

Successful elimination of PRRS virus from an infected farrow-to-finish herd by vaccination

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ABSTRACT: Porcine reproductive and respiratory syndrome (PRRS) virus represents a major threat to the swine industry worldwide. This study describes the transmission of a European strain of PRRS-1 to a pig farm leading to the spread of the virus to different age categories of pigs and the development of clinical signs in pregnant sows and piglets. Porcine reproductive and respiratory syndrome aetiology was confirmed by serological tests and by virus isolation and subsequent sequencing. Repeated mass vaccination using modified live virus was used to synchronise the level of PRRS-specific immunity in all age categories of animals and to hinder virus circulation in the herd. Four months after the second mass vaccination, regular modified live virus vaccination of sows and gilts was implemented. Introduction of sentinel animals demonstrated cessation of virus circulation and the success of the control programme.

Keywords: control programme; pig; mass vaccination; serology

Porcine reproductive and respiratory syndrome (PRRS) is a disease which causes severe economic losses in pig herds worldwide. PRRS virus-induced disease manifests itself mainly as reproductive disorders in sows (infertility, abortions, unbalanced litters of piglets) or respiratory disease in nursery and finishing pigs (interstitial pneumonia). Clinical signs are influenced by the virulence of the strain and by the immune status and management practices of the herd. PRRS-1 virus (formerly the European strain of PRRS virus) is particularly widespread in European countries. Its prevalence at the herd level reaches up to 90% in some countries (Beilage and Batza 2007), including the Czech Republic.

Eradication of the virus from positive herds and maintaining virus-free status is costly and requires adoption of consistent control measures. In the past, several programmes applicable to herds have been described leading to successful eradication of the virus from herds after their closure, vaccination

or destocking and restocking (Dee and Joo 1997; Dee et al. 1998; Mortensen et al. 2002; Gillespie and Carroll 2003; Yang et al. 2008; Corzo et al. 2010). These programmes involve, among other steps, consistent removal of the reservoir and potential sources of the virus in herds. PRRS virus can exist in endemic form in a herd if there is a group of animals which are not immune against the circulating strain (Allende et al. 2000; Evans et al. 2010). From this aspect, piglets are at serious risk as they are particularly susceptible to infection and at the same time can shed the virus for up to several weeks (Stevenson et al. 1993). A subsequent follow-up and successful maintenance of a virus-free status is dependent on a large number of other factors such as herd size, the presence of other pig herds in the neighbourhood and availability of PRRS-free semen and gilts (Mortensen et al. 2002; Pitkin et al. 2009).

The aim of our work was to determine the feasibility of eliminating PRRS infection from the

herd using standard vaccination with a modified live vaccine combined with increased biosecurity measures.

MATERIAL AND METHODS

Herd description. The nucleus herd with 750 sows practiced internal replacement of gilts and had been verified to be free of PRRS for about three years following its repopulation. Breeding gilts were used for internal replacement and for the market as well. The farm with a farrow-to-finish herd was located 500 m outside a village with no other swine herds in the vicinity. The outbreak of PRRS was observed in the farrowing unit.

Serological examination. Serological testing in the monitored herd was carried out using a HERDCHEK* PRRS X3 (IDEXX Laboratories, Inc., Maine, USA) according to the manufacturer's instructions. A total of 160 pig sera were collected at different stages of PRRS infection in the herd and during the vaccination period and examined. Furthermore, 58 sentinel animals were tested for their virus-free status, and, after the herd restocking, a total of 100 newly introduced gilts were serologically tested.

Detection of the virus. Total RNA from serum samples of sera was extracted using the NucleoSpin[®] RNA II Kit (Macherey-Nagel, Macherey-Nagel, Duren, Germany) according to the instructions of the manufacturer. RNA was eluted in 60 µl RNase-free water and immediately used for RT-PCR amplification. The remaining RNA was stored at –80 °C.

