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ELIMINATION OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS) WITH SERUMIZATION, NATURAL EXPOSURE AND VACCINATION ON SIX PIG FARMS IN SLOVENIA

MARINA ŠTUKELJ, ZDRAVKO VALENČAK[1]

SUMMARY: Porcine reproductive and respiratory syndrome (PRRS) is currently the most important swine disease worldwide. A variety of strategies have been described for PRRS eradication. The study involved six one-site small pig farms where the reproductive problems were observed. The owners were acquainted with strict biosecurity and herd closure for at least 200 days. Serum samples were tested with IDEXX X3 PRRS ELISA for antibodies detection and with one step RT-PCR (Qiagen, Germany). Production results after serumization improved 3 months after on both farms. Herds were also without virus. On farm 1 new boar without quarantine and new gilts were introduced in to the breeding herd. Eleven months after serumization production results decreased and same strain of PRRS virus was present in herd. Breeding herd on farm 2 is 13 months without PRRS virus. Only in group of growers of 10 weeks PRRS virus persists but the subtype of it is new. After natural exposure on both farms number of seropositive pigs was decreased and fatteners were seronegative. Both farms were also without PRRS virus. After vaccination on farm 5 production results improved and also number of high seropositive animals decreased. Fourteen months after vaccination number of seropositive pigs increased and production results decreased. Results from second vaccinated farm were similar. Natural exposure with implementation the strict biosecurity protocols and improvement of management are the key factors for successful eradication of PRRS. At the moment serumization and vaccination are the methods with limited success.

Key words: *PRRS, elimination, serumization, natural exposure, vaccination.*

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[1]Marina Štukelj, MSc, teaching assistant, and Zdravko Valenčak, PhD, assitant professor, University in Ljubljana, Faculty of Veterinary Medicine, Institute of Pig Health protection, Gerbičeva 60, 1000 Ljubljana, Slovenija.

Corresponding author: Marina Štukelj, e-mail: Marina.Stukelj@gmail.com, phone: (01) 477 91 00.

INTRODUCTION

Porcine reproductive and respiratory syndrome (PRRS) is economically the most important pig disease that occurs worldwide. Reproductive disorders 12%, increased mortality 43% and decreased feed efficiency 45% of total loss are consequence of PRRS (Zimmerman, 2008). Data based on ccalculations from the literature shows that losses due to PRRS outbreak on the farm with 50 breeding sows totals 20,363 EUR of which 55.30 EUR per farrowing/sow, which totals for farm with 50 sows 6.913 EUR, 4.60 EUR per weaner which totals 5.750 EUR and 7 EUR per prefattener and fattener which totals 7.700 EUR. Calculation is not absolute and it varies based on the phase of PRRS (acute or endemic) (Neumann et al., 2005). In United Status of America they believe that production of pig with PRRS is not economically effective and so they started with programs of PRRS eradication. Chile for instance succeeded to completely eradicate PRRS in 9 years by this method (Zimmerman, 2008). There are still few countries free of PRRS: Australia, New Zealand, Finland, Norway, Switzerland and Sweden. Due to enormous losses there are many attempts worldwide of control, elimination or eradication of PRRS (Jenny and Dee, 2006).

PRRS is a reason of frustrations both for pig producers as well as for veterinarians from the very first outbreak on. There are many successes in understanding of epidemiology, improvements in diagnosis and effective programs of disease control. But there are still many unsolved questions. With new knowledge about PRRS we are able to prevent, control and eradicate PRRS but in spite of all known measures of controlling, diagnostic and available vaccines achieving the goal is still difficult. Reasons are infection with few different subtypes of virus, homologous protection, permanently infected pigs, bad management and not known routes of infection (Štukelj, 2012). After PRRS outbreak there are various ways of intervention which depend on the size of a farm and prevalence of disease. We can either do nothing or we can start with control or elimination and eradication (Torremorell and Christanson, 2002; Taylor, 2006; Zimmerman et al., 2006).

