

AN ATTEMPT TO ELIMINATE PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS) BY SERUM INOCULATION ON SMALL PIG FARM

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Summary: The great heterogeneity among porcine reproductive and respiratory syndrome virus (PRRSV) isolates is probably the main obstacle to its effective control using current commercial vaccines, since the induced immunity by one strain is specific only to this strain. Exposure of all breeding pigs to the PRRSV circulating on the farm is an option for elimination of PRRS in breeding herd. Adoption of strict biosecurity measures is essential. The objective of this study was to eliminate the PRRS from a farrow-to-finish small pig farm (130 breeding pigs) by serum inoculation. The owner was acquainted with the biosecurity measures (strict biosecurity protocols and herd closure for at least 200 days). Breeding pigs were immunized with serum obtained from weaners. The number of high positive breeding pigs decreased from six months after the II. serum inoculation till the end of the study, but the prevalence of antibody were almost the same comparing the sampling before serum inoculation to last sampling 13 months after the II. serum inoculation. The breeding herd were free of virus during all testing, but PRRSV circulated in the two-month old weaners. The owner did not implement herd closure and other required biosecurity measures and a new strain of PRRSV was introduced. Hence, serum inoculation proved to be unsuccessful for the elimination of PRSS from the farrow-to-finish farm. Implementation of biosecurity measures in field conditions is a much more difficult challenge than what was expected at the beginning.

Key words: control; immunization; pig; PRRS; serum inoculation

Introduction

Porcine reproductive and respiratory syndrome (PRRS) is a viral infection endemic in most swine-producing countries and leads to major economic losses (1). The disease is characterized by reproductive failure, including late-term abortions, early farrowing, stillbirths, weak born piglets and increased mortality in neonates, nursery and growing pigs, and respiratory tract illness that can be especially severe in neonatal and nursery-

age pigs (2). PRRSV infection is difficult to control due to large heterogeneity among the isolates. A variety of strategies have been described for PRRS elimination, including total depopulation/repopulation, partial depopulation (3), isowean (4) or segregated early weaning (5), test and removal (6), mass vaccination with unidirectional pig flow and herd closure (7). Elimination of a disease is disappearance of all clinical cases of a specific disease (8) which is the consequence of desistance of virus replication and circulation in the population of pigs. No single strategy for elimination will work on infected farms; therefore, the program must be individually designed based

on the unit's pig flow and facility design as well as serological results (9).

PRRS elimination is a long term goal, and the first step is stabilization of the breeding herd. Herd stability is defined as a herd which lacks clinical signs and in which a virus is not actively circulating and transmitting between pigs (9). Stabilization can be achieved with simultaneous immunization of the breeding herd (10). Immunization can be achieved with commercial vaccines, serum inoculations and natural exposure. It appears that currently available vaccines may not be effective in protecting against infections with genetically different strains of PRRSV (11). Numerous studies have shown some cross-protection against different strains which are reflected only in the reduction of clinical signs and lesions but not in elimination of the virus (12). Moreover, inoculation with a homologous strain provides a high level of protection against the same or nearly the same virus strain (13). It is, however, readily accepted that homologous immunity is more protective than heterologous immunity. In fact serum inoculation is the intentional immunization of pigs with the strain of PRRS virus originating from the same, infected farm (homologous herd strain). This method consists of intramuscular injection of complete breeding herds with serum derived from acutely infected pigs that contain the particular farm-specific PRRSV (14). Shibata et al. (15) showed that, after exposure to a homologous PRRSV strain, pigs subsequently challenged with that strain did not develop clinical signs, and virus replication was reduced in both the titer and the length of infection.

In addition, herd closure is also required to achieve herd stability. In the period of herd closure new pigs cannot be introduced to the farm. This applies also to internal replacements of gilts to the breeding herd (7). The success of PRRS elimination depends on biosecurity practices and cooperative work (9). Consequently, one very important measure is to follow strictly biosecurity protocol, which includes preventing direct routes of spread as well as indirect and miscellaneous routes, as authored by Pitkin et al. in the American Association of Swine Veterinarians Foundation (AASV) website (www.aasv.org/aasv/PRRSV_BiosecurityManual.pdf).