Detection of viral RNA was performed with RT-PCR using the commercially available EZ-PRRSV MPX 4.0 Master Mix (IDEXX Laboratories, Inc., Maine, USA) according to the manufacturer's instructions. Detection of viral RNA was mainly performed at the early stage of PRRS infection with the aim of confirming the results of serological examinations and to characterise the virus in detail by sequencing. The first examination was performed in a total of 10 samples of blood serum derived from newborn piglets originating from five litters showing the most common signs of the disease, and from 58 sentinel animals prior to and after their use in reproduction.

Virus sequencing and phylogenetic analysis. RNA was first transcribed into cDNA using the Transcriptor First Strand cDNA Synthesis Kit

(Roche, Mannheim, Germany) according to the manufacturer's instructions in a reaction volume of 20 µl. The resulting cDNA was subjected to amplification of the ORF5 gene of PRRS virus. Amplification of ORF5 for sequencing was performed using a nested PCR assay with previously described primers (Pesente et al. 2006). Gel-purified PCR products were sequenced (Macrogen, the Netherlands). ORF5 sequences of PRRS-1 virus field strains were analysed using Geneious v. 10 software. A phylogenetic tree was constructed using the neighbour-joining method. A boot-strap value of 1000 replicates was applied for robust phylogeny estimation.

The source of sentinel animals and gilts for repopulation. Sentinel animals originated from a PRRS virus-free herd. Before the introduction into the farrow-to-finish herd included in the control programme, sentinel animals were serologically tested for the presence of PRRS-specific antibodies. Furthermore, direct detection of the virus in serum samples and nasal swabs was carried out in each animal.

Program for control and elimination of PRRS virus. Our main aim was to stabilise the PRRS-positive herd using repeated mass vaccination with modified live virus vaccine (Porcillis PRRS, MSD AH) in order to reduce disease occurrence and to stop virus shedding and circulation in the herd. The mass vaccination was applied two times, on day 150 after the beginning of the outbreak and four months later.

Four months after the second mass vaccination, a new vaccination schedule for the stud herd of sows and gilts was established: Breeding gilts were vaccinated with the first dose at 22 weeks of life, and with the second dose at 25 weeks of life, using the same modified live virus (MLV) so that immunity could develop before the insemination. The sows were revaccinated two weeks after parturition, i.e., three weeks before insemination. Virus shedding was monitored by virus isolation and serological examination of sentinel animals.

The effect of vaccination was further supported by improved biosecurity measures and a closed herd management strategy which included the removal of all finishers from the farm. Additional measures to combat the outbreak of infection, e.g., strict control of staff and animal flow, determination of clean and infected (black and white) zones and improved welfare, were also applied.

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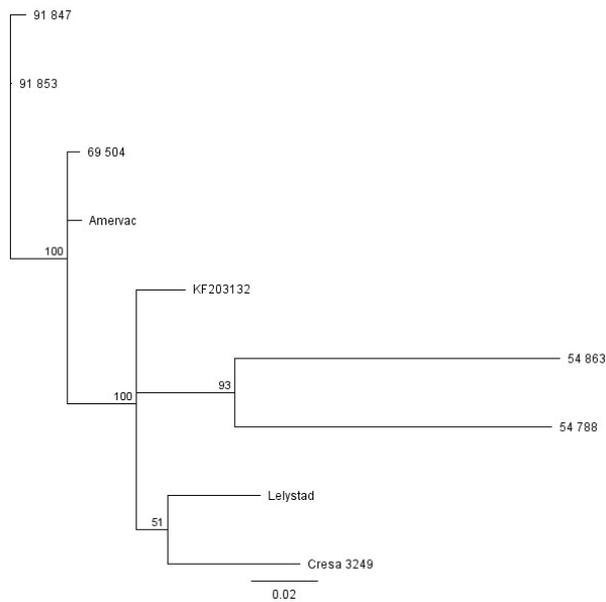


Figure 1. Phylogenetic tree based on alignment of ORF5 nucleotide sequences of two PRRS-1 isolates described in this study, four reference strains and three strains recently isolated in the Czech Republic. The neighbour-joining method was used. The percentage of bootstrap support higher than 50% for each node is displayed. The scale representing nucleotide substitution per site is displayed

RESULTS

During the first five months after the onset of PRRS infection induced by the PRRS-1 virus strain in the herd of sows, the spread of infection was observed in all age categories, including sows in the reproductive cycle (pregnant or postpartum), their suckling piglets, weaned piglets in the nursery period weighing up to 28 kg, gilts in gilt developer units and fattening pigs.

