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Detection of PRRSV circulation in herds without clinical signs of PRRS:

Comparison of five age groups to assess the preferred age group and sample size

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Short title: PRRS-virus circulation in swine herds without signs of PRRS

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Key words: PRRSV, porcine reproductive and respiratory syndrome virus, rt-PCR, virus circulation, seroprevalence.

Abstract

A cross-sectional study was conducted to find the most effective diagnostic approach to detect circulation of porcine reproductive and respiratory syndrome virus (PRRSV). The study was performed in 10 Dutch swine herds, with sows and fattening pigs or breeding stock. Herds did not experience clinical signs of PRRS during the last 6 months before sampling, but a PRRSV infection was confirmed at most 2 years before sampling. Blood samples were collected from 5 age groups: sows during early and late gestation, weaners at 9 weeks of age, fatteners or breeding stock at 16 and 22 weeks of age. For each category, 20 serum samples were examined; in total 100 serum samples per herd. Samples were analysed for PRRSV antibodies with ELISA (n=1002), and rt-PCR when ELISA S/P-ratios were above 1.5 (n = 307) or below 0.4 (n=187; random selection from each age group). A logistic regression analysis was used to obtain factors associated with the probability of virus detection in a pig (PCR positive test result). Herd, ELISA-result, and age group were included as explanatory variables. Variables remained in the model when statistically significant. ELISA results showed that none of the herds could be considered to be free of PRRSV infection. Mean PRRSV seroprevalence in unvaccinated animals varied between 18% to 82%, and mean PRRS-virus prevalence varied between 0% and 41%. In only one of the 10 herds, no PRRS-virus could be detected. The odds of finding PRRS-virus in blood samples were 8.6 (95% CI, 5.3-13.9) in pigs of 9 weeks of age and 4.6 (95% CI, 3.0-7.0) in pigs of 16 weeks of age, compared with fatteners of 22 weeks of age. This result indicates that 9- to 16-week-old pigs are the preferred age group to detect PRRS-virus, in herds without clinical signs of PRRS. We concluded that PRRS-virus circulation could be detected in 8 out of 9 of the study-herds, with a relatively low number of blood samples. Testing 12 blood samples in both rt-PCR and ELISA, with 6 samples in pigs 9 weeks of age and 6 samples in pigs 16 weeks of age, will lead to a cost-efficient first evaluation of the PRRSV infection-status in herds without clinical signs of PRRS.

1. Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is known to play an important role in reproductive problems in sows, as well as in respiratory problems in weaners, fatteners and rearing pigs. The economic impact of PRRS has been quantified in North America (Neumann et al., 2005), and figures on the economic impact of PRRS in the Netherlands are similar (Brouwer et al., 1994). Although the economic impact of PRRS in the Netherlands was not recently re-examined, the costs of respiratory and reproductive diseases initiated or enhanced by PRRSV are still considerable, and one of the reasons for Dutch farmers to consider the control or eradication of this virus.

Often, diagnostic tests are used to assess the PRRSV infection status of herds. Several serological tests are available for the diagnosis of PRRSV-infections (Mattheu et al. 2006; Wellenberg, 2006.). For routine settings, both ELISA test methods and reversed transcriptase PCR are mostly used. In herds with no clinical signs of PRRS, these diagnostic tests can be used to determine whether virus circulation is still present. The farmer can use this information to focus on the control or eradication of PRRSV. The decision of farmers to control or eliminate PRRSV can either be made shortly after an outbreak of PRRS, or in endemic situations without clinical signs of PRRS. In both cases, more information on the actual PRRSV circulation on herd level is required to support decisions of farmers to implement management measures. The absence of virus circulation in herds could be the starting point to control or eliminate PRRSV from the herd. However, information is lacking on the most effective method, regarding age groups and sample size, to examine PRRS-virus circulation within herds without clinical signs of PRRS. The objective of this study was to determine the preferred age group for sampling, and the required sample size, in order to detect PRRS-virus circulation in herds without clinical signs.

