EFFECTS OF PORCINE CIRCOVIRUS TYPE 2 (PCV-2) VACCINATION ON REPRODUCTIVE PARAMETERS UNDER DANISH FIELD CONDITIONS

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SUMMARY

A field study of PCV-2 vaccination in sows and gilts was carried out. The study contained 1 vaccinated (vac.) and 1 non vaccinated (non vac.) group in 16 consecutive batches. The outcome of 549 artificial inseminations (AI) was studied to test whether farrowing rates were independent of PCV-2 vaccination before AI. Differences in farrowing rates were not statistically significant. However a tendency to statistical significant difference in farrowing rates was found between vaccinated sows (85,9 %) and non vaccinated sows (77,3%) for parity 1 and parity 2. First parity and second parity sows had 1,05 live born piglet more in vaccinated group versus non vaccinated group. For all parities the difference was 0,6 live born piglet more. This study indicates that control of PCV-2 in gilts, first parity and second parity sows may decrease return to estrus due to implantation failure with positive effect on farrowing rate and number of live born piglets pr. sow. This study further indicates that presence of PCV-2 in the blood at levels around 1E+04 copies/ml could be related to reproductive porcine circovirus diseases (PCVD´s) but not post weaning multisystemic syndrome (PMWS). Further studies are needed to confirm the findings of this study.

INTRODUCTION

PCV-2 has been shown to be the causative agent in the disease syndrome PMWS (Allan and others 2004) alone or in association with other pathogens or immunostimulants (Allan and others 1999; Allan and others 2000; Krakowka and others 2007). However other PCV-2 related diseases now known as PCVD´s has also been described (Allan and others 2003; Segales and others 2004; Chae 2005; Jensen and others 2006; Opriessnig and others 2006b). Among these are reports on reproductive failure of the sow (West and others 1999; Ladekjaer-Mikkelsen and others 2001; O'Connor and others 2001; Farnham and others 2003; Brunborg and others 2007; Høgedal and others 2008). In many of these cases no PMWS occurred. PCV-2 has been reported to be transmitted transplacental (Pensaert and others 2004; Yoon and others 2004) and even being shed by the sow in colostrum (Shibata and others 2006). Inoculation of the sow with PCV-2 at different stages of gestation results in return to estrus, foetal death and mummification, stillborn or weak born piglets (Johnson and others 2002b; Nielsen and others 2004; Mateusen and others 2004; Pensaert and others 2004; Mikami and others 2005; Park and others 2005).
Sows have been reported to be PCV-2 viremic in field conditions at levels from 0,7-7,6 % detected by polymerase chain reaction (PCR) in serum (Calsamiglia and others 2007). Under experimental conditions viremia is shown to persist more than 50 days in inoculated sows (Pensaert and others 2004), however pigs in the field may stay viremic for months (Carasova and others 2007).