Detection of PRRS virus

The first detection of the virus was carried out from 10 serum samples collected from neonatal piglets at an early stage of the disease outbreak. The presence of PRRS virus was found in a total of six piglets originating from five different litters.

ORF5 phylogenetic analysis

A phylogenetic tree (Figure 1) was constructed based on nucleotide sequences of ORF5 of two new isolates (labelled as 91 847 and 91 853), four reference strains (Amervac, Lelystad, Cresa 3249, KF203132) and three strains isolated recently in the Czech Republic (69 504, 54 863, 54 788). Genotype I strain PRRS virus was identified on the basis of the sequencing of the ORF5 gene. The homology of the isolated strains to field or vaccine strains is shown in Table 1. Two new isolates were closely related to the Amervac vaccine strain and one recent Czech strain (69 504) but differed from the Lelystad strain and Cresa strain.

Serological profile of the herd and virus detection during the disease outbreak

Serological tests were carried out two months after the beginning of the outbreak and at the peak of clinical manifestation of PRRS infection at five months after the outbreak. Out of a total of 50 pigs of different ages, 24 were serologically positive. The overall prevalence in the herd in this period was 48%. Seropositivity in each category is shown in Table 2. At the peak of the outbreak (day 150), 32 out of 50 tested pigs were found to be positive. The prevalence thus increased to 64%.

Table 1. Comparison of pairwise nucleotide distances between PRRS-1 ORF5 from vaccine virus strains and strains detected in the field

	69 504	Amervac	91 847	91 853	KF203132	Lelystad	Cresa3249	54 863	54 788
69 504	ID	99.07	97.22	98.15	95.99	94.44	91.98	86.11	86.42
Amervac	99.07	ID	97.28	97.83	96.01	94.20	92.21	85.49	86.42
91 847	97.22	97.28	ID	99.50	93.89	92.57	91.58	84.88	85.80
91 853	98.15	97.83	99.50	ID	94.39	93.07	91.58	84.88	85.80
KF203132	95.99	96.01	93.89	94.39	ID	95.38	93.23	87.96	86.73
Lelystad	94.44	94.20	92.57	93.07	95.38	ID	93.56	86.11	83.95
Cresa3249	91.98	92.21	91.58	91.58	93.23	93.56	ID	85.49	85.80
54 863	86.11	85.49	84.88	84.88	87.96	86.11	85.49	ID	84.26
54 788	86.42	86.42	85.80	85.80	86.73	83.95	85.80	84.26	ID

After four months, the second wave of mass vaccinations was performed in all animals in the herds of sows and gilts, except for weaned piglets and fattening pigs (fattening was completed). Serological examination of 50 animals of different age categories in the herd after the second mass vaccination showed a 100% prevalence of PRRS-1 specific antibodies (Table 2).