The objective of this study was to eliminate PRRS from a small farrow-to-finish pig farm with herd closure, improved biosecurity and serum inoculation.

Materials and methods

Farm

The study was carried out from June 2010 until March 2012 on one farrow-to-finish farm consisting of four boars and 130 breeding sows. Six months after the second round of serum inoculations, the owner reduced the number of breeding sows to 88 due to the lower price of pigs on the Slovenian market and not due to our request as a measure for the elimination of PRRSV. Semen originated from their four boars. Serum inoculation was performed twice on the farm: the first being after the conformation of PRRS and the second three months after I. serum inoculation.

Herd closure

The introduction of new pigs to the farm was prohibited for 200 days. Also in this period gilts from the farm could not enter the breeding herd.

Biosecurity measures

The owner was acquainted with obligatory measures: strict biosecurity protocols (entering the farm after changing clothes; having personnel aid in the changing of coveralls and boots; the washing of hands; using footbaths; maintaining individual responsibility for each pig category; use of the all in/all out system; one age category of pigs in one room; one way pig flow; the cleaning and disinfection of pens, pig equipment kept on the farm; deratization and disinsection).

Preparation of inoculum for serum inoculation of breeding pigs

The weaners at age 8 to 14 weeks of age were bleeding and tested by RT-PCR. Inoculum was prepared from positive serum samples. The PRRSV positive serum samples were pooled. To one part of each pool four parts of RPMI-1640 medium (Gibco, Germany) were added and mixed with 1% of Antibiotic-Antimycotic (100x), (Invitrogen, Germany). The inoculums contained 10^2 to 10^4 TCID₅₀ PRRSV/ml. All breeding pigs were inoculated intramuscularly with 2 ml/pig on the same day.

Samplings procedure

All together 704 blood samples were collected for serology and 456 for molecular testing. The sequencing of PRRSV positive samples were performed 6 times.

Enzyme-linked immunosorbent assay (ELISA)

The HerdChek, IDEXX Laboratories, PRRS X3 ELISA test was used for detecting antibodies in serum samples. The ELISA was performed according to the manufacturer's instructions. Sample results were divided in four groups: samples with S/P less than 0.4 (negative), samples with S/P between 0.4-1 (low positive), samples with S/P between 1 and 2 (positive) and samples with S/P more than 2 (high positive).

Detection of PRRSV with gel-based RT-PCR and direct sequencing of PRRSV positive samples

Total RNA was extracted from 140 µl of serum samples using the QIAamp® viral RNA mini kit (Qiagen, Germany) according to the manufacturer's instructions. 456 samples were

tested individually or as pools (maximum 5 samples in pool) by one-step RT-PCR (One-Step RT-PCR Kit, Qiagen, Germany) using sequences based on the open reading frame 7 (ORF7), which detect Type 1 and Type 2 PRRSV strains respectively (16, 7). The PRRS strain VR-2332 (Type 2) and the Lelystad viruses (Type 1) were used as positive controls. Reaction mixtures without RNA served as negative controls. Fifteen PRRSV positive samples were directly sequenced in both directions using the MacroGen sequencing service (MacroGen, South Korea) and the RT-PCR amplification primers. For each sample, 258 nucleotide long sequences were aligned with the published data using BLAST (available at <http://www.ncbi.nlm.nih.gov/>) at the National Centre for Biotechnology Information (NCBI), and PRRSV sequences obtained were compared using the sequence analysis software Lasergene® (DNASTAR Inc., Madison, WI, USA).