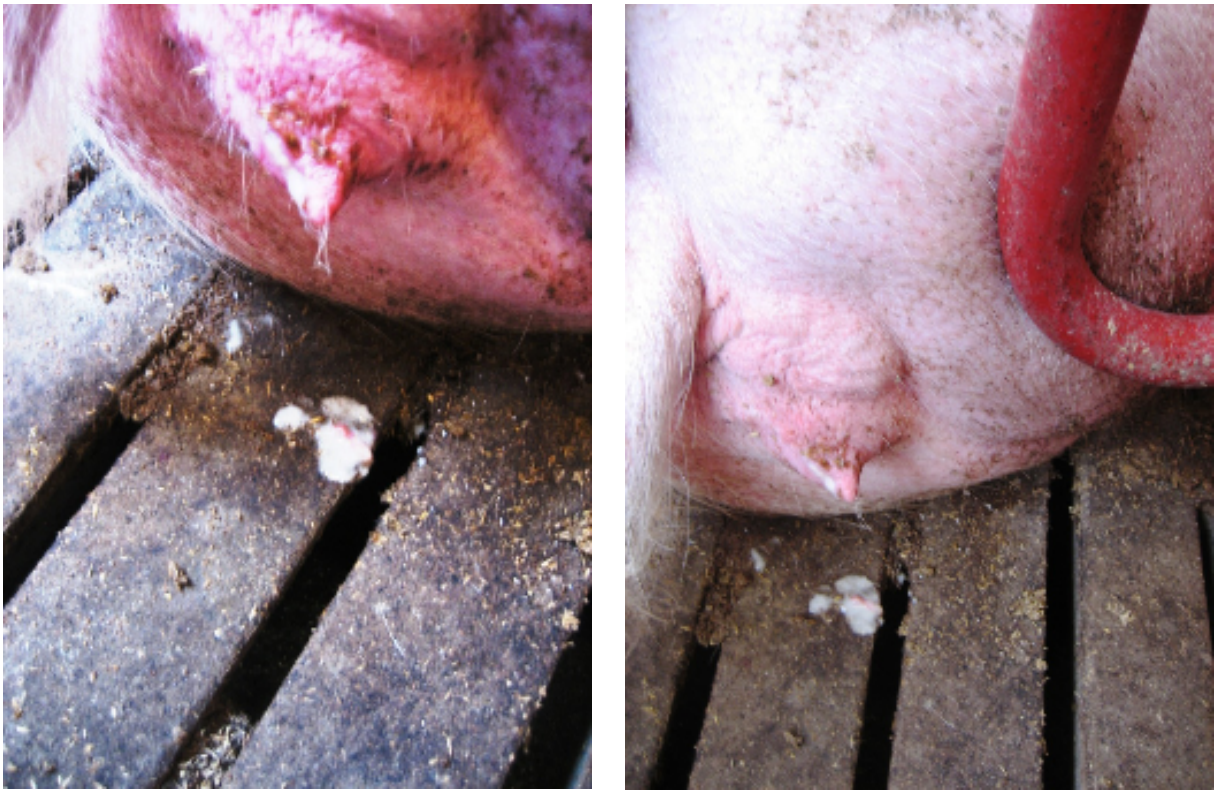
MATERIAL AND METHODS

The purpose of this study was to evaluate the effects of the vaccination of gilts and sows with an inactivated PCV-2 vaccine (Circovac®, Merial) before AI in a herd with a history of low farrowing rate (defined as below 85%) but without clinical signs of PMWS under Danish field conditions. In the selected herd PCV-2 was present in gilts and sows during the AI period. The null hypothesis was that farrowing rate is independent of PCV-2 vaccination before AI.

Selection and description of the herd

A 600 sow multiplying farm was selected. All genetics are Danbreed. All sows in the farm were Landrace x Landrace, and offspring predominantly Landrace x Yorkshire; however some were Landrace x Landrace for internal replacement.
The farm is a 2-site farm, with sows and gilts from 14 weeks of age (replacement gilts) at site 1, weaning piglets at 4 weeks of age to site 2. Site 2 contains pigs from 4 weeks of age to approximately

30 weeks of age. Replacement gilts are moved back from site 2 to site 1 at approximately 14 weeks of age and kept in separate rooms until entering the AI room.
Pregnant sows are housed loose in groups, however with sows kept confined in single boxes 4 weeks after AI. The herd has weekly batch management and 1 farrowing room pr. batch (separate airspace). 1 buffer farrowing room also exists for nursing sows and nursing piglets.
Health surveillance in this multiplying herd is performed for diseases according to the Danish SPF system every month (SPF SUS). The health status of the herd is SPF with presence of Actinobacillus Pleuropneumonia serotype 2 and Mycoplasmae Hyopneumonia. No clinical sign of PMWS has been found, and mortality rates are continuously monitored and reported to Danish Pig Production (DPP).
The herd has been free of porcine respiratory and reproductive syndrome virus (PRRSV) based on monthly serology, and has been correctly and consistently vaccinated against porcine parvovirus (PPV). No other known pathologies leading to reproductive disorders have been identified in the herd. Treatment of vaginal discharge with oral antibiotics has not been perceived as curative. Vaginal discharge was observed to contain material perceived as foetal membranes 3-5 weeks post AI.



