Thesis for a doctoral dissertation (PhD)

Genomic epidemiology of domestic PRRS infections

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Budapest, 2023

Introduction

Porcine Reproductive and Respiratory Syndrome (PRRS), caused by the Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), is a major significant infectious disease affecting pigs industry globally. The virus belongs to the Arteriviridae family, Betaarterivirus genus within Nidovirales. The presence of PRRSV in Hungary can be traced back to the 1990s, although its definitive identification was established in 1995 through serological monitoring (Hornyák et al., 1996). Subsequent viral isolation in 1999 provided evidence of the European genotype of PRRSV in the country (Medveczky et al., 2001). PRRS was initially designated a notifiable disease in Hungary by the Animal Health Code (FVM) Decree 41/1997 (28.V.7.), a status which ceased after June 1, 2001, due to a lack of PRRS regulation across European Union (EU) member states. During this transitional phase, the virus spread more rapidly within Hungary due to the absence of official measures upon infection confirmation. Hungary's accession to the EU further facilitated disease spread, as infected livestock could enter from Western European nations without undergoing official veterinary inspection (Balka et al., 2009).

The PRRS became a notifiable disease again in Hungary from 1 January 2006 onwards, based on Act CLXXVI of 2005. The National PRRS Eradication Plan came into force in 2014 with the aim to assess the infection in domestic pig herds, to optimise the rearing of fattening pigs with lower use of drugs, mainly antibiotics, and to achieve PRRSV-free status. In preparation for the eradication, comprehensive studies were carried out between 2010 and 2013 in order to accurately record and classify the PRRS infection in small and large breeding herds as well as among fattening pigs, and to classify the identified PRRSV strains into genetic clades based on sequencing (Szabó et al., 2020).

Previous studies have shown that PRRSV first appeared in feral pigs and later started to adapt in domestic pigs. In Europe, many feral pigs are infected with the European strain of the virus (Stadejek et al., 2013), but this has not been confirmed by diagnostic methods in feral pig herds in Hungary (NÉBIH ÁDI).

The virus can also be spread through the saliva, nasal secretions, urine, semen and occasionally whith faeces of infected animals. The infection can be transmitted by direct contact with pigs or indirectly by contaminated persons or fomites. Furthermore, use of artificial insemination facilitates rapid and long-distance transmission of PRRSV. Animals can be infected by intranasal, oral, intramuscular, intrauterine and intravaginal routes. Depending on the route of virus entry, different amounts of virus are required for infection and disease development (Zimmerman, 2019). Virus particles can also be transported by attachment to airborne particles, which influence virus spread. The particles determine the distance the virus can be transported. PRRSV has been detected in particle sizes ranging from 6×10^2 (0.4-

0.7µm) to 5.1x104 RNA copies/m3 (9.0-10.0µm). The virus can spread to 9.1 km from pig herds (Alonso et al., 2015).

PRRS virus is observed with equal frequency in pig herds of different age classes. It can cause respiratory disorders in young pigs and reproductive disorders (abortion, early farrowing, early death, stillbirth) in breeding pigs. The absence of clinical signs does not refer to virus free status (Zimmerman, 2019). Therefore, diagnostic tests and continuous monitoring of different age classes are particularly important. The presence of infection can be tested by indirect and direct methods. Indirect virus detection is performed by serological (e.g. ELISA) tests, where we can detect the products of pathogen-induced immune reactions, i.e., antibodies. The antibodies produced by the antigens of the virus can be detected in the blood sera of infected animals, so that ELISA can be used to determine the infection in herds as well as immunological status of different age groups. Direct virus detection can be carried out in order to identify infection in the acute phase of the disease. In the different age groups, the virus can only be detected in blood serum for a certain time following infection, 4-6 weeks in piglets and 1-2 weeks in boars and sows. The viral RNA can be detected by reverse transcription-polymerase chain reaction (RT-PCR) (Zimmerman, 2019), for which ORF7 is the most suitable conserved gene sequence.

Blood samples from different age groups are tested by serological (ELISA) and molecular biological (PCR) methods to determine the time of infection. This procedure is used to develop a farm-specific eradication protocol, which includes not only a vaccination scheme but also internal disease control measures. These include, for example, restrictions on animal movements, the use of disinfectants, the entry of workers into the holding and restrictions on movement within the holding between different animal facilities, the wearing of appropriate clothing, the tying of work equipment to sheds, etc.