Control of the disease. One possible measure is control of the disease. The goal here is reduction of losses due to virus circulation in breeding herd and stopping of vertical and horizontal shedding to achieve stabilization of breeding herd and development of specific immunity against farm's strain of virus with improvement of production results. This can be achieved by using only controlled negative semen, with acclimatization of gilts before introducing them to the farm or with vaccination (Zimmerman et al., 2006; Batista et al., 2004; Pesente et al., 2006; Vashisht et al., 2008; Scortti et al., 2006; Martelli et al., 2007; Kimman et al., 2009; Cesar et al., 2010).

Because PRRS virus is immunosuppressive there is increased manifestation of endemic diseases on the farm (streptococcal meningitis, porcine multisystem wasting syndrome, enzootic pneumonia, Glässer disease, parasitosis). Higher incidence of mentioned diseases leads to use of more antibiotics. Control of PRRS includes also control of endemic and secondary diseases.

Elimination and eradication. Elimination of the disease means that there are no clinically visible signs of the disease but there can still be present virus or specific antibodies. Eradication of the disease means absence of clinical signs, pato-anatomical signs and also absence of both the specific agent (virus) and specific antibodies. Elimination, eradication or disease free status can be achieved only through implemented strict biosecurity measures.

The first step in PRRS elimination is to find how the virus was introduced (Torremorell and Christianson, 2002). The next step is double closure of the farm which means both no introduction of new pigs on the farm and no introduction of farm's own gilts to the breeding herd for at least 200 days (Zimmerman et al., 2006; Cho and Dee, 2006). Strict biosecurity measures should be followed (Chappell et al., 2010; Pitkin et al., 2011).

Method of elimination/eradication of PRRS:

- depopulation/repopulation
- test and removal
- immunization: natural exposure, vaccination and serumization.

Depopulation/repopulation. Total depopulation is very radical and rather expensive method but very successful in fighting PRRS. With this method all pigs are removed from the farm, farm is cleaned and disinfected and only after that new negative pigs are introduced (Torremorell and Christianson, 2002; Corzo et al., 2010; Zimmerman et al., 2006; Cho and Dee, 2006).

Test and removal of positive pigs. This method can be used in case of low prevalence or when only few pigs are positive. Pigs are tested for antibodies and virus. The testing is finished when last positive pig is removed from the farm (Zimmerman et al., 2006; Dee, 2004; Cho and Dee, 2006).

*Immunization.*The goal of immunization is stabilization of breeding herd which means that all breeding pigs have present antibodies against PRRS virus but all are without PRRS virus. Immune sows protect their piglets with colostrum (Zimmerman et al., 2006).

Natural exposure. In small herds we can wait until all breeding pigs become immune to the virus through natural exposure. The time of immunization can differ. Negative effects of the disease are decreasing after the certain time (Corzo et al., 2010; Torremorell and Christianson, 2002).

First measure is herd closure for six months (Dee, 1998). Natural exposure is based on the fact that PRRS virus cannot exist in population in which all pigs have present specific antibodies. All breeding pigs have to be exposed to PRRS virus infection. The end result is that all breeding pigs are immune and no pig is excreting PRRS virus (Dee, 2009; Zimmerman et al., 2006).

Vaccination. On the market there are only two PRRS vaccines: against European (genotip I) and against American (genotip II) strains. In the period of 2009 – 2011 in Slovenia we have proved several strains of genotip I. In year 2011 also genotip II was proved. All registered vaccines for genotip I have one strain of PRRS virus (strain Lelystad). With live vaccine immunity is better but because new strain of PRRS virus is introduced in to the herd an outbreak of PRRS can be induced. Beside that in PRRS the protective immunity is homologous (only against herd strain) or against the strain which is genetically close to vaccine strain. Immunity against inactivated PRRS vaccines is weak but new PRRS outbreak is impossible (Kimman et al., 2009; Corzo et al., 2010; Stadejek et al., 2011).