Results

PRRS was confirmed on the farm via testing of 10 animals which showed positive or high positive results in ELISA. Before I. serum inoculation 13 (9.7%) samples were negative, 24 (17.9%) were low positive, 43 (32.1%) were positive and 54 (40.3%)

Table 1: Number of tested sera for serology (ELISA for detection of PRRS antibodies), PRRSV detection (RT-PCR method for PRRSV genome detection) and times of sequencing

Sampling	No. of tested sera by ELISA		No. of tested sera with RT-PCR		Sequencing of PRRSV positive samples	
	Breeding pigs	Pigs	Breeding pigs	Pigs	Breeding pigs	Pigs
Confirmation of PRRS	10	-	-	-	-	-
Before I. serum inoculation	134	-	-	15	-	yes
3 months after I. serum inoculation	134	-	-	15	-	yes
3 months after II. serum inoculation	133	-	133	-	-	-
6 months after II. serum inoculation	88	20	88	20	-	yes
10 months after II. serum inoculation	20	30	20	30	-	yes
13 months after II. serum inoculation	97	13	97	13	-	yes
17 months after II. serum inoculation	-	25	-	25	-	yes

Footnote: In the "Pigs" column, all categories from weaning pigs to fatteners are included

Table 2: Results of PRRSV Detection By RT-PCR and Sequencing of PRRSV

Sampling	Results of RT-PCR		Age of positive pigs (weeks)	Identification number of sequence	Nucleotide identity to 08066t/2010
	Breeding pigs	Pigs			
Before I. serum inoculation	-	positive	10	08066t/2010 06088t/2010	100% 100%
3 months after I. serum inoculation	-	positive	8-12	Meol/2010	98.4%
3 months after II: serum inoculation	negative	-	-	-	-
6 months after II: serum inoculation	negative	positive	10	2768-81/2011 2768-83/2011 2768-84/2011 2768-86/2011 2768-87/2011 2768-89/2011	99.6% 97.7% 99.6% 98.1% 97.7% 98.1%
10 months after II. serum inoculation	negative	positive	10	Meol15/2011 Meol19/2011	97.3% 96.9%
13 months after II: serum inoculation	negative	positive	10	Meol20/2011 Meol21/2011	99.2% 99.2%
17 months after II. serum inoculation	-	positive	10	0803-1/2012 0803-2/2012	99.2% 99.2%

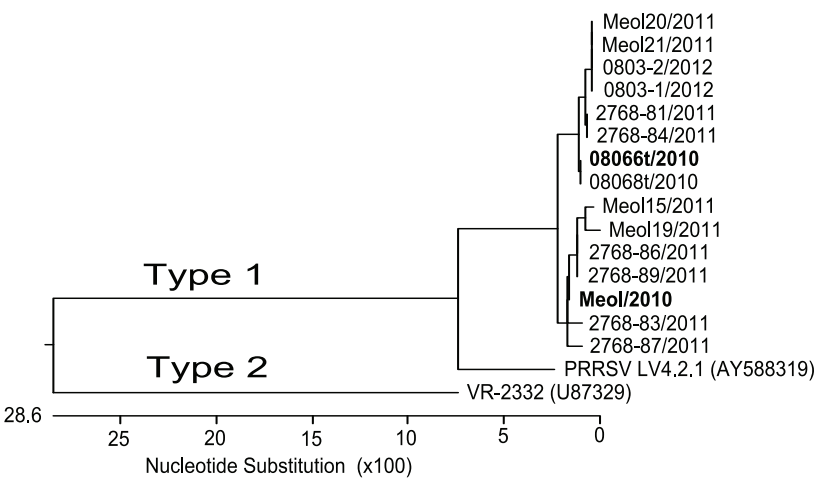


Figure 1: The Phylogenetic Tree of 15 Sequenced PRRSV Samples. The strains 08066t/2012 and Meol/2010 which were used for serum inoculation are presented in bold