The basic principle for the eradication of infected pig herds is strict compliance with the external and internal disease control rules of the farms, laboratory monitoring between different age groups of the herd and immunisation against PRRS (including pregnant and non-pregnant breeding sows, gilts, breeding boars, piglets during lactation and fattening pigs). The identification of PRRSV ORF5 and ORF7 sequences provides very important information on the spread of PRRSV, the potential for infection and the mode of transmission (Bálint et al, 2021). The visualisation of epidemiological relationships significantly increases the possibility for practitioners to analyse epidemiological processes and molecular biology-based results using virological methods (Szabó et al., 2020). The evaluation of sequence data is of particular importance for assessing virus spread within and between farms in the PRRS epidemic.

The primary objective of PRRSV control is to reduce and eliminate economic losses, to eliminate the virus, and to maintain the status of currently negative pig herds and prevent the emergence of new recombinant strains in infected herds. Preventive measures by livestock

keepers are also important, such as quarantining imported animals, following transport and disinfection protocols, and maintaining biosecurity of premises. Among the specific control options, vaccination with inactivated vaccines has been observed to provide immune response and protection in naïve pigs. Live viral (MLV) vaccines are more efficacious in developing appropriate immune response, but they do not provide complete protection against gebetically heterologous strains. Their use can also be hazardous (Botner et al., 2000), since the virus has a high genetic variability, which can lead to mutations and recombination, and thus the virus can regain virulence. In spite of the above drawbacks, vaccination is currently the most effective solution available to date to mitigate the economic damage caused by PRRSV (Nan et al., 2017).

Aims of the studies

The aim of this research is to develop a diagnostic method to distinguish whether the suspected PRRSV viremia is caused by a live modified vaccine virus or a wild virus used for vaccination. In addition to this, regular laboratory PRRSV PCR testing can be used to monitor the spread of PRRSV strains in Hungary. The research may contribute to the improvement of the knowledge on genetic stability of the vaccine strain applied in Hungarian pig farms.

The discriminatory (DIVA) PCR method developed in the present research may contribute to reduce the circulation of the virus in pig herds through a more efficient in-house diagnosis of PRRSV and may contribute to the efficiency of a PRRS eradication programme in a given herd. The development of the DIVA PCR was of paramount importance, which we implemented in our own research, as this method allows sensitive and rapid discrimination of wild PRRS virus from attenuated vaccine strains.

In addition, the research could help to identify and track PRRS virus strains in our country, to further analyse and compare their evolution and phylogenetic relationships, to determine their geographical distribution and to define their epidemiological role, using existing data.

Our laboratory receives weekly blood serum samples from several PRRS infected farms on weekly basis. Most of these herds also use live virus vaccination. By analysing the ORF5 and ORF7 sequences of the samples, which can be traced back several years, we can obtain an accurate picture on the evolution of wild virus strains in response to vaccine-induced selection pressure, the emergence of new variants that may co-exist in the farm, and the genetic stability of the vaccines used.

For pig farms using live virus vaccines, it is essential to be informed as soon as possible whether the virus identified in the age group is of vaccine or wild virus origin. The so-called discriminatory PCR (DIVA PCR) can be used to determine whether a wild virus or a vaccine virus is present in the animal. This discriminatory PCR screening, based on the identification of the ORF5 sequence, may have a preventive character for infected herds. In our work, we aimed to demonstrate the importance and role of the use of discriminatory PCRs in the eradication process. We would like to draw attention to the possible emergence of new recombinant virus strains with high virulence and their importance.

Material and Methods

The infected herds were sampled according to the regulations of the PRRS National Eradication Plan 4.0 (95% prevalence and 2% confidence interval) developed under their eradication programme on a weekly/monthly basis to monitor the status of the herd. The samples were collected by Eurofins Vetcontrol Ltd (formerly known as M.A.H. Food-Controll Ltd. Vetcontrol Veterinary Diagnostic Department) and the Veterinary Diagnostic Directorate of NÉBIH for diagnostic and monitoring tests. In general, blood and organ samples (e.g., lungs, lymph nodes) were processed. Blood samples were collected from suckling and weaned piglets, from fattening and finishing pigs and from breeding animals (boars, sows, gilts). In addition to regular monitoring, animals showing reproductive signs and respiratory disorders were also sampled (organ samples from selected pre-fattening and fattening animals and aborted foetuses) for screening for PRRSV.

Sequence analysis of live viral vaccines is based on similarity network phylogenetic analysis (Bálint et al, 2020) was used to identify the most similar sequences to the virus in each vaccine. The virus vaccines included in the study were Porcilis MLV® PRRS vaccine (manufacturer: MSD Animal Health, Madison, NJ, USA), Unistrain PRRS vaccine (formerly Amervac) (manufacturer: Laboratorios Hipra, S.A., Amer, Spain), Reprocyc PRRS EU vaccine (manufacturer: Boehringer Ingelheim Vetmedica GmbH, Ingelheim am Rhein, Germany).