Four months after the second mass vaccination, i.e., 10 months after the control programme launch, a new vaccination schedule for the stud herd of sows and gilts was established: Breeding gilts were vaccinated with the first dose at 22 weeks of life, and with the second dose at 25 weeks of life, using the same MLV so that the immunity could develop before insemination. The sows were revaccinated two weeks after parturition, i.e., three weeks before insemination. As a check of the efficacy of the programme, serological monitoring was carried out in the fattening pigs aged 16–20 weeks 15 months after the programme launch. Serological testing did not reveal antibodies in any of the 60 animals from three restocking cycles (data not shown). Therefore, two sentinel gilts were introduced into the gilt developer. Repeated examinations of these animals did not reveal either the PRRS virus or anti-PRRS virus antibodies over the course of two months. Subsequently, a further five sentinel animals were introduced into the herd from a PRRS-negative herd. Over the course of a two-month exposure, neither PRRS virus nor PRRS virus-specific antibodies were detected in their sera. An additional 20 sexually mature sentinel gilts were introduced into the herd of sows 24 months after the beginning of the control programme. Monitoring the occurrence of PRRS virus and antibodies yielded negative results throughout the period until parturition. This was consistent with the negative results found in a further two groups comprising a total of 33 sentinel gilts introduced into the herd

26 and 27 months after the beginning of the control programme. The obtained results indicated that the field PRRS virus did not circulate either in the subpopulation of sows or gilts.

DISCUSSION

The spread of PRRS virus in an immunologically naive farrow-to-finish herd with a closed herd turnover was particularly rapid between farrowing and nursery stables, all of which were in the same halls, and, furthermore, were connected by corridors with gestational pens for 80 pregnant sows and gilts. Individual buildings for the housing of fattening pigs, gilt developer units and insemination units were located outside this complex. Whereas strict measures were implemented to ensure external biosecurity, most of the rules for ensuring internal biosecurity were not followed (Fablet et al. 2016). Besides that, at the time when PRRS virus infection was confirmed, a change of ownership occurred. For the new owner who intended to make use of the genetic background, the depopulation of the infected herd was not a feasible option. The strategic objective of the control programme was to achieve PRRS-negative status in the herd using a live attenuated vaccine. Out of the two commercial vaccines available on the market at that time, Porcilis PRRS inj. was chosen. The manufacturer states that this MLV vaccine can be used also in cases when pregnant sows were previously infected with the PRRS-1 virus field strain (Beilage and Beilage 2004; Scotti et al. 2006; Beilage and Batza 2007). Based on the prognosis of immunity development after infection with the field strain, the programme was initiated at a time when stabilisation of the situation was expected with respect to the closed operation system, i.e., five months from the onset of infection (Schugart and Kriegler 2013). Both the first and second wave of mass vaccinations

Table 2. Results of serological testing at different times during the outbreak

Age category	First sampling (day 60)		Before 1 st vaccination (day 150)		After 2 nd mass vaccination (day 270)	
	tested pigs (<i>n</i>)	positivity (%)	tested pigs (<i>n</i>)	positivity (%)	tested pigs (<i>n</i>)	positivity (%)
New-born piglets	10	20	10	20	50	100
Nursery pigs	10	60	10	80	50	100
Fattening pigs	10	40	10	60	50	100
Sows	10	60	10	80	50	100
Gilts	10	60	10	80	50	100

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four months later were carried out without increasing the number of abortions. Before the second wave of vaccinations, all fattening pigs were removed from the farm to decrease the circulation of PRRS virus. The negative serological test results obtained in these fattening pigs provided the first evidence for the effectiveness of the chosen vaccination procedure in the 16-month period from the start of the programme's implementation. These pigs had not been vaccinated, but were in contact with revaccinated mothers in the second week of life. Subsequently, sentinel animals (gilts, boars) were used to monitor the circulation of the PRRS field strain virus. Repeated examinations of these animals did not show the presence of either the virus in the blood or antibodies in the blood serum. At the request of the farmer, the next steps of the programme were postponed, e.g., the herd vaccination was terminated. The breeder was considering the further use of the herd of sows included in the control programme, which in terms of genetics did not provide him with the necessary future utility. Therefore, the breeder decided to wait until he would have a sufficient number of new gilts of a different genetic line to allow restocking of the herd without a production break. The final decision on this course was made because the field virus was not likely to be circulating in the herd any more as indicated by the results of monitoring. To confirm this assumption, three groups comprising a total of 53 breeding sows were gradually introduced into the herd 25 weeks after from the beginning of the programme. The results of monitoring PRRS virus in these animals were negative after four months (RT-PCR and ELISA assays), which was the basis for initiating the process of herd restocking without a production break. Turnover in the herd was gradually performed over a period of seven months and started with the one-time introduction of a large groups of gilts into the isolation facility, which was located on the farm at a beeline distance of 60–80 m from the farrowing and rearing houses. After two months of isolation and examination which gave negative results, the gilts were introduced into the herd, thereby replacing the sows culled from the original herd. This resulted not only in PRRS virus eradication from the original herd, but also in its repopulation using gilts from a PRRS virus-free herd 38 months after the beginning of the control programme's implementation. The repopulated herd was PRRS virus-free from the beginning and no circulation of the virus occurred during the subsequent two years. This study provides evidence