2. Materials and methods

2.1 Herds and animals

Ten herds were selected on the absence of clinical signs of PRRS for a period of at least six months. This selection was based on information of the herd veterinarian and the farmer. All herds were selected on the basis of a confirmed circulation of PRRSV, and consisted of a combination of sows

and fatteners or breeding stock. Most of these herds used a vaccination scheme for PRRSV, mainly in the sows, only one farm vaccinated piglets (Table 2). On average, these herds consisted of 330 (150 – 750) sows and 1400 (850 – 2800) fatteners and/or breedingstock. In all ten herds, a cross-sectional sampling of one hundred blood samples was carried out from January to April 2005. Twenty samples were collected from each of the following five age groups; sows in early gestation, sows in late gestation, piglets at 9 weeks of age, and young fattening or breeding pigs at 16 weeks and 22 weeks of age.

2.2 Test systems and analyses

Sera were tested for the presence of PRRSV antibodies by ELISA (IDEXX HerdCheck PRRS 2XR ELISA, Maine, USA). All sera with an ELISA S/P ratio of > 1.5 ($n= 307$) were analysed for the presence of PRRS-virus by conventional reverse-transcriptase-PCR (rt-PCR) (Van Maanen et al., 2006) to monitor prolonged virus circulation in infected animals. In addition, 20 sero-negative serum samples per herd (S/P ratio < 0.4) were chosen at random, and tested by rt-PCR ($n= 187$) to look for virus circulation in animals expected to be negative for PRRSV or infected recently.

2.3 Statistical analysis

A logistic regression analysis was carried out in SPSS 15.0 (SPSS Inc., 1989-2006) to obtain factors associated with the probability of virus detection in a pig (PCR positive test result). Herd, ELISA result and age group were included as explanatory variables. Variables remained in the model when statistically significant at $P \leq 0.05$. In order to find manageable and predictable sample sizes for veterinary practice, stratification by means of sampling on age group level was examined. Based on the predicted prevalence of the final logistic regression model for each age group, the required sample size was calculated to detect at least one PRRSV PCR or antibody positive animal with 95% confidence, assuming a random spread of virus and antibodies in the animals present in the different age groups. This calculation was done to obtain the age group with the highest prevalence and the smallest required sample-size.

The antibody prevalence was only based on the unvaccinated pigs: weaners, fatteners and in one herd also the sows. One herd vaccinated all pigs; this herd was not included for calculation. The ELISA was assumed to have a sensitivity of 97.6% and a specificity of 98.6 % (Wellenberg, 2006), and the rt-PCR a 100% sensitivity and specificity (van Maanen et al., 2006).

3. Results

3.1 Seroprevalence, virus prevalence and estimated sample size

In total, 1002 blood samples were screened for the presence of PRRSV antibodies. In all herds antibodies against PRRSV were found. The mean seroprevalence in unvaccinated pigs on herd level was 53%, and between herds it varied between 18% and 82%. The seroprevalence (in unvaccinated pigs) was the highest in fatteners and/or breeding stock of 16 and 22 weeks old. Based on herd level prevalence, the calculated sample size to detect seropositive pigs on herd level varied between 3 and 16 samples. The calculated samples sizes to detect PRRSV circulation by antibody detection in the different age groups, varied between 3 and 15 (Table 1). In 9 of 10 examined herds, the presence of PRRS-virus was detected by rt-PCR. The virus prevalence on herd level varied between 0% and 41%. Based on herd level prevalence, the calculated sample size to detect virus on herd level, varied between 6 and 237 samples. To estimate the required sample-size on age group level, the virus prevalence on age group level was assessed (Table 1). The highest virus prevalence was found in the weaners at 9 weeks of age and fatteners at 16 weeks of age. In these groups, the calculated samples sizes were 9 and 10 samples. For each of the 10 study herds, more detailed results on the virus prevalences for the age groups with the highest prevalences, 9 and 16 weeks, are presented in Table 2. The range in virus prevalence is not further examined for the sows and fatteners of 22 weeks of age, as the needed sample size for virus-detection was too high for practical use, considering the low virus prevalence in these age groups. Virus prevalences in the 10 examined herds ranged from 0 to 100% in 9 week old pigs and from 0 to 85% in 16 week old pigs (Table 2).