Picture 1. Vaginal discharge with foetal membranes

Type of study

The study is a non blinded field study with 2 parallel randomized groups of gilts and sows (vaccinated and non vaccinated / control group). No placebo was used in the control group. Sows and gilts were randomly assigned to groups depending on their unique herd ID (ear tag even and uneven numbers). Vaccination was carried out by 1 trained staff member in the farm. Circovac® was applied to sows and gilts as primary (6 weeks before AI) and secondary vaccination (3 weeks before AI) before insemination and as a booster 3 weeks before farrowing according to the product label. The study has been registered and approved by The Danish Medicines Agency.

Herd data collection

A serological profile based on unstabilized blood in 64 animals was carried out 2 months prior to the first AI included in the study, and analyzed by commercial tests at DTU - Danish Veterinary Institute. PCV-2 was detected by enzyme linked immunosorbent assay (ELISA) antibodies and quantitative real-time PCR. PPV antibodies were detected by ELISA. Gilts for bleeding were selected according to age groups. Sows were selected in order to represent different parities with and without reproductive

pathologies. The serological profile was used to confirm that PPV vaccination was carried out correctly in the herd, and to give an idea of PCV-2 circulation in the herd. Recordings for each individual sow and gilt were kept and computerized for 16 consecutive weekly batches (the length of 1 gestation cycle). Data is contained in herd paper records and Agrosoft® WinSvin software (Agrosoft A/S). All data was finally pooled in an Excel (Anonymous) sheet to form the final database.

Statistical analysis

Statistical analysis was performed. Each AI was considered as an independent event. However sows or gilts with 3 or more consecutive returns to estrus were not included in the analysis unless specified, as they were defined as statistical outliers and not independent events. Statistical analysis has been performed in Excel ™ (Anonymous) and StatCalc (Anonymous2008) by Yates corrected Chi square test, F-test for equal variances and T-test for differences in means.

RESULTS

Serology

Serological analysis was performed on 64 sows and gilts from 6 weeks of age to 160 weeks of age. Titres for PPV show peaks in titres around first and second vaccination 24- 28 weeks of age. PPV antibody peaks shows boosting of titre due to revaccination occurring at intervals of approximately 22 weeks after last conception corresponding to the reproductive cycle in the farm.

