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Vaccination of Sows against Porcine Circovirus Type 2 (PCV2) in a Subclinically Infected Herd Does Not Impact Reproductive Performance

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Abstract: Porcine circovirus type 2 (PCV2) vaccination in piglets has become one of the crucial and indisputable procedures in modern swine production. The role of PCV2 vaccination in breeding animals is less explored. In the present study, the evaluation of the impact of sow vaccination on reproductive parameters was performed in a high health status, PCV2 subclinically infected herd of 3200 sows. The comparison of the number of liveborn, stillborn, and weak born piglets between groups of sows vaccinated on 1 or 28 days after weaning, or nonvaccinated, did not show any statistically significant differences. Although in the tested farm the vaccination of sows appeared to have no effect on reproductive performance, the results should not be generalized. Careful and individualized analysis of diagnostic and production data is crucial in economizing infectious disease control programs in sow herds and pig populations.

Keywords: PCV2; PCV2-RD; vaccination; reproductive parameters

1. Introduction

Porcine circovirus type 2 (PCV2) is a small, nonenveloped member of the family *Circoviridae*. The virus is ubiquitous in domestic swine and is involved in an aetiology of a wide range of disease syndromes causing significant economic losses in the swine industry [1]. Most commonly, PCV2 causes subclinical infections in pigs (PCV2-SI). However, in the presence of certain infectious or noninfectious triggers, the course of infection may drift towards a range of clinical forms, collectively referred to as porcine circovirus diseases (PCVD) [2]. The vast majority of published PCV2 pathogenicity studies present its key role in the development of systemic disease characterised by severe impairment of the immune system (PCV2-SD, previously known as postweaning multisystemic wasting syndrome—PMWS) as well as enteric disease (PCV2-ED), lung disease (PCV2-LD), and reproductive disease (PCV2-RD) [2]. Lymphoid depletion and lymphopenia in peripheral blood are consistent features in pigs with clinical PCVD [3]. The mechanism underlying the outcome of PCV2-RD is poorly understood. The evidence from experimental data showed that porcine embryos are susceptible to infection, which might lead to embryonic death, stillbirth, or foetus mummification [4]. There are several well-documented cases of experimentally induced PCV2-RD following transuterine inoculations of foetuses [5], oronasal inoculation of pregnant sows [6], intrauterine inoculation of PCV2-negative sows [7], and embryo exposure [8].

The efficacy of vaccination against PCV2 has been broadly studied and confirmed in numerous studies. All commercial PCV2 vaccines are well-known to reduce the viremia, viral load in lymphoid tissues, and virus shedding [9–19].

Immunisation of piglets, usually undertaken around weaning age in farms with clinical manifestation of PCVD, leads to significant improvement of production results, mainly reduced mortality and more unified growth rates [20–22]. Additionally, vaccination could be economically justified also in subclinically infected herds [1]. Thus, PCV2 vaccination in piglets has become one of the crucial and indisputable procedures in modern swine production [23].

The role of the PCV2 vaccination in breeding animals is less explored. Currently, only Circovac® (Ceva Santé Animale, Libourne, France) is licensed for application in sows in the EU. However, Ingelvac CircoFLEX® (Boehringer Ingelheim Vetmetica GmbH, Ingelheim am Rhein, Germany) license permits the vaccination of all pigs from three weeks of age (WOA). Therefore, both vaccines can be legally applied in gilts and sows. Vaccination of sows was originally proposed to increase the level of maternal immunity passed to their progeny [18,24,25]. Despite some concerns regarding potential interference between maternal immunity and the vaccine antigen in young piglets [22,24], reduced prevalence of PCV2 viremia in vaccinated pigs born from vaccinated sows was reported [12,19,22,26].

Less is known about the impact of PCV2 vaccination on reproductive performance of sows. PCV2-RD was described only in very high health status or newly established farms, and in gilts fully sensitive to the PCV2 infection [27,28]. Recently, some concerns regarding the reappearance of PCV2-RD problem have arisen. Due to the common vaccination and general decrease of infectious pressure, replacement animals may remain naive until late age and get infected upon introduction into the breeding herd [29]. This may lead to reproductive problems at all gestation stages and infection of piglets in utero [4,30]. However, the impact of the latter on piglet health was not quantified.