3.2 Results of rt-PCR-testing on ELISA-negative samples or samples with S/P-ratio > 1,5

The sera tested for PRRSV antibodies with ELISA were divided in three classes based on S/P-ratio's; S/P-ratio < 0.4 (N = 364); S/P-ratio higher than 0.4 but lower than 1.5 (N = 331); S/P-ratio higher than 1.5 (N = 307). The rt-PCR was performed on a random selection of samples with ELISA S/P-ratio below 0.4 (n=187) and all samples with ELISA S/P-ratio higher than 1.5. The results were: 23 of 187 (12.2 %) serum samples with S/P-ratio <0.4, and 46 of 307 (15.3 %) serum samples with S/P ratio >1.5 were positive by rt-PCR. In total, 69 (13.9%) of the 494 tested serum samples were rt-PCR positive.

3.3: Logistic regression analysis for the presence of virus.

In Table 3, the results of the logistic regression analysis for the presence of virus are summarized. The logistic regression model for detection of virus circulation included serostatus as an independent variable. Weaners of 9 weeks of age had 8.6 (95%CI 5.3-13.9) times higher odds for virus presence than pigs of 22 weeks of age (= reference group in this study). Fatteners of 16 weeks of age had 4.6 (95% CI 3.0-7.0) times higher odds compared to pigs of 22 weeks of age. Sows were the age group with the lowest odds ratio (0.06 95%CI 0.02-0.18) for detection of virus. Seropositive pigs were 3 times (95%CI 2.1-4.1) more likely to be virus positive than seronegative pigs.

3.4. Sampling scenarios in age groups of 9 and 16 weeks

Based on the prevalences in our study (Table 2), we made a comparison between sampling scenarios in order to detect higher (at least 40%) or lower levels (at least 22%) of virus circulation in the preferred (non-vaccinated) age groups of 9 and 16 weeks of age. The highest number of farms was detected by sampling 6 or 12 pigs from both of these two age groups, both in rt-PCR and in ELISA (8 of 9 herds; 88%). However, no difference was found in the number of PRRSV-positive herds in case 6 or 12 blood samples were collected from both age groups (12 or 24 samples in total). Sampling of only one of these two age groups, by taking 6 or 12 blood samples, resulted in the detection of 7 of 9 (77%) infected farms with PRRSV circulation

Discussion

The objective of this study was to determine the preferred age group, and the required sample size in 5 selected age groups, in order to detect PRRS-virus circulation in herds without clinical signs of PRRS. The selected 5 age groups did not include piglets of 3-6 weeks of age. Although virus circulation in this agegroup can be present, the comparison of PCR-results with ELISA-results would not be possible due to the presence of maternal antibodies in this age group.

In this study, PCR and ELISA were used as diagnostic methods. The actual virus circulation can be determined by using PCR-technique (Van Maanen et al., 2006; Wellenberg, 2006). Although the period of viraemia, the time frame to detect the virus by PCR in blood samples, can persist for several weeks in young pigs (Albina et al. 1998), the time window to detect PRRSV infections by ELISA test methods may be longer than by PCR (Fano et al., 2007). However, detection of antibodies does not provide information on the actual circulation of virus. Compared to serological tests, the advantage of using PCR methods is that this test method is independent of, and not influenced by the presence of either maternal antibodies, or antibodies induced by vaccination. In addition, PCR can be used to demonstrate early infections. Ten Dutch swine herds without clinical signs of PRRS but with histories of PRRSV infections were included in the study. Although the 10 examined herds are not representative for all Dutch farming systems, they were representative for the 218 herds with both sows and fatteners or breeding stock of an average size (330 sows and 1400 fatteners or breeding stock). Although no clinical signs of PRRS were recorded during the last 6 months, none of these 10 herds could be classified as a “PRRSV-free herd”. Antibodies against PRRSV were detected in unvaccinated pigs in all herds. Because all pigs were 9 weeks or older, the detected antibodies against PRRSV are considered not to be of maternal origin (Nodelijk et al., 2003). To detect PRRS-virus circulation in low prevalent herds, large sample sizes are needed. In order to use smaller sample sizes, and reduce costs, we examined not only the virus prevalences and seroprevalences on herd level but also within different age groups (Table 1). Based on the virus prevalences and seroprevalences found in this study, the sample size could be calculated for each age group. For detection of virus, the highest odds ratio's, were found for the age groups of 9 and 16 weeks. The overlapping confidence intervals for the odds ratios of these two groups indicate that both age groups are preferred for the detection of PRRSV circulation by rt-PCR. The narrower confidence interval of the odds ratio in the age group of