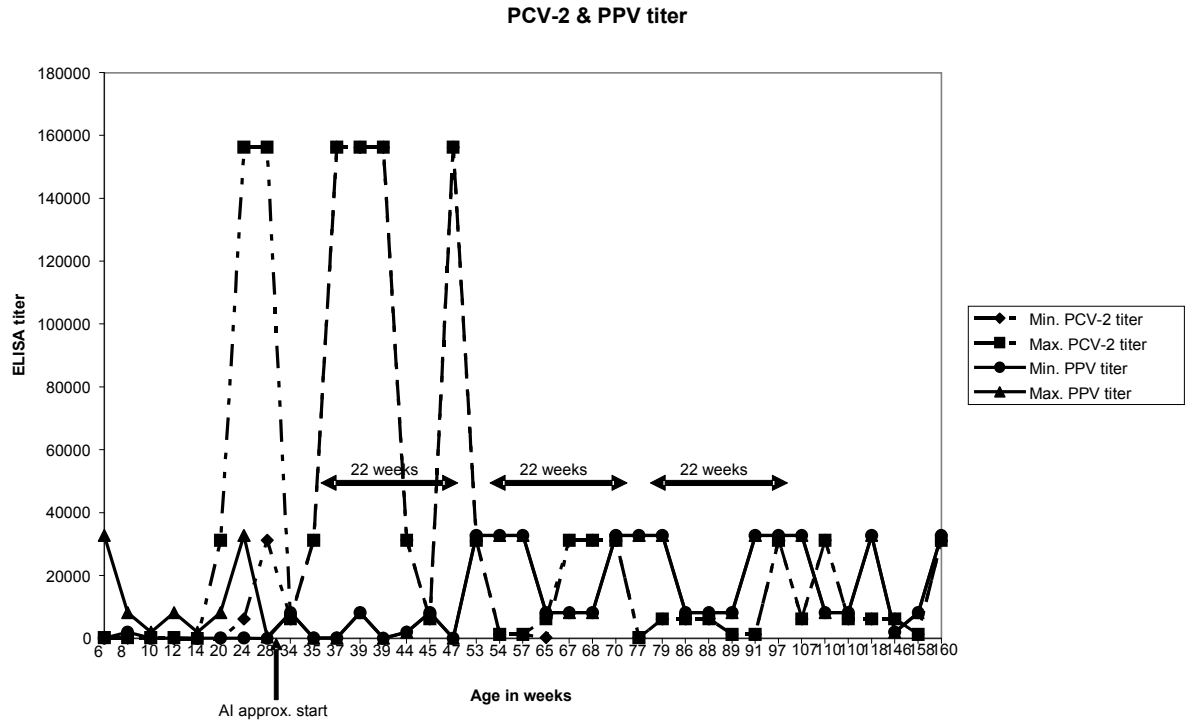


Figure 1 Serological profile

PCV-2 titres start to evolve from 14 weeks of age, and reach the highest level before start of AI. Titres persist during first pregnancy and beyond second AI. The seroconversion takes place after movement of gilts from site 2 back to site 1. 17 of 18 gilts from 14 weeks of age to 28 weeks of age were PCR positive for PCV-2 in serum (94% positive). Quantitative PCR detects PCV-2 DNA copies. The highest numbers of copies pr. ml serum is observed before 30 weeks of age. Older sows show sporadic levels of PCV-2 by PCR in the blood. 7 of 30 sows were PCR positive for PCV-2 by PCR (23% positive). 6 of these were first parity (2 with 3 and 4 returns to estrus) and 1 was second parity. Peaks in PCV-2 titre could suggest some cyclic exposure (figure 1), however detection of PCV-2 in serum by PCR is related to gilts, first and second parity sows.