Immunity protection after vaccination is complete only when there is no PRRS strain on the farm but the genetically close to vaccine strain (Martelli et al., 2007; Lager et al., 1997; Kimman et al., 2009). Complete protection can be achieved with herd vaccine.

Serumization. The easiest way to achieve homologous protection is serumization. Serumization is speeding the natural immunization. Blood of pigs with virus is used for

inoculation of all breeding pigs in one day (Štukelj et al., 2011).

Partial depopulation. This method is used as additional measure in PRRS eradication to stop the shedding of virus on the farm. Partial depopulation means that we remove different categories of pigs from the farm. With PRRS weaners are the critical category (Zimmerman et al., 2006; Torremorell and Christianson, 2002).

MATERIAL AND METHODS

Farms. Farm 1 has 2 boars and 72 breeding sows, farm 2 has 4 boars and 130 breeding sows. Serumization was performed once on farm 1 and twice on farm 2 in three months period. Farm 3, farm 4 and farm 6 have each 15 breeding sows. Farm 5 has 20 breeding sows. The method of natural exposure was used on farm 3 and 4, and vaccination with live attenuated PRRS vaccine strain Lelystad was performed on farm 5 and 6. On all six farms double closure for six months was accepted. In this time import of new pigs to the farm and also replacement of gilts from their own farm to the breeding herd was prohibited. All farms are free of classical swine fever and Aujeszky disease.

Sampling. On all farms PRRS was proved with first sampling. At second sampling blood was taken from all breeding pig for determination of prevalence and to choose the appropriate method of elimination. Samplings were repeated every 3 months to control the process of elimination.

Table 1. Number of tested sera for detection of antibodies against PRRSV and number of tested sera for virus detection.

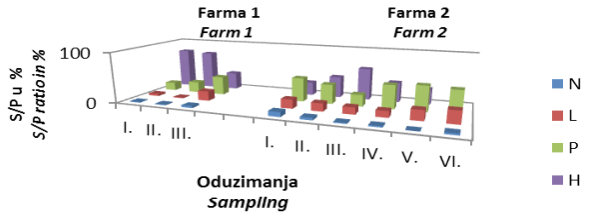
Tabela 1. Broj uzoraka za antitela protiv virusa PRRS i broj pregledanih uzoraka na prisutnost virusa PRRS

Farm *Farma*	No. of samples tested with ELISA *Broj uzorka ispitan u ELISA ELISA*	No. of samples tested with RT-PCR *Broj pregledanih uzoraka s RT-PCR*
1	292	40
2	519	271
3	64	20
4	31	10
5	94	-
6	80	20

Methods. Detection of antibodies by ELISA. Sera were tested with IDEXX PRRS ELISA (HerdChek, IDEXX Laboratories Westbrook, Maine, USA). S/P ratios were calculated according to test manual and were divided in N negative, L low positive – S/P less than 1, P positive S/P between 1 and 2, 4 – high S/P more than 2. Detection of nucleic acid of PRRS virus by RT-PCR in ORF 7 region of viral genome. Samples were tested by reverse transcription and polymerase chain reaction (RT-PCR) which enables detection of EU and USA strains of PRRS virus in well conserved region ORF 7 (Donadeu et al., 1999).

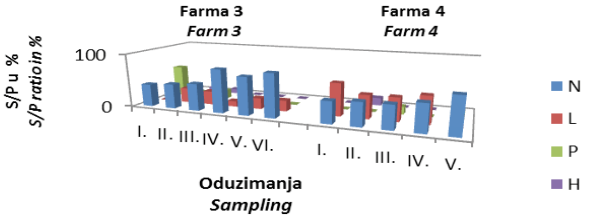
RESULTS

On farm 1 three months after serumization improvement of production results and trend of stabilization of breeding herd were visible. Six months after serumization number of high positive samples decreased. Before third sampling new boar and fatteners without quarantine were added to the herd. Two months after third sampling production results were significantly lower. On farm 2 three months after serumization six breeding sows were still negative and 21 were with low S/P values, so we decided for second serumization. At third sampling, 3 months after second serumization, improvement of production results and trend of stabilization of breeding herd were visible. At fourth sampling, six months after serumization, breeding herd stabilization was evident (S/P values were lower, some pigs were negative) and production results were improved. 18 months after second serumization number of negative increased and number of high positive decreased. On all farms PRRS virus was from genotip I.