Previous study showed that gilts and sows up to second parity have the greatest chance to spread PCV2 and contribute to the virus' persistence in conventional sows herds [31]. Therefore, the vaccination of gilts is often practiced to eliminate the risk of PCV2-RD in first litters. However, anecdotal field observations indicate that also the vaccination of sows can improve some reproductive parameters, even in endemically infected sow herds where the level of viremia is low or below the PCR detection limit. Nevertheless, strong scientific data to verify this hypothesis remain scarce. Thus, the objective of the present study was to assess the impact of sow vaccination against PCV2 on reproductive parameters in a large-scale and high-performing commercial sow farm.

2. Materials and Methods

2.1. Herd Description

The study was conducted in a high-performing, porcine reproductive and respiratory syndrome virus (PRRSV)-negative sow farm (3200 DanBred sows) located in Northern Poland. The farm used cross-breeding: ♀ (♂ Landrace × ♀ Yorskshire) × ♂ Duroc to produce feeder pigs. Replacement gilts were multiplicated at a different location and periodically transported (respecting quarantine rules) from the nucleus farm belonging to the same company and having the same health status and production standards. The standard PCV2 vaccination protocol in the farm involved immunization of 4-week-old piglets, 31-week-old replacement gilts, as well as sows in every cycle, just after weaning. The production parameters were maintained at a high level before the trial: 17.21 liveborn per litter, 1.40 stillborn per litter, 0.40 mummified per litter, 33.56 weaned per sow per year, and 91.5% farrowing rate. At the beginning of the study, parity distribution was as follows: parity 0–16%, parity 1–19%, parity 2–19%, parity 3–15%, parity 4–12%, parity 5–10%, parity 6–8%, parity 7–1%.

Prior to the study, sera were collected from 25 random piglets (in 1, 7, 14, 21, 28 days after birth), 30 replacement gilts (in 7, 10, 14, 18, 23, 33 WOA), and 20 pregnant sows of different age groups. The samples were pooled by five and tested by real-time PCR in order to assess PCV2 circulation in the population. The results showed low PCV2 viral loads with cycle threshold (Ct) of 32.0 and

36.0 in samples collected from 2-week-old piglets and 33-week-old replacement gilts, respectively, which confirmed that PCV2 subclinical infections were present in the herd [2,12].

2.2. Study Design

In order to assess whether maintaining or a modification of the protocol of sow vaccination was beneficial for the production parameters, six weekly groups of weaned sows (from November 2017 to January 2018) were randomly divided into three subgroups, to which different PCV2 vaccination protocols were applied in the next reproduction cycle. Animals in group A (183 sows) were vaccinated intramuscularly the day after weaning with 1 mL of CircoFLEX®. Group B (175 sows) remained unvaccinated, and group C (180 sows) was vaccinated at 28th day after weaning. The vaccination timing in group C was chosen to analyse the possibility of prevention of embryo losses using PCV2 immunisation after conception but during (or shortly after) the implantation period. The study was a part of standard health management of the herd, so the agreement of the local ethics committee was not required.

The proportion of all animals selected for the trial according to their parity was: parity 1–24%, parity 2–21%, parity 3–18%, parity 4–13%, parity 5–14%, parity 6–10%. Replacement gilts were excluded from the trial. The number of liveborn, stillborn, mummified, and weak piglets were recorded for each group. Piglets born weak were defined as animals presenting low viability and/or lower birth weight comparing to their littermates. The data were collected in real-time by farm personnel using commercial software (Cloudfarms, Cloudfarms s.r.o., Slovakia) installed on handheld devices.