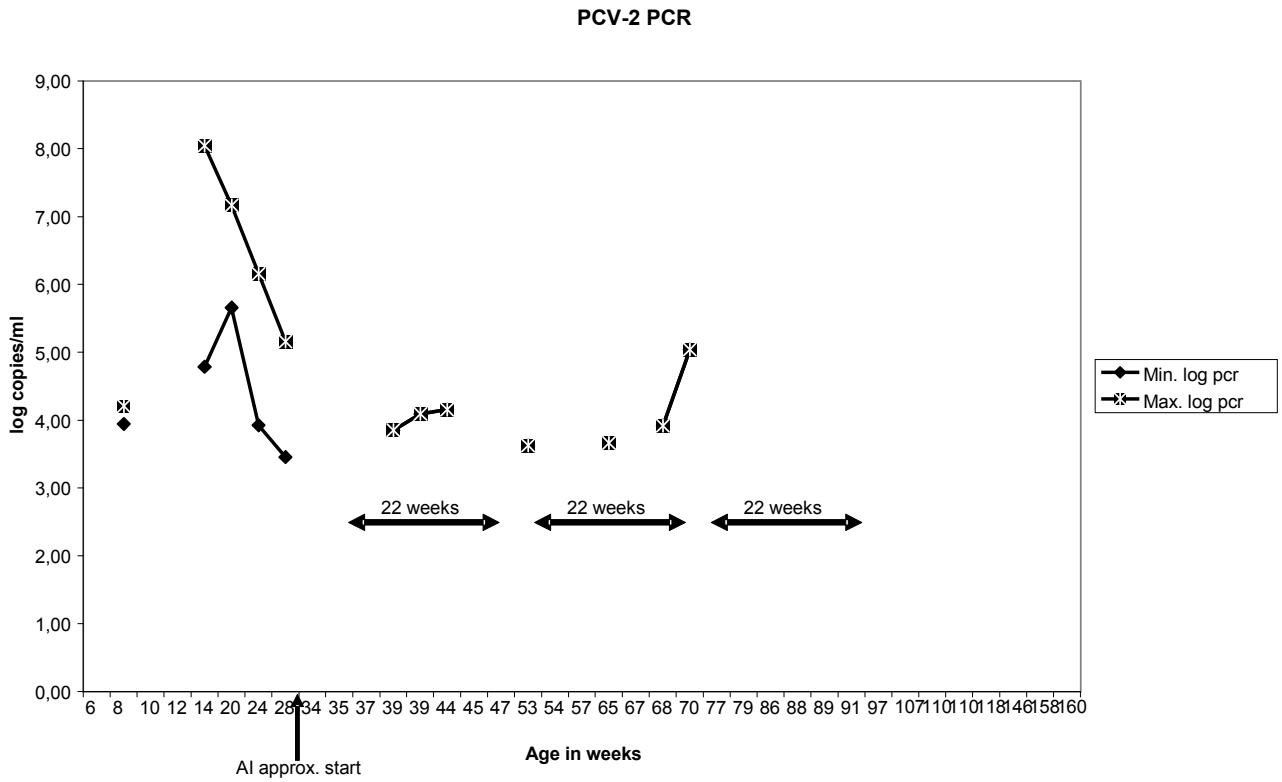


Figure 2 log PCV-2 copies/ml serum

Farrowing rates

A total of 549 AI´s was performed resulting in 431 farrowings. 15 sows or gilts had more than 3 consecutive returns to estrus, and despite 3 consecutive returns 4 of these sows actually did farrow (1 unvaccinated and 3 vaccinated sows). 11 sows were not culled despite 3 or more returns to estrus. These 15 sows are omitted from calculations of farrowing rates. Culling before farrowing is included in the calculation of farrowing rate.

Table 1 Results of AI´s

Parity		AI	Return to estrus	Culled before farrowing	3. returns not culled	Farrowings
All	Vac	248	28	14	8	198
	Non vac.	301	38	27	3	233
	Sum	549	66	41	11	431

Table 2 Results for sows by parity

Parity		AI	Return to estrus	Culled before farrowing	3. returns not culled	Farrowings
1.	Vac	77	10	3	1	63
	Non vac.	119	23	3	1	92
2.	Vac	57	2	3	5	47
	Non vac.	59	5	9	1	44
3.	Vac	45	6	1	0	38
	Non vac.	44	1	4	0	39
4.	Vac	21	2	1	0	18
	Non vac.	31	3	4	1	23
>5.	Vac	48	8	6	2	32
	Non vac.	48	6	7	0	35
1. & 2.	Vac	134	12	6	6	110
	Non vac.	178	28	12	2	136

Table 3 Farrowing % by parity

Yates corrected Chi square-test				Relative risk	Cofidence limits	
Farrowing %		%	P-value	RR	Lower 95%	Upper 95%
All parities	Vac	82,5	0,26	0,80	0,57	1,14
	Non Vac.	78,2				
Parity 1	Vac	82,9	0,51	0,78	0,43	1,41
	Non Vac.	78,0				
Parity 2	Vac	90,4	0,08	0,40	0,15	1,03
	Non Vac.	75,9				
Parity 3	Vac	84,4	0,79	1,37	0,47	3,99
	Non Vac.	88,6				
Parity 4	Vac	85,7	0,66	0,61	0,18	2,10
	Non Vac.	76,7				
Parity >5	Vac	69,6	0,90	1,12	0,59	2,13
	Non Vac.	72,9				
Parity 1 & 2	Vac	85,9	0,08	0,62	0,37	1,03
	Non Vac.	77,3				

A tendency (p=0,08) of statistical significant difference in farrowing rate of 8,6% is observed for the pooled first and second parity sows. According to the serological profile this group of sows could be at risk for PCV-2 exposure and PCV-2 infection.