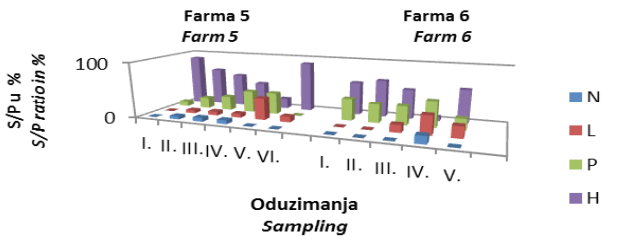
Graph 1. Results of serology of breeding herd from farm 1 and 2 after serumization
Graf 1. Rezultati seroloških testiranja krmača na farmi 1 i 2 posle serumizacije

Results of molecular testing on farm 1 and 2 proved absence of PRRS virus 3 month after serumization. On farm 1 eleven months after serumization same type of PRRS virus was proved. On farm 2 13 months after the serumization PRRS virus was not detected in breeding herds. Virus was persistently detected only in weaners at age 10 weeks. Ten months after second serumization new subtype of PRRS was proven by sequencing.



Graph 2. Results of serology of breeding herd from farm 3 and 4 after natural exposure
Graf 2. Rezultati seroloških testiranja krmača na farmi 3 i 4 posle prirodnog prekuženja

On farm 3 and 4 in samplings after natural exposure increasing of negative and also consequently decreasing of positive and low positive samples was evident. On both farms fatteners were also tested for antibodies and were respectively negative. On farm 3 and 4 virus was not detected.



Graf 3. Rezultati seroloških testiranja krmača na farmi 5 i 6 posle vakcinacije

Graph 3. Results of serology of breeding herd from farm 3 and 4 after vaccination

On farm 5 vaccination of breeding herd was performed twice in period of 3 months. Blood was taken before first vaccination (sampling I), before second vaccination (sampling II), two months after second vaccination (sampling III), five months after second vaccination (sampling IV), 10 months (sampling V) and 14 months after second vaccination (sampling VI). In table 4 it is visible that number of high positive was decreasing and number of positive was increasing and production results were improving until sampling VI. 14 months after vaccination number of high positive sample increased and production results drastically decreased.

On farm 6 pigs were vaccinated for substantial time before first sampling. Samplings were performed the following way: sampling II 1 month, sampling III 2 months, sampling IV six months and sampling V 11 months after our vaccination. Up until sampling V number of high positive samples was decreasing and number of positive samples was increasing and production results were improved. 11 months after vaccination number of high positive samples increased and production results decreased.

On farm 5 molecular testing was not performed. On farm 6 all 20 tested weaners of age 6 to 15 weeks were positive by RT-PCR before second sampling.

DISCUSSION

Closure of farm for at least 200 days is necessary for a successful elimination of PRRS by any method (Yeske, 2008). Very important is also to abide strict biosecurity measures (Dee, 1998).

The best protection against PRRS virus is homologous, which can be achieved either by serumization or natural exposure. On farm 1 and 2 three months after serumization we succeed with herd stabilization (all pigs with unified titres and without virus). On farm 1 they didn't abide closure rules, so new outbreak occurred. On farm 2 closure is accepted but all in all out system is not practiced and so virus is persisting in category of weaners.

On farm 3 and 4 positive effect was visible 3 months after closure (Graph 3). With each testing number of negative samples was increasing. From literature antibodies are persisting for 300 days (Zimmerman et al., 2006) but from our study it looks that antibodies can persist for a year and eight months.

