven percent of the tested sows had been vaccinated (53% Porcilis® PCV, 37% Ingelvac® CircoFLEX, 10% Circovac®). It is important to understand whether sows have been vaccinated to prevent *Circovirus* infection or that sow were diagnosed with circo-associated problems and were therefore vaccinated. In

the first case there will probably be a negative association between PCV2 antibody titers and reproductive disorders. In the second case there will probably be a neutral or positive association. However, no certainty can be given about the actual outcome, therefore use of vaccination was one of the exclusion criteria.

Sow blood samples were collected within two weeks post-partum. Positive titers can have three causes. Firstly, the sow can be vaccinated. Secondly, the sow can have had a field infection prior to gestation. Thirdly, the sow can have experienced a field infection during gestation. The first and second cause do not lead to reproductive disorders *(Madson et al., 2009).* The third cause can lead to reproductive disorders *(Park et al., 2005; West et al., 1999),* or to no reproductive disorders, because piglets themselves can produce neutralizing antibodies too in late gestation *(Madson et al., 2009; Saha et al., 2014).* Since there is much indistinctness, this is another reason to exclude farms with unclear or positive vaccination status from further analysis.

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) can cause reproductive disorders like mummified fetuses, weak live-born piglets, abortion, premature farrowing and stillborn piglets, similar to the reproductive disorders caused by *Porcine Circovirus type 2 (Goyal., 1993; Rossow et al., 1995).* Since PRRSV and *Porcine Parvo Virus* (PPV) can cause similar problems as PCV2, the reproductive disorders described by both farmers and veterinarians could be caused by any of these viruses. A reason for differences in association between sow herd PCV2 status and the incidence of reproductive disorders can therefore be the PRRSV- or PPV status. The current study looked at the effect of the farms' PRRSV status on the presence of reproductive disorders and at the possible role of PRRSV as a confounder. PRRSV did not significantly effect the presence of reproductive disorders on the tested Dutch swine herds. The logistic regression model gave $p = 0.177$. Neither was PRRSV a significant confounder. The Pearson chi-square test gave $p = 0.277$.

Current study described and reflected a select group of factors that are known to influence the prevalence of reproductive disorders. However, there are more additional factors that can directly or indirectly influence the prevalence of reproductive disorders. Two examples are given.

One factor could be the pig breed used on the farms. Experimental studies have suggested a higher susceptibility to PMWS in Landrace pigs compared to Piétrain, Duroc and Large White pigs *(Opriessnig et al., 2006; Opriessnig et al., 2009).* Field studies have found a higher susceptibility in pure bred conventional Yorkshire and Landrace boars than in pure bred conventional Hampshire boars *(Wallgren et al., 2009).* This difference in susceptibility may also be observed in PCV-RD. Since no data about the sow and boar choice on the questioned farms was available, some of the reported reproductive disorders were possibly to be assigned to the herds' breed.

The other factor is the presence of fungal toxins in feed. It has been suggested that ochratoxine A can act as an important trigger for PCV2 infections *(Gan et al., 2015).* It was not known what quantities of ochratoxine was in the foods of the researched sow and gilt herds. It is unknown what effect admixture of ochratoxine has on the hypothesis of the current study. Possibly the prevalence of reproductive disorders increases. Possibly the antibody status of the herds increases. However, due to this

uncertainty, no reliable conclusions can be drawn about the effect of ochratoxine on the hypothesis in this study.

Conclusion

The results of this study suggest that there is no significant association between the serological PCV2 status of sow herds and reproductive disorders in the Netherlands. This study was able to point out some critical factors that might have influenced the outcome of the question. The next paragraph may present useful leads for further research.

Recommendations

If a new study on associations between the serological PCV2 status of sow herds and reproductive disorders in these herds would be performed, some changes in the research protocol could be made to improve the analysis. In the current study a group of factors that are known to influence the prevalence of reproductive disorders are analyzed. However, the inclusion and exclusion rules for farms to enroll in the study were not very well described and therefore multi-interpretable. Besides that, factors that can directly or indirectly influence the prevalence of reproductive disorders are not well controlled for.

The easiest way to ensure that farm test-results are independent within the farm, would be to sample each farm only once and preferably in the same period of time to reduce seasonal influences.

Farms should have similar serological status for other pathogens that can alter the prevalence of reproductive disorders like PPV and PRRSV. By preference either all farms perform PCV2 vaccination or no farms perform PCV2 vaccination for sows and gilts. If farms with PCV2 vaccination are used, it must be clear whether the vaccination is used to prevent PCV2 clinical symptoms or to lower the prevalence of the clinical symptoms that are already present on the farm. Farms should preferably all have negative PRRSV serology and negative PPV serology.

Furthermore, there should be a clear distinction between the two dependent variables: a group with well described reproductive disorders and a group with clearly no reproductive disorders. A way to create these groups is by having a decisive anamnesis protocol. In the current study, the anamneses were recorded by way of a questionnaire that asked about reproductive disorders on the farm like abortion, premature birth, weak piglets, infertility, and repeat breeders. The issues that were questioned were good, but for a more reliable result the farmer should be presented a list on which he can score the number of cases for a set period of time. In addition, the farmer should also note the parity of the sow, so that the possible influence of the parity can be researched and so that gilt blood samples can be linked to gilt reproductive disorders.

To record whether seroconversion occurs during insemination or during gestation due to intercurrent infection, blood samples can be taken a couple of days prior to the insemination and two weeks postpartum

The blood samples can be analyzed by the in house Alfalise test, but no cut-off value should be used. A relation between the antibody titer and the prevalence of reproductive disorders can be calculated if test results are interpreted as a continuous variable instead of a categorical variable.

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