16 weeks shows that this group gives a more reliable outcome. The virus prevalences in the age groups of 9 and 16 weeks of age were the highest, with estimated mean virus prevalences of 30% and 27% (Table 1). As stated in Materials and Methods, paragraph 2.2, PCR-testing was not done in animals with ELISA S/P-ratios from 0.4 to 1.5, which may have influenced the virus prevalence. However, because viraemia in young pigs is known to last for longer periods (Zimmermann et al. (2006a; Albina et al. 1998), comparable virus prevalences are to be expected, when pigs with S/P-ratios from 0.4 to 1.5 are tested with rt-PCR. The range in virus prevalence between herds ranged from 0 to 100% in 9 week old pigs and ranged from 0 to 85% in 16 week old pigs (Table 2). This pattern is in accordance with those described earlier by Evans et al. (2008) and Zimmermann et al. (2006b). They characterised the spread of PRRSV within herds as two different patterns: either at least 90% of weaners are infected at 8.5 weeks of age, or 20% to 40% were infected at 10-12 weeks of age. These findings support the use of a virus prevalence of 20% as a threshold-value in distinguishing highly infected groups of pigs from groups where virus circulation is less prominent. Therefore, a sample-size of 12 samples seems appropriate to detect virus circulation in over 22% of the pigs with 95% confidence when monitoring for active virus circulation. Given the above mentioned percentages of infected pigs, and the reproduction ratio R estimated to be 3 (Nodelijk et al., 2000) this number of samples will be sufficient when monitoring for new outbreaks too. When comparing sampling-scenarios, it appears that the use of 12 or even 6 samples from each of the non-vaccinated age groups of 9 and 16 weeks (24 or 12 in total), will result in a high detection rate of virus circulation in the farms examined in this study. For a first evaluation of the PRRSV infection-status of herds with sows and fatteners or breeding stock, samples should be taken from both age groups for both rt-PCR and ELISA. In contrast, PRRSV infection in farm 4 will not be detected by using these sampling scenarios. A higher number of samples would be required to detect a seroprevalence of 15% or lower in the age groups of 9 weeks, or to detect a virus prevalence of 20% and lower in the age group of 16 weeks (Table 2). As 1:4 pooling of samples for rt-PCR-testing was established to be feasible (van Maanen et al. 2006), costs for examination of PRRS-virus circulation within herds can be reduced (Van Maanen, personal communication). In case of vaccination of piglets, PCR is the only option to detect virus circulation in herds by examining blood samples from weaners and young fatteners or breedingstock.

The results of this study indicate that the use of antibody tests alone, was not enough to determine PRRS-virus circulation. In a random selection of seronegative samples, 12,2 % of these samples were PRRS-virus positive by rt-PCR. This finding supports the use of the rt-PCR test method as additional test to detect virus circulation. Based on the information received from the study farms, we hypothesized that the positive rt-PCR reactions were the result of circulating field virus and not vaccine virus. The rt-PCR positive age groups were not vaccinated with PRRS-MLV vaccines or at least 6 weeks before sampling (one farm).

We conclude that for a first evaluation of PRRSV-circulation on herds with sows and fatteners or breeding stock, a total of 12 samples is sufficient. The collection of 6 samples from both 9 and 16 week old pigs, and analysing them using rt-PCR and ELISA, is the most efficient method and leads to the detection of 8 out of 9 of farms with virus circulation.