2.3. Statistical Analyses

The Kolmogorov–Smirnov test of goodness-of-fit was used to assess the normality of each dependant variable tested. Considering the non-normal distribution of the data (No. of piglets born, No. of piglets born alive, No. of piglets born dead, No. of piglets mummified, No. of piglets born dead and mummified, No. of weak piglets), further analysis was performed based on a nonparametric Kruskal–Wallis one-way ANOVA. The influence of the treatment and parity on the percentage of sows with piglets born dead and mummified was compared between groups with a Chi-square test. The level of statistical significance was set at p-value = 0.05.

3. Results

In total, reproduction parameters were obtained from 538 sows of 2nd–6th parity which gave birth to 10,465 piglets. In group C (sows vaccinated 28 days after weaning) the highest average number of liveborn (17.99 ± 3.0) and the lowest (1.83 ± 1.9) average number of stillborn were observed (Table 1). Interestingly, the lowest average number of mummified (0.55 ± 0.9), as well as piglets born weak, (2.25 ± 3.0) was recorded in unvaccinated group B (Table 1). Surprisingly, the worst results were observed among sows vaccinated on the first day after weaning (Table 1). However, the differences between the groups were only numerical and not statistically significant ($p > 0.05$).

The comparison of reproduction parameters in sows of different parity also did not show statistically significant differences between the groups ($p > 0.05$) (Figure 1). Neither the treatment nor the parity influenced the percentage of sows with piglets born dead and mummified (data not shown).

Table 1. Reproduction parameters in all (2nd–7th parity) sows observed during the trial. *n*: number of sows in each group, SD: standard deviation.

Group	*n*	Vaccination against PCV2	Liveborn Piglets		Stillborn Piglets		Total Born		Mummified Piglets		Born-Weak Piglets	
			Total	Average ± SD	Total	Average ± SD	Total	Average ± SD	Total	Average ± SD	Total	Average ± SD
A	183	1 day after weaning	3127	17.09 ± 4.4	384	2.10 ± 2.4	3511	19.19 ± 4.4	158	0.86 ± 1.9	498	2.72 ± 3.4
B	175	-	3029	17.31 ± 3.1	357	2.04 ± 2.1	3386	19.35 ± 3.5	97	0.55 ± 0.9	394	2.25 ± 3.0
C	180	28 days after weaning	3238	17.99 ± 3.0	330	1.83 ± 1.9	3568	19.82 ± 3.1	137	0.76 ± 1.3	450	2.50 ± 3.3