Post farrowing Parameters

Distribution of post farrowing parameters was checked (figure 3 & 4). No abortions were observed during the study. No mummified piglets were recorded. Cross fostering took place between sows irrespective of group assignment.

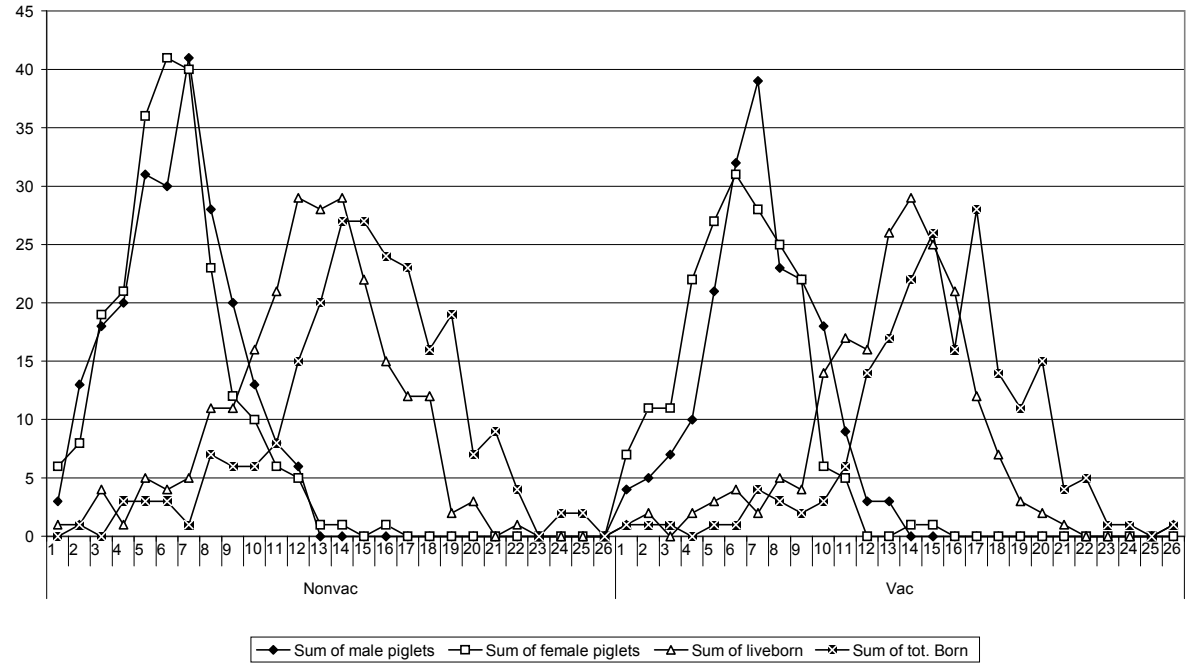


Figure 3 Normal distributed post farrowing parameters

Table 4 T-test performance after farrowing all parities.

Test of 431 farrowings						
All parities		**Mean**	**Standard deviation**	**F-test**	**P(T<=t) one-tail**	**P(T<=t) two-tail**
Total born	Non Vac.	14,91	3,99	0,54	0,31	0,15
	Vac	15,30				
Live born	Non Vac.	12,48	3,69	0,91	0,05	0,10
	Vac	13,08				
Female piglets	Non Vac.	6,16	2,59	0,77	0,41	0,82
	Vac	6,11				
Male piglets	Non Vac.	6,35		0,84	0,01	0,01
	Vac	6,97				

Sows with more than 3 returns to estrus are included in the analysis (4 sows that actually farrowed), a statistical difference of 0,6 live born piglet is observed between groups in the one tailed test. The difference in live born piglets is due to significantly more male piglets in the vaccinated group.