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Table 1: Sample size (with 95% confidence intervals) per age group based on the estimated virus- and seroprevalence to detect PRRS virus and antibodies with 95% confidence in a group of 1000 pigs by PCR with 100% sensitivity and specificity, and by ELISA with 97.6% sensitivity and 98.6% specificity. The seroprevalence is based on unvaccinated weaners and fatteners (or breeding stock) in 9 herds, and in sows from one herd.

Age groups	Virus prevalence					Sero-prevalence				
	N	Mean	95% confidence	Sample	95% confidence	N	Mean	95% confidence	Sample	95% confidence
			interval	size	interval sample			interval	size	interval sample
		(%)	(%)	(n)	size (n)		(%)	(%)	(n)	size (n)
Sows	185	0,5	0-1.6	450	170-1000	40	53	37-69	7	3-11
Piglets 9 weeks	92	30	21-40	9	6-13	178	29	23-35	15	12-19
Fatteners/ breedingstock 16 weeks	120	27	19-35	10	7-15	180	61	54-68	6	3-7
Fatteners/ breedingstock 22 weeks	97	8	3-14	36	20-94	180	69	62-76	3	3-6

Table 2: Virus prevalence and seroprevalence in weaners of 9 weeks of age and fatteners or breeding stock of 16 weeks of age for each of the 10 herds. Except for herd 10, all weaners and fatteners or breeding stock were unvaccinated.

Included are the used vaccinationschemes in each herd. S = vaccination of sows; G = vaccination of gilts; P = vaccination of piglets

Weaners 9 weeks of age									Fatteners/breeding stock 16 weeks of age					
Used Vaccination			Virus prevalence			Sero-prevalence			Virus prevalence			Sero-prevalence		
Herd nr	Type PRRSV-vaccine	Used Scheme	Number of samples tested	Virus prevalence %	Standard error %	Number of samples tested	Sero-prevalence %	Standard error %	Number of samples tested	Virus prevalence %	Standard error %	Number of samples tested	Sero-prevalence %	Standard error %
1	None	-	6	100	0	20	50	11	13	85	10	20	70	10
2	EU-MLV	S + G	10	0	0	20	5	5	17	0	0	20	90	7
3	EU-MLV	S	4	0	0	20	40	11	10	0	0	20	5	5
4	EU-KV	S	9	0	0	20	15	8	10	20	13	20	0	0
5	EU-KV	G	5	0	0	20	50	11	14	7	7	20	95	5
6	EU-KV	S	14	7	7	18	0	0	13	31	13	20	75	10
7	EU-KV	S	14	71	12	20	90	7	12	0	0	20	100	0
8	EU-MLV	S	8	100	0	20	5	5	10	50	16	20	20	9
9	US-MLV	S	7	0	0	20	0	0	7	14	13	20	95	5
10	EU-MLV	S + P	15	20	10	20*	45*	11	17	47	12	20*	90*	7
Total	-	-	92	30	5	178	29	3	120	27	4	180	61	4

*Herd 10 vaccinated the piglets; these results have been excluded in the calculation of sero-prevalence.

Table 3: Results of the logistic regression analyses on virus presence in 10 Dutch swine herds.

Variable	Category of variable	Regression coefficient	Standard Error	P-value	Odds ratio	95% confidence interval
Intercept		-2.54	0.29	<0.01	-	-
ELISA result	Seronegative	Ref.*	-	-	1.0	-
	Seropositive	1.09	0.33	<0.01	2.9	2.1-4.1
Age Group	Pigs 22 weeks of age	Ref.*	-	-	1.0	-
	Sows (all groups together)	-2.76	1.07	0.01	0.06	0.02-0.18
	Piglets 9 weeks of age	2.15	0.48	0.00	8.6	5.3-13.9
	Pigs 16 weeks of age	1.52	0.43	0.00	4.6	3.0-7.0

Ref*: in this study used as the reference group.