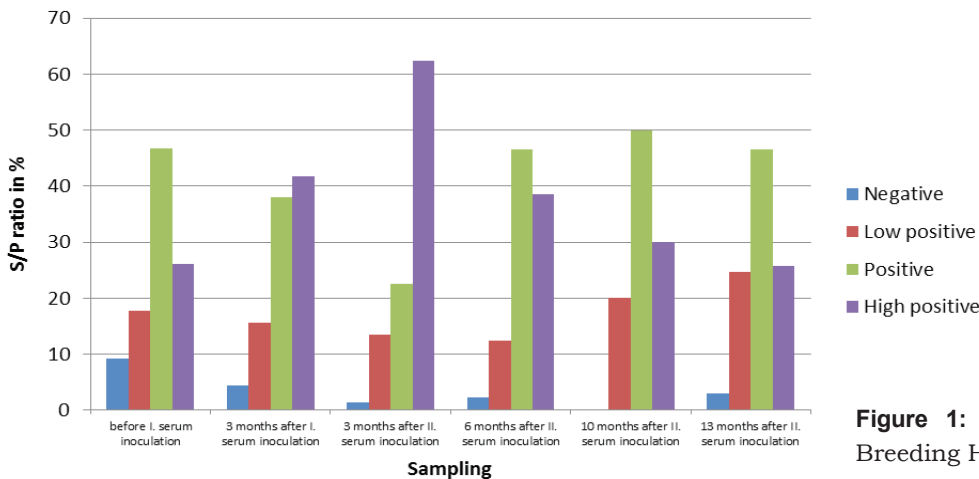


Figure 1: Serological Results of the Breeding Herd

Table 3: Results of PRRSV Detection By RT-PCR and Sequencing of PRRSV

Biosecurity measures	Required	Implemented
Herd closure (introducing replacement gilts into the breeding herd)	yes	no
Herd closure (introducing new pigs to the farm)	yes	yes
Entering the farm after changing clothes	yes	no
One age category of pigs in one room	yes	yes
One way pig flow	yes	yes
Changing of coveralls between pig category	yes	no
Changing of boots between pig category	yes	no
Washing hands between pig category	yes	no
All in/ all out in farrowing and fattening units	yes	no
Individual responsibility for each pig category	yes	no
Cleaning and disinfection of pens	yes	no
Footbath	yes	yes
Pig equipment kept on the farm	yes	no
Deratization and disinsection	yes	yes

were high positive (figure 1). Three months after the I. serum inoculation 6 (4.5%) breeding pigs were still negative and 21 (15.7%) breeding pigs were low positive therefore the second serum inoculation was performed. Three months after II. serum inoculation 2 (1.5%) samples of breeding pigs were negative; 18 (13.5%) were low positive; 30 (22.5%) positive and 83 (62%) high positive, which points to improved immune response of breeding herd. Six months after II. serum inoculation the number of high positive pigs decreased to 35 breeding pigs (39.8%); the number of positive breeding pigs increased to 39 (44.3%); and the percentage of low positive breeding pigs remain at the same level (13.6%) compared to prior sampling. Six months after II. serum inoculation we also checked the status of weaners. Two were negative; three, low positive; seven, positive; and six were high positive which indicate a persistent circulation of the wild type of PRRSV. Samplings at 10 and 13 months after II. serum inoculation still present a trend of decreasing high positive and positive pigs in the breeding herd while the low positive increased. One possible reason is that the breeding herd developed protective immunity; and, in spite of the new introduction of the homologous PRRSV, the titers of antibodies (high positive and positive) decreased. The last sampling—17 months after II. serum inoculations— was performed only in weaners and fatteners to check if PRRS was

eliminated from the herd. Results showed the following: 2 negative, 4 low positive, 7 positive and 12 high positive which indicated persistent circulation of the PRRSV.