that PRRS infection is very difficult to control, even in the case of vaccination. Attenuated live vaccine appears to be generally the most suitable means of obtaining robust immunity of pigs against infection with PRRS virus (Heller et al. 2011; Werdeling et al. 2012). Our study confirms that successful vaccination against PRRS virus within a virus eradication programme can be achieved with current MLV vaccines as the gold standard. Another means of increasing the effectiveness of PRRS control programmes in infected herds in the future may be the introduction of new types of vaccines such as ReproCyc PRRS EU registered in the CR and EU in 2016. This will enable immunisation of healthy pregnant sows in infected herds (Stadler et al. 2016). With this in mind, we cannot see any reason for using autovaccines with homologous isolates of the PRRS virus. It is far more important to focus on an effective biosecurity system to prevent the introduction of a new PRRS virus into a herd included in a control and eradication programme (Mortensen et al. 2002). In another case of a PRRS virus control programme, we identified a biosecurity system failure due to the infiltration of virus into the isolation facility which was used for the adaptation of PRRS-negative gilts. Therefore, two years after the programme was launched, the final decision was made to depopulate the PRRS-positive farrow-to-wean herd, because PRRS virus control and eradication programmes are difficult to implement in an ongoing production situation with external replacement of gilts.

We can say that an important factor that enabled successful control in the PRRS-infected farrow-to-finish herd was that the farm has its own onsite gilt development unit. Similar conclusions were drawn also by Bottoms et al. (2012). Restocking carried out after PRRS virus eradication proved to be very efficient with regard to piglet production, which was significantly increased and comparable with other herds. However, the production break necessitated by the process of depopulation and subsequent repopulation resulted in the loss of almost an entire year's production. Nevertheless, despite this setback in our case, not only were we able to regain a competitive market position, we were also able to create conditions for reconstructing the housing technologies for all categories of PRRS virus-free pigs. As a result of our activities, the host farm now numbers amongst the most successful in the country.