Subsequently, the percentage similarity of these sequences to ORF5 and ORF7 sequences was evaluated. The ORF5 and ORF7 regions of the live virus vaccine PRRSV strain tested in GenBank were assigned to each Hungarian sequence/sample from 2014 onwards according to epidemiological data (veterinary information on the origin of the sample, immunisation, etc.). The aim of the sequence analysis study was to investigate how the ORF5/ORF7 sequence of the given vaccine virus evolves after immunization compared to the reference sequences of the vaccines used in Hungary. We also hypothesized that this study would help us to develop DIVA Taqman rRT-PCR.

The primers and assays for DIVA PCR were designed based on the identified sequences of MLV vaccine strains in the respective farms using Molecular Evolutionary Genetics Analysis version 6.0 (MEGA 6.0) software. Primers and assays were designed using Primer3 software. The targeted region was the ORF5 gene, the most variable region of the PRRSV genome. The middle region of ORF5 was found to be suitable for primer and assay design in the generation of the PRRSV discriminative TaqMan rRT-PCR assay. The forward and reverse primers were designed to match both the vaccine and wild-type PRRSV sequences.

The forward and reverse primers contained two and one degenerate nucleotide, respectively, which allowed binding to Porcilis MLV® and the farm-specific PRRS wild-type virus, respectively. In contrast, two different TaqMan probes were each designed to match either vaccine and/or wild-type virus sequences. The two assays differed by only four nucleotides to prevent binding to non-target sequences. The ORF5 of the MLV's was identical to the original Porcilis PRRS MLV®.

Practical application of DIVA PCR was performed in four pig farms in Hungary, involving pigs that participated in the PRRS eradication programme. Between 2017 and 2019, 38 447 samples from different age groups were tested using a commercially available ViroReal PRRSV RT-PCR Kit (Ingenetix, Austria), involving pig farms that carried out their immunisation programme with live virus vaccines and used Porcilis MLV® for live vaccines. All samples that tested positive with the commercially available kit were tested with the developed DIVA PCR. These tests can identify wild-type PRRSV and MLV vaccine. DIVA Real Time PCR was used to obtain information on the PRRS status of the age groups. The method was able to predict whether the age group under investigation was infected with wild PRRSV before a given technological step in the colonies. If a sow tested positive by farm-specific PCR for PRRSV, her piglets were culling before 2-4 weeks of selection.
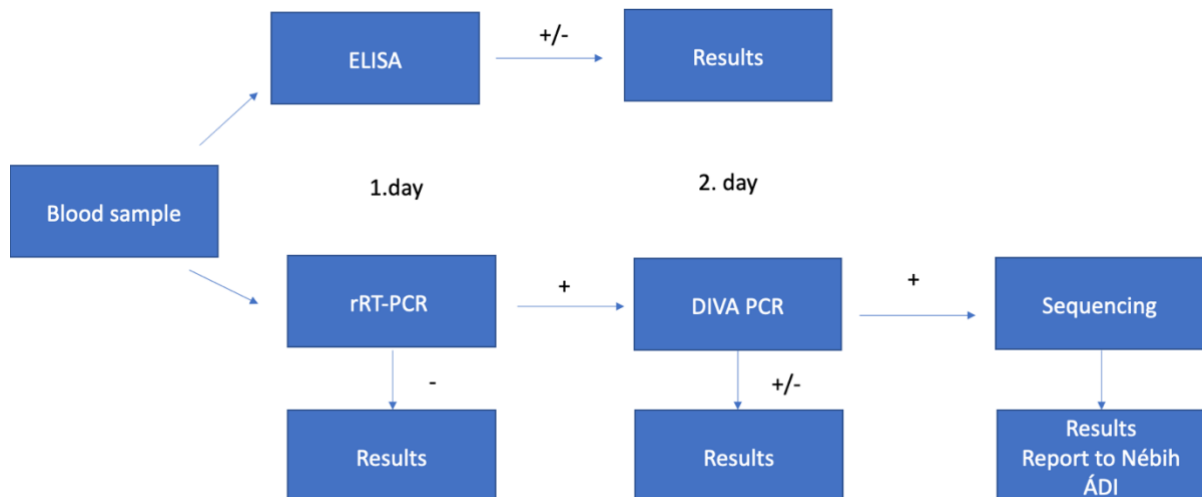

Figure 1: Test protocol for sites participating in the PRRS decontamination programme.