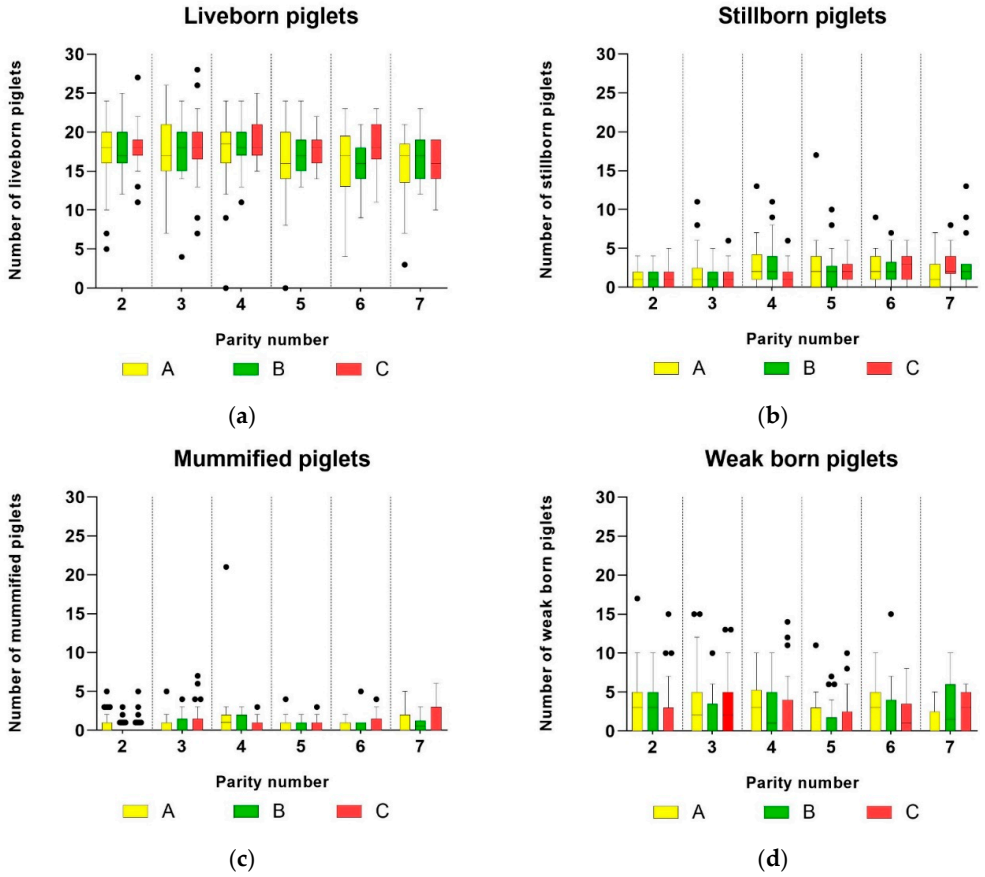

Figure 1. Comparison of number of liveborn piglets (**a**), stillborn piglets (**b**), mummified piglets (**c**) and weak-born piglets (**d**) in sows of different parity from three tested groups: A (sows vaccination 1 day after weaning), B (unvaccinated) and C (sows vaccination 28 days after weaning). The box-and-whiskers plot was constructed based on Tukey's method. The horizontal lines show median values.

4. Discussion

Vaccines registered for controlling PCVD are proved to be highly effective [9–19]. Vaccination of gilts is often practised prior to the first insemination to limit the risk of PCV2-RD. Vaccination of sows is mainly aimed to increase the level of maternal immunity transferred to piglets to protect them against PCV2 infection until their vaccination. However, some veterinary practitioners claim that sows vaccination against PCV2 can lead to improvement of reproductive parameters such as the number of liveborn piglets or survivability of piglets, even in the case of subclinical and undetectable infection in immune dams. Such claims are supported by some field studies which demonstrated improvement of breeding performance after PCV2 vaccination in standard PCV2-positive sow herds [20,32].

In the present study, the evaluation of the impact of sow vaccination with CircoFLEX® vaccine on reproductive parameters was performed in the high health status, PCV2 subclinically infected herd of 3200 sows. The comparison of the number of liveborn, stillborn, and weak born piglets between groups of sows vaccinated on 1 or 28 days after weaning, or nonvaccinated, did not show any statistically significant differences. This is in agreement with the observations reported by Kurmann et al. [25]. In that study conducted within 14 months, the reproduction parameters (gestation rate, numbers of mummified, aborted, weak born, and liveborn piglets, and litter size) did not change and did not differ between the vaccinated and unvaccinated dams.

On the other hand, Pejsak et al. [32] described spectacular improvement of reproduction parameters in a conventional 1200 head sow farm, such as an increase of farrowing rate from 65% to 87% in two years after the implementation of PCV2 vaccination in sows. However, the conclusion of the beneficial

impact of the vaccination was based solely on the analysis of the reproduction parameters and was not supported by any laboratory diagnostic data.

Another study by Oliver-Ferrando et al. [20] indicated the potential benefits of sows vaccination in a subclinically infected herd. The authors described significant improvement of the number of liveborn piglets and the number of weaned piglets per litter in vaccinated sows of the second parity. Moreover, the number of mummified piglets per litter was decreased [20]. Although the earlier study showed that sows up to the second parity are the most prone to a PCV2-related negative impact on reproduction due to infection and vertical transmission, our study did not show any benefit of the vaccination in this group of sows. Most probably, the reason of this discrepancy was the fact that in the present study gilts were vaccinated before introduction into the breeding herd, in contrast to the lack of regular practice of gilts and sows immunization in the farm described by Oliver-Ferrando et al. [20].