REFERENCES

- Allende R, Laegreid WW, Kutish GF, Galeota JA, Wills RW, Osorio FA (2000): Porcine reproductive and respiratory syndrome virus: Description of persistence in individual pigs upon experimental infection. *Journal of Virology* 74, 10834–10837.
- Beilage EG, Beilage TG (2004): Safety and efficacy of herd vaccination with Porcilis ((R))-PRRS in persistently infected farrow-to-finish herds. *Praktische Tierarzt* 85, 911–916.
- Beilage EG, Batza HJ (2007): PRRSV-eradication: an option for pig herds in Germany? *Berliner und Munchener Tierarztliche Wochenschrift* 120, 470–479.
- Bottoms K, Poljak Z, Dewey C, Deardon R, Holtkamp D, Friendship R (2012): Investigation of strategies for the introduction and transportation of replacement gilts on southern Ontario sow farms. *BMC Veterinary Research* 8, doi: 10.1186/1746-6148-8-217.
- Corzo CA, Mondaca E, Wayne S, Torremorell M, Dee S, Davies P, Morrison RB (2010): Control and elimination of porcine reproductive and respiratory syndrome virus. *Virus Research* 154, 185–192.
- Dee SA, Joo HS (1997): Strategies to control PRRS: a summary of field and research experiences. *Veterinary Microbiology* 55, 347–353.
- Dee SA, Joo HS, Park BK, Molitor TW, Bruna G (1998): Attempted elimination of porcine reproductive and respiratory syndrome virus from a seedstock farm by vaccination of the breeding herd and nursery depopulation. *Veterinary Record* 142, 569–572.
- Evans CM, Medley GF, Creasey SJ, Green LE (2010): A stochastic mathematical model of the within-herd transmission dynamics of porcine reproductive and respiratory syndrome virus (PRRSV): Fade-out and persistence. *Preventive Veterinary Medicine* 93, 248–257.
- Fablet C, Marois-Crehan C, Grasland B, Simon G, Rose N (2016): Factors associated with herd-level PRRSV infection and age-time to seroconversion in farrow-to-finish herds. *Veterinary Microbiology* 192, 10–20.
- Gillespie T, Carroll A (2003): Methods of control and elimination of porcine reproductive and respiratory syndrome virus using modified live vaccine in a two-site production system. *Journal of Swine Health Production* 11, 291–295.
- Heller P, Schagemann G, Schroder C, Stampa E, Ohlinger V, Wendt M (2011): Elimination of PRRS virus (EU-type) from a farrow-to-finish breeding farm by vaccination with Ingel-vac PRRS MLV under unfavourable conditions. *Praktische Tierarzt* 92, 416–422.
- Mortensen S, Stryhn H, Sogaard R, Boklund A, Stark KD, Christensen J, Willeberg P (2002): Risk factors for infection of sow herds with porcine reproductive and respiratory syndrome (PRRS) virus. *Preventive Veterinary Medicine* 53, 83–101.
- Pesente P, Rebonato V, Sandri G, Giovanardi D, Ruffoni L, Torriani S (2006): Phylogenetic analysis of ORF5 and ORF7 sequences of porcine reproductive and respiratory syndrome virus (PRRSV) from PRRS-positive Italian farms: A showcase for PRRSV epidemiology and its consequences on farm management. *Veterinary Microbiology* 114, 214–224.
- Pitkin A, Deen J, Dee S (2009): Use of a production region model to assess the airborne spread of porcine reproductive and respiratory syndrome virus. *Veterinary Microbiology* 136, 1–7.
- Schugart K, Kriegler W (2013): PRRSV eradication. *Tierarztliche Umschau* 68, 542–545.
- Scortti M, Prieto C, Martinez-Lobo FJ, Simarro I, Castro JM (2006): Effects of two commercial European modified-live vaccines against porcine reproductive and respiratory syndrome viruses in pregnant gilts. *Veterinary Journal* 172, 506–514.
- Stadler J, Zoels S, Eddicks M, Kraft C, Ritzmann M, Ladinig A (2016): Assessment of safety and reproductive performance after vaccination with a modified live-virus PRRS genotype 1 vaccine in pregnant sows at various stages of gestation. *Vaccine* 34, 3862–3866.
- Stevenson GW, Vanalstine WG, Kanitz CL, Kaffaber KK (1993): Endemic Porcine reproductive and respiratory syndrome virus-infection of nursery pigs in 2 swine herds without current reproductive failure. *Journal of Veterinary Diagnostic Investigation* 5, 432–434.
- Werdeling F, Mues G, Offenberg S, Zantz S, Fiebig K (2012): Development and control of an acute PRRS field virus infection in an endemic PRRS positive breeding herd after vaccination with a modified live vaccine. *Praktische Tierarzt* 93, 722–728.
- Yang JS, Moon HJ, Lee CS, Park SJ, Song DS, Kang BK, Choi JU, Park BK (2008): Elimination of porcine reproductive and respiratory syndrome virus from a seedstock breeding farm and a supplying boar stud by a modified test and removal method. *Veterinary Record* 162, 333–337.

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