Considering the observed difference in farrowing rates when pooling data from first and second parity sows, analysis was carried out regarding parameters related to number of piglets for parity 1 and parity 2 sows.

Table 5 Piglets born from first & second parity sow

Test of 246 farrowings						
Parity 1+2		Mean	Standard deviation	F-test	P(T<=t) one-tail	P(T<=t) two-tail
Total born	Non Vac.	13,89	3,94	0,75	0,06	0,11
	Vac	14,69				
Live born	Non Vac.	12,04	3,65	0,82	0,01	0,02
	Vac	13,09				
Female piglets	Non Vac.	5,94	2,61	0,42	0,14	0,28
	Vac	6,30				
Male piglets	Non Vac.	6,15	2,54	0,49	0,02	0,05
	Vac	6,79				

A statistical difference of 1,05 live born piglet is found between groups of parity 1 and parity 2 sows. The difference in live born piglets is due to significantly more male piglets in the vaccinated group.

Analysis of piglet mortality the first 5 days after farrowing

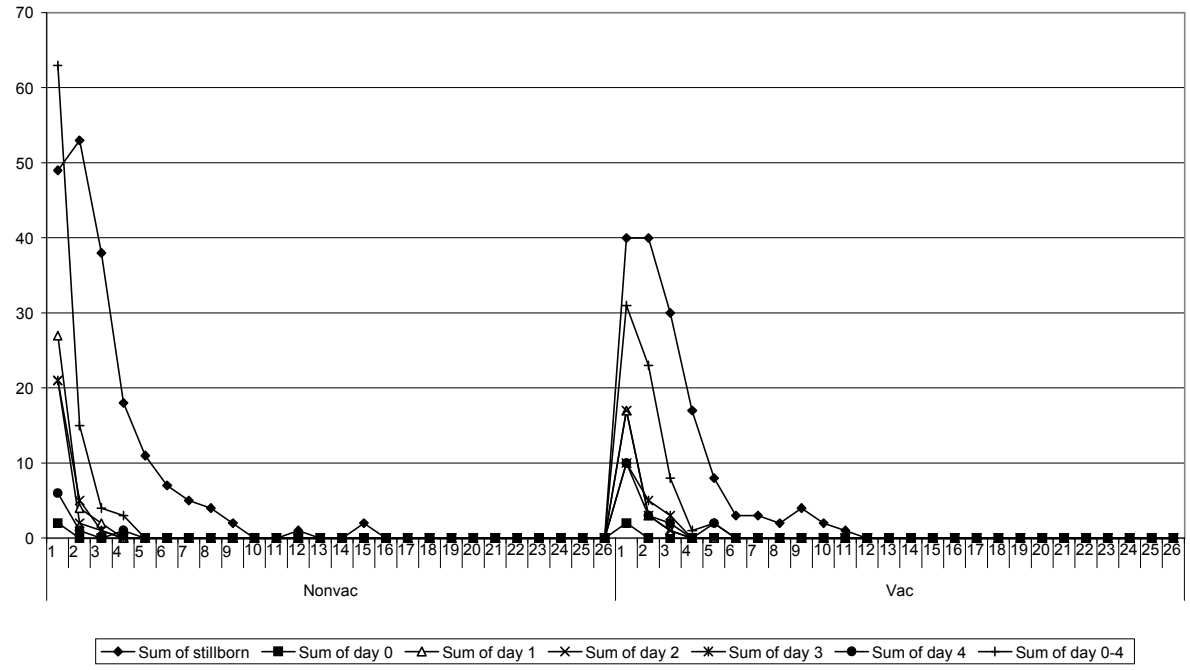


Figure 4 Not normal distributed mortality data

Table 6 Stillborn piglets all parities

Yates corrected Chi square-test		Stillborn piglets			Relative risk	Cofidence limits	
All parities		>=5	<5	P-value	RR	Lower 95%	Upper 95%
	Non Vac.	32	201	0,61	1,18	0,72	1,95
	Vac	23	175				

No overall difference in number of stillborn piglets between groups is detected with cut off at 5 stillborn piglets.