Thus, it can be concluded that the vaccination of only replacement gilts in the 4 and 31 WOA was apparently adequate to protect against any impact PCV2 might have had on the reproductive performance of the analysed herd.

Considering the list price of a PCV2 vaccine, 1 USD/dose, the annual cost of sow vaccination in every cycle in a herd of 1000 heads is about 2300 USD, excluding work. So, in herds with proven lack or very limited PCV2 circulation in sows, the economic benefit of the vaccination for reproductive performance is questionable. Thus, based on the results of the study, the decision was taken to stop PCV2 vaccination in sows in the tested farm, but to maintain it in replacement gilts. Nevertheless, it has to be underlined that sow vaccination should be considered in herds where, despite piglet vaccination, the infection (clinical or subclinical) occurs soon after weaning [23].

The limitation of this study is that it was performed on only one farm, populated by highly prolific genetics. Also, the impact of sow vaccination on piglet mortality before weaning was not analyzed, but considering the very limited PCV2 detection rate in the herd up to 28 days of age, such impact of the virus was unlikely to occur.

5. Conclusions

To sum up, any investments to improve the reproductive performance of sow herds must involve careful disease monitoring and differential diagnosis, including noninfectious factors. In the present study conducted in a high health herd subclinically infected with PCV2, the vaccination of sows appeared to have no effect on reproductive performance. The results underline the role of careful and individualized analysis of diagnostic and production data in order to economize and fine-tune PCV2 and other infectious diseases control programs in sow herds and pig populations.

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References

1. Alarcon, P.; Rushton, J.; Wieland, B. Cost of Post-Weaning Multi-Systemic Wasting Syndrome and porcine circovirus type-2 subclinical infection in England—An economic disease model. *Prev. Vet. Med.* **2013**, *110*, 88–102. [CrossRef] [PubMed]
2. Segales, J. Porcine circovirus type 2 (PCV2) infections: Clinical signs, pathology and laboratory diagnosis. *Virus Res.* **2012**, *164*, 10–19. [CrossRef] [PubMed]