Results from sequencing show that subtypes of PRRS in Slovenia are not closely related to Lelystad virus. (Toplak et al., 2010) Relevance of homologous protection was visible also in our situation when six month after vaccination a new outbreak occurs. In many pigs there was no seroconversion also after numbers of repeated vaccination. Af-

ter vaccination there was only a short improvement of production results and alleviation of clinical signs. Corzo et al. (2010) reported on alleviation of clinical signs also in case of heterologous type of virus. Vaccination is suitable for control and elimination of farm strain (Gillespie and Carrol, 2003). In our study we proved improvement of production results but virus was still present on the farm after vaccination.

CONCLUSION

Natural exposure with implementation of biosecurity rules and improvement of management are key factors in elimination and eradication of PRRS. All above mentioned measures are necessary for a successful pig production regardless of the herd health status. Serumization and vaccination are at this moment only methods with limited success.

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ELIMINACIJA SVINJSKOG REPRODUKTIVNOG I RESPIRATORNOG SINDROMA (PRRS) SERUMIZACIJOM, PRIRODNIM PREKUŽENJEM I VAKCINACIJOM NA ŠEST FARMI U SLOVENIJI

MARINA ŠTUKELJ, ZDRAVKO VALENČAK

Izvod

Svinjski reproduktivni i respiratorni sindrom (PRRS) je najskuplja komercijalna svinjska bolest koja se javlja u svijetu. Kod izbijanja bolesti imamo na razpolaganje različite načine preduzimanja u odnosu na veličinu farme i prevalencu bolesti, možemo, da ne obavljamo nikakve radnje, ili poduzimamo različite akcije poput kontrole bolesti, eliminaciju bolesti i eradikaciju bolesti. Odabrali smo 6 farmi sa problemima u reprodukciji. Na svim farmama bilo je dvojako zatvaranje uzgoja za najmanje 6 meseci. Krvni uzorci su pregledani testom IDEXX PRRS ELISA. Dokaz nukleinskih kiselina PRRS virusa s RT-PCR metodom. Na farmi 1 i 2 je tri meseca nakon serumizacije došlo do poboljšanja proizvodnih rezultata i do trenda prema stabiliziranju uzgojnog stada, 6 meseci nakon serumizacije smanjio se broj visoko pozitivnih krmača i također su se poboljšali proizvodni rezultati. Rezultati molekularnog testiranja na farmi 1 i 2 potvrdili su nakon 3 meseca odsustvo PRRS virusa u priplodnom stadu. Jedanaest meseci nakon vakcinacije na farmi 1 još jednom smo dokazali prisutnost istog podtipa virusa. Na farmi 2 u uzgojnom stadu već godinu i mesec nismo dokazali PRRS virus a virus se održava u kategoriji zalučene prasadi u dobi od 10 tedana (novi podtip virusa). Na farmi 3 i 4 proveli smo prirodno prekuženje. Dobiveni rezultati pokazuju da nakon svakog oduzimanja povećan je broj negativnih krmača. Na obje farme testirani su i tovljenici na prisutnost protutela i svi su bili bez protutela protiv PRRS virusa. Na farmi 3 i 4 nismo dokazali prisutnost virusa. Na farmi 5 i 6 izvedena je bila vakcinacija krmača i stalno se smanjivao broj visoko pozitivnih krmača i povećao broj pozitivnih a također su poboljšani proizvodni rezultati. Jedanaest meseci nakon vakcinacije ponovo je došlo do porasta visoko pozitivnih krmača i pogoršanja proizvodnih rezultata. Prirodno prekuženje pridržavanjem bio-sigurnosih zahteva i poboljšanje managementa kod unutarnje bio-sigurnosti su ključni čimbenici eliminacije i eradikacije PRRS. Serumizacija i vakcinacija su trenutačno metode s ograničenim uspehom.

Ključne reči: PRRS eliminacija, serumizacija, prirodno prekuženje, vakcinacija.

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