During the whole period of the study, the breeding herd was negative for PRRSV by the RT-PCR method in all samplings. Sample 08066t/2010 (Fig 1) was used for the I. serum inoculation of the entire breeding herd (134 breeding pigs). A second serum inoculation of the entire breeding herd (134 breeding pigs) was carried out using the detected PRRSV strain Meol/2010. Observed sequence homology between detected strains in herd and topology from phylogenetic tree obtained from fifteen sequenced positive samples in the period of study suggested that the second strain was introduced into the farm between I. serum inoculation and II. serum inoculation.

The owner implemented only 5 of the required biosecurity measures, and the rest of the measures were neglected.

Discussion

To be successful, any PRRS herd elimination strategy must stop replication of the virus within a population of breeding pigs and this will prevent infection of neonates (18). PRRSV circulates in

endemically infected herds because, at any given time, animals are in various stages of infection and immunity (19). Considering the heterogeneity of PRRS serotypes and the importance immunity of homologues, serum inoculation can be a successful measure to eliminate PRRS from the farm (20). With simultaneous serum inoculation of homologues PRRSV strain (strain 08066t/2010) of the breeding herd, we tried to stop the circulation of PRRSV in the breeding herd which would lead to stabilization of breeding herd and which resulted in, as much as possible, uniform S/P ratios (between 1 to 2) and production of negative fatteners. Three months after I. serum inoculation, 4.5% breeding pigs were still negative and 15.7% positive, which was too high for the number of breeding pigs to ensure good protection. We decided to use the II. serum inoculation (strain Meol/2010) in order to ensure the stoppage of shedding the PRRSV. From the beginning of the study we implemented one additional method, herd closure. PRRSV elimination through herd closure is based on the fact that naturally developed immunity eliminates virus infection from the farm (7, 21). With serum inoculation we try to enhance the development of homologous immunity which did not prove as good a protection, according to our serological results. One good candidate for the elimination of the PRRSV is the three-site farm (7); moreover, the success rate is above 85% for farms with segregate production (22), keeping in mind that our study was performed on farrow-to-finish farm. According to the results of RT-PCR method and sequencing, we confirmed the introduction of a new closely-related strain PRRSV after I. serum inoculation. The observed sequence homology between 08066t/2010 strain (used for I. serum inoculation) and Meol/2010 strain which we used for II. serum inoculation was 98.4% (table 3, figure 1). Both strains were detected also 6, 10 and 13 months after II. serum inoculation in weaners age of 10 weeks, confirming long period of circulation of strains in farm, although the breeding herd was negative in all testing during the study. The results of serology 6 months after II. serum inoculation show a trend of decreasing high positive breeding pigs which continued until the end of the study. On the other hand, the prevalence of antibody is almost the same comparing the sampling before serum inoculation (90.3%) to sampling 13 months after II. serum inoculation (96.9%). Merely the percentage of

high positive breeding pigs decreased from 40.3 to 25.8. We expected the number of high positive breeding pigs to be much lower or non-existent. The results 17 months after serum inoculation indicated by the periodical introduction of PRRSV into the breeding herd resulted in persistently high S/P ratios.

The results of RT-PCR of weaners aged 8 weeks from testing 6, 10, 13 and 17 months after II. serum inoculation were negative, but the virus constantly persisted in group of 10 week old weaners. Pigs born from PRRSV infected dams maintain maternal antibody until 4 to 8 weeks of age using the indirect ELISA (23). Thus it is obvious that after decreasing maternal immunity, the weaners got infected. Shortly, 25% of breeding pigs were high positive 13 months after II. serum inoculation and at the age of 10 weeks, the virus was persistently circulating among the weaners. In this category of weaners, they were in various stages of infection and immunity. While some developed antibodies, the virus replicated in others due to not following the all-in/ all-out protocol. Newly incoming weaners were infected from prior weaners that remained in the room. In every visit to the farm we checked if the biosecurity measures were implemented according to our written guidelines. On the basis of owner assurance, the owner followed all required measures. But the facts presented a completely different picture. He equivocated on lack of time and personnel. We established that only 5 of the 14 required biosecurity measures were followed. Probably, it is very difficult to change the daily routine, which takes one more time and energy. The owner introduced his own replacement gilts into the breeding herd. In the case of introducing the negative replacement gilt, that animal can be a source of virus replication and transmission. The herd closure was not implemented as proposed at the beginning of the study and consequently this can be one of the reasons that the breeding herd could not reach stabilization. So the first goal in the process for achieving the elimination of PRRSV was not accomplished. Although some breeding pigs were identified as negative during the study, when we analysed the individual data the same animals did not remain negative. Hence this suggested that we did not stop the circulation of the virus in breeding herd despite all of the negative results of RT-PCR. One very important measure was the all-in/all-out protocol which