3. Opriessnig, T.; Meng, X.J.; Halbur, P.G. Porcine circovirus type 2 associated disease: Update on current terminology, clinical manifestations, pathogenesis, diagnosis, and intervention strategies. *J. Vet. Diagn. Investig. Off. Publ. Am. Assoc. Vet. Lab. Diagn.* **2007**, *19*, 591–615. [CrossRef]
4. Kim, J.; Jung, K.; Chae, C. Prevalence of porcine circovirus type 2 in aborted fetuses and stillborn piglets. *Vet. Rec.* **2004**, *155*, 489–492. [CrossRef]
5. Johnson, C.S.; Joo, H.S.; Direksin, K.; Yoon, K.J.; Choi, Y.K. Experimental in utero inoculation of late term swine fetuses with porcine circovirus type 2. *J. Vet. Diagn. Investig.* **2002**, *14*, 507–512. [CrossRef]
6. Park, J.S.; Kim, J.; Ha, Y.; Jung, K.; Choi, C.; Lim, J.K.; Kim, S.H.; Chae, C. Birth abnormalities in pregnant sows infected intranasally with porcine circovirus 2. *J. Comp. Pathol.* **2005**, *132*, 139–144. [CrossRef]
7. Rose, N.; Blanchard, P.; Cariolet, R.; Grasland, B.; Amenna, N.; Oger, A.; Durand, B.; Balasch, M.; Jestin, A.; Madec, F. Vaccination of porcine circovirus type 2 (PCV2)-infected sows against porcine Parvovirus (PPV) and Erysipelas: Effect on post-weaning multisystemic wasting syndrome (PMWS) and on PCV2 genome load in the offspring. *J. Comp. Pathol.* **2007**, *136*, 133–144. [CrossRef]
8. Mateusen, B.; Maes, D.G.; Van Soom, A.; Lefebvre, D.; Nauwynck, H.J. Effect of a porcine circovirus type 2 infection on embryos during early pregnancy. *Theriogenology* **2007**, *68*, 896–901. [CrossRef]
9. Karuppannan, A.; Opriessnig, T. Porcine Circovirus Type 2 (PCV2) Vaccines in the Context of Current Molecular Epidemiology. *Viruses* **2017**, *9*, 99. [CrossRef]
10. Segales, J.; Urniza, A.; Alegre, A.; Bru, T.; Crisci, E.; Nofrarias, M.; Lopez-Soria, S.; Balasch, M.; Sibila, M.; Xu, Z.; et al. A genetically engineered chimeric vaccine against porcine circovirus type 2 (PCV2) improves clinical, pathological and virological outcomes in Postweaning Multisystemic Wasting Syndrome affected farms. *Vaccine* **2009**, *52*, 7313–7321. [CrossRef]
11. Woźniak, A.; Miłek, D.; Baska, P.; Stadejek, T. Does porcine circovirus type 3 (PCV3) interfere with porcine circovirus type 2 (PCV2) vaccine efficacy? *Transbound. Emerg. Dis.* **2019**, *66*, 1454–1461. [CrossRef] [PubMed]
12. Woźniak, A.; Miłek, D.; Matyba, P.; Stadejek, T. Real-time PCR detection patterns of porcine circovirus type 2 (PCV2) in Polish farms with different status of vaccination against PCV2. *Viruses* **2019**, *11*, 1135. [CrossRef] [PubMed]
13. Park, K.H.; Oh, T.; Yang, S.; Cho, H.; Kang, I.; Chae, C. Evaluation of a porcine circovirus type 2a (PCV2a) vaccine efficacy against experimental PCV2a, PCV2b, and PCV2d challenge. *Vet. Microbiol.* **2019**, *231*, 87–92. [CrossRef] [PubMed]
14. Czyzewska-Dors, E.; Dors, A.; Pomorska-Mol, M.; Podgórska, K.; Pejsak, Z. Efficacy of the Porcine circovirus 2 (PCV2) vaccination under field conditions. *Vet. Ital.* **2018**, *54*, 219–224.
15. Afghah, Z.; Webb, B.; Meng, X.; Ramamoorthy, S. Ten years of PCV2 vaccines and vaccination: Is eradication a possibility? *Vet. Microbiol.* **2017**, *206*, 21–28. [CrossRef]
16. Feng, H.; Blanco, G.; Segales, J.; Sibila, M. Can Porcine circovirus type 2 (PCV2) infection be eradicated by mass vaccination? *Vet. Microbiol.* **2014**, *172*, 92–99. [CrossRef]
17. Dvorak, C.M.T.; Yang, Y.; Haley, C.; Sharma, N.; Murtaugh, M.P. National reduction in porcine circovirus type 2 prevalence following introduction of vaccination. *Vet. Microbiol.* **2016**, *189*, 86–90. [CrossRef]
18. Chae, C. Commercial porcine circovirus type 2 vaccines: Efficacy and clinical application. *Vet. J.* **2012**, *194*, 151–157. [CrossRef]
19. Fraile, L.; Sibila, M.; Nofrarías, M.; López-Jimenez, R.; Huerta, E.; Llorens, A.; López-Soria, S.; Pérez, D.; Segalés, J. Effect of sow and piglet porcine circovirus type 2 (PCV2) vaccination on piglet mortality, viraemia, antibody titre and production parameters. *Vet. Microbiol.* **2012**, *161*, 229–234. [CrossRef]
20. Oliver-Ferrando, S.; Segales, J.; Lopez-Soria, S.; Callen, A.; Merdy, O.; Joisel, F.; Sibila, M. Exploratory field study on the effect of Porcine circovirus 2 (PCV2) sow vaccination on serological, virological and reproductive parameters in a PCV2 subclinically infected sow herd. *BMC Vet. Res.* **2018**, *14*, 1–10. [CrossRef]
21. Cline, G.; Wilt, V.; Diaz, E.; Edler, R. Efficacy of immunising pigs against porcine circovirus type 2 at three or six weeks of age. *Vet. Rec.* **2008**, *163*, 737–740. [PubMed]
22. Martelli, P.; Saleri, R.; Ferrarini, G.; De Angelis, E.; Cavalli, V.; Benetti, M.; Ferrari, L.; Canelli, E.; Bonilauri, P.; Arioli, E.; et al. Impact of maternally derived immunity on piglets' immune response and protection against porcine circovirus type 2 (PCV2) after vaccination against PCV2 at different age. *BMC Vet. Res.* **2016**, *12*, 77. [CrossRef] [PubMed]
23. Segales, J. Best practice and future challenges for vaccination against porcine circovirus type 2. *Expert Rev. Vaccines* **2015**, *14*, 473–487. [CrossRef] [PubMed]