Table 7 Piglets dying during first 5 days of life

Chi-square test		dead piglets			Relative risk	Cofidence limits	
All parities		>1	0	P-value	RR	Lower 95%	Upper 95%
	Non Vac.	85	148	0,49	1,11	0,86	1,44
	Vac	65	133				

No significant difference in piglet death during the first 5 days of life is detected between vaccinated and nonvaccinated sows.

DISCUSSION

The use of quantitative PCR (QPCR) for detection of PCV-2 viremia has been accepted as useful on herd level, but not in the individual animal for assigning PMWS diagnosis (Grau-Roma and others 2008). In this herd antibody levels for PCV-2 steadily inclines from 14 weeks and stays at a high level up to 50 weeks of age thus with individual variance. Antibody titres are not necessarily correlated to protection, but might as well be associated to high viremic load (Meerts and others 2005). QPCR are in the range 1E+05 to 1E+08 from 14 weeks to 28 weeks of age. However levels at 1E+04 to 1E+05 were observed in sows of 37 weeks to 70 weeks of age. This observation is similar to other findings (Carasova and others 2007). The clinical relevance of presence of PCV-2 in sows has not been described. The embryo becomes susceptible for PCV-2 infection after "hatching" the zona pellucida (Mateusen and others 2004), and presence of PCV-2 virus in the sow up to 50 weeks of age might possibly affect conception in first parity and second parity sows and gilts, and thus explain the observed overall difference of 4,3 % in farrowing rates in this study. However for first and second parity sows the observed difference was 8,6 % (P=0,08). Ideally the study should be made in first parity sows only to avoid confounding from the last gestation cycle. The observed improvement in farrowing rate is less than the estimated 7,6 % PCV-2 viremic sows in 7 Spanish herds (Calsamiglia and others 2007). The sample size in this study is too low to detect a statistical significant difference of 8 %. The needed sample size would be 361, thus making the study impossible in most commercial herds unless the herd is recently established. This study indicates that presence of PCV-2 in the blood at levels around 1E+04 copies/ml could be related to reproductive PCVD but not PMWS.
For first parity and second parity sows the statistical difference of +1,05 live born piglet is stronger than considering all parities (+0,6 live born piglet). Under Danish field conditions a before-after analysis of productivity records from 15.000 sows in 34 herds revealed a statistically significant difference at +0,24 piglets 3 months before vaccination versus 3 months after vaccination (Kunstmann and Lau 2008). These findings could suggest PCV-2 interacts with the implantation of the embryo in the uterus or is related to later embryonic death under field conditions. The pathogenesis has been shown experimentally (Pensaert and others 2004). The observation of foetal membranes in vaginal discharge could support PCV-2 as causative agent in early embryonic death and implantation failure, and is supported by the improvement in number of live born piglets as well as the trend in improved farrowing rates in the vaccinated group. Experimental infection of sows with PCV-2 by the intra nasal or the intra uterine route has been performed during the later stages of pregnancy (Nielsen and others 2004; Pensaert and others 2004; Park and others 2005) but more studies in sows are needed during earlier stages of pregnancy. The negative effect of PCV-2 is probably related to gilts and young sows.
The recording of piglet mortality from day 0 to day 5 shows no overall indication of piglets from non vaccinated animals to be more likely to die. Others have observed decreased viability of piglets related to exposure late in pregnancy by PCV-2 (Johnson and others 2002; Nielsen and others 2004).. However as cross fostering takes place a litter or sow effect may not be seen (Calsamiglia and others 2007).
This study indicates that control of PCV-2 in gilts, first parity and second parity sows may decrease return to estrus due to implantation failure with positive effect on farrowing rate and number of live born piglets pr. Sow, however further studies are needed to confirm this.

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