was not followed and thus resulted in poor hygiene due to non-vacant pens being thoroughly cleaned and disinfected. It follows that both pigs and pens were the source of PRRSV. Moreover, additional staff were not appointed to a single pig category and did not change coveralls or boots between pig categories, nor wash hands between pig categories. Hence these factors were the reason as well as the route of transmission of PRRSV between categories and between facilities. Pig equipment was not kept on the farm but rather brought to the farm without prior sterilisation (tattooing pliers). From the results of serology, molecular testing and biosecurity measures, we can conclude that the owner did not follow the required biosecurity measures nor carry out strict herd closure which proved to be the reasons for the unsuccessful elimination of PRRSV from the farm. In order to eliminate PRRSV from the farm, the proposed measures should be strictly followed and additional measures, immunization of fatteners and partial depopulation should be implemented since we are dealing with a one-site farm. Dee et al. (3) reported that partial depopulation and strict biosecurity measures can stop the circulation of PRRSV in weaners.

Thus it can be concluded that the serum inoculation did not prove itself as a successful measure for elimination of PRRSV from the farrow-to-finish farm and implementation of herd closure and biosecurity measures in field conditions is a much more difficult challenge than expected. Nonetheless, further study focusing on the education of farmers must be undertaken.

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POSKUS ELIMINACIJE PRAŠIČJEGA REPRODUKCIJSKEGA IN RESPIRATORNEGA SINDROMA NA MANJŠI FARMI Z INOKULACIJO SERUMA

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Povzetek: Poglavitni razlog za velik genetski raznoolikost virusov PRRS je verjetno neučinkovita kontrola bolezni s komercialnimi cepivi, ki vsebujejo samo en sev virusa, saj je zaščita po preboleli okužbi homologna. Ena izmed možnosti za eliminacijo PRRS je prekužitev plemenske črede s farmskim sevom virusa. Za uspešno eliminacijo je nujno upoštevati biovarnostne zahteve. Namen študije je bil eliminirati virus PRRS iz manjše farme (130 plemenskih prašičev) z inokulacijo seruma. Rejec se je obvezal, da bo izvajal stroge biovarnostne ukrepe in zaporo reje vsaj za 200 dni. Plemensko čredo smo imunizirali s pozitivnim serumom tekačev. Šest mesecev po drugem vnosu seruma je število visoko pozitivnih prašičev padlo in trend padanja se je nadaljeval do konca študije, vendar pa je prevalenca protiteles pred serumizacijo v primerjavi s prevalenco na koncu študije (13 mesecev po vnosu seruma) ostala skoraj enaka. Plemenska čreda je bila v vseh testiranih negativna na prisotnost virusa, virus pa smo stalno dokazovali pri kategoriji tekačev, starih 10 tednov. Rejec se ni držal zapore reje in ostalih predpisanih biovarnostnih zahtev, saj je med drugim vnesel na farmo nov sev virusa PRRS. Eliminacija PRRS z inokulacijo seruma zato ni bila učinkovita. Ugotovili smo, da je izvajanje biovarnostnih zahtev v praksi za rejca zelo velik izziv.

Glavne besede: kontrola; imunizacija; prašiči; PRRS; vnos seruma