24. Opriessnig, T.; Patterson, A.R.; Madson, D.M.; Pal, N.; Ramamoorthy, S.; Meng, X.-J.; Halbur, P.G. Comparison of the effectiveness of passive (dam) versus active (piglet) immunization against porcine circovirus type 2 (PCV2) and impact of passively derived PCV2 vaccine-induced immunity on vaccination. *Vet. Microbiol.* **2010**, *142*, 177–183. [CrossRef]
25. Kurmann, J.; Sydler, T.; Brugnera, E.; Buergi, E.; Haessig, M.; Suter, M.; Sidler, X. Vaccination of dams increases antibody titer and improves growth parameters in finisher pigs subclinically infected with porcine circovirus type 2. *Clin. Vaccine Immunol.* **2011**, *18*, 1644–1649. [CrossRef]
26. O'Neill, K.C.; Hemann, M.; Gimenez-Lirola, L.G.; Halbur, P.G.; Opriessing, T. Vaccination of sows reduces the prevalence of PCV-2 viremia in their piglets under field conditions. *Vet. Rec.* **2012**, *171*, 425. [CrossRef]
27. West, K.H.; Bystrom, J.M.; Wojnarowicz, C.; Shanz, N.; Jacobson, M.; Allan, G.M.; Haines, D.M.; Clark, E.G.; Krakowka, S.; McNeilly, F.; et al. Myocarditis and abortion associated with intrauterine infection of sows with porcine circovirus 2. *J. Vet. Diagn. Investig.* **1999**, *11*, 530–532. [CrossRef]
28. Hansen, M.S.; Hjulsager, C.K.; Bille-Hansen, V.; Haugegaard, S.; Dupont, K.; Hogedal, P.; Kunstmann, P.; Larsen, L.E. Selection of method is crucial for the diagnosis of porcine circovirus type 2 associated reproductive failures. *Vet. Microbiol.* **2010**, *144*, 203–209. [CrossRef]
29. Segales, J. Current challenges of porcine circovirus 2 prevention and control. In Proceedings of the 25th International Pig Veterinary Society Congress, Chongqing, China, 11–14 June 2018; pp. 62–63.
30. Dieste-Pérez, L.; van Nes, A.; van Maanen, K.; Duinhof, T.; Tobias, T. The prevalence of PCV2 viremia in newborn piglets on four endemically infected Dutch sow farms is very low. *Prev. Vet. Med.* **2018**, *153*, 42–46. [CrossRef]
31. Eddicks, M.; Koeppen, M.; Willi, S.; Fux, R.; Reese, S.; Sutter, G.; Stadler, J.; Ritzmann, M. Low prevalence of porcine circovirus type 2 infections in farrowing sows and corresponding pre-suckling piglets in southern German pig farms. *Vet. Rec.* **2019**, *184*, 189. [CrossRef]
32. Pejsak, Z.; Kusior, G.; Pomorska-Mól, M.; Podgórska, K. Influence of long-term vaccination of a breeding herd of pigs against PCV2 on reproductive performance. *Pol. J. Vet. Sci.* **2012**, *15*, 37–42. [CrossRef] [PubMed]