Results

In the ORF5 sequence analysis of vaccines, 832 ORF5 sequences were analysed. We found that there were 618 ORF5 sequences from Porcilis MLV® that had at least 98% similarity to the strain in the Porcilis PRRS vaccine. Of these sequences, 328 (53.1%) were 100% identical to the ORF5 sequence of the vaccine strain (including the Porcilis PRRS vaccine strain), 264 (42.7%) had a similarity between 99.1 and 99.9%, and 26 (4.2%) had a similarity between 98 and 99%. Of the Amervac vaccine esters, 179 were found to be at least 98% similar to the strain in the Unistrain PRRS vaccine. Four of these sequences (including the Amervac and Unistrain vaccine strains and two international sequences: DQ345725.1 (MLV vaccine) and JQ040769.1 (clone AN0708EU_1)) had 100% ORF5 sequence similarity. Seventy (39.1%) sequences showed 99% similarity, while 105 (58.7%) sequences showed less than 99% but more than 98% similarity. None of the sequences detected in the Hungarian pig herds were 100% identical to the ORF5 sequence of the PRRSV strain in the Unistrain PRRS vaccine, as farm-specific clades were formed. 4 of the sequences were not derived from the immunization of the herd but were sequences of the original virus in the vaccine. In the case of Reprocyc vaccine, 35 ORF5 sequences showed at least 98% similarity to the virus strain in the ReproCyc PRRS EU vaccine. Three of these sequences (8.6%), including the Reprocyc vaccine strain, were identical to the ORF5 sequence of the vaccine strain. For 11 (31.4%) and 21 (60%) sequences, the similarity ranged from 99.1 to 99.9%, and from less than 99% to more than 98%, respectively. In this case also, farm-specific clades are formed. There were 3 sequences that were the sequence of the vaccine virus (1 from Reprocyc, 1 from Reprocyc PRRS- Flex vaccine and 1 from Genbank). For the ORF7 analysis of the vaccines, a total of 478 different PRRSV ORF7 sequences from domestic and GenBank databases were analysed for sequences that were more than 98% similar to the standard vaccine virus sequence. There were 88 sequences that could be analysed, of which 51 were Porcilis MLV® 29 were Amervac and 8 were Reprocyc ORF7 sequences. For the ORF7 sequence analysis, we had less data available, so the resolution of the results was much lower, but confirmed our previous results. It can be concluded that the ORF7 gene is a more conserved sequence.

The standard curves were set with Porcilis MLV® and PRRSV 30040/2017 NÉBIH strains, using the results of the tenfold RNA dilution tests. Tenfold dilutions of 10^{10} and 10^0 RNA copy number/µl RNA free water were performed for each sample. All reactions were performed in three parallel measurements and the average data of the cycle threshold (Ct) values were used to design the standard curves, also adjusted with tenfold RNA dilutions. The PCR efficiency (E) was 1, while the correlation efficiency (R^2) was 0.99 in both cases. A

comparison of the sensitivity of the method with commercially available PRRSV Real-Time PCR showed a difference of 1-3 Ct in favour of the commercial kit depending on the concentration of the protocol.
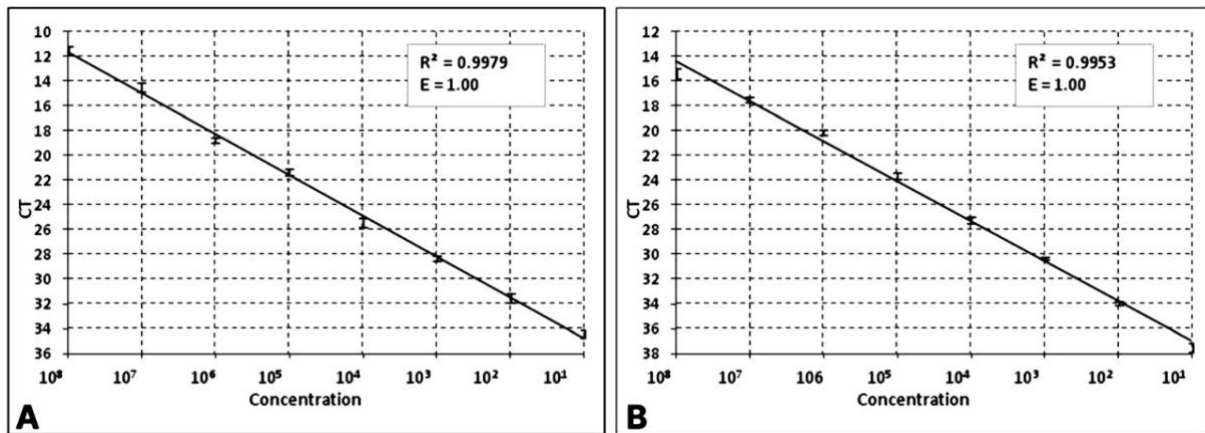

Figure 2: Standard curves generated using 10-fold dilutions of template RNA prepared from the Porcilis MLV (A) and PRRSV 30040/2017 NEBIH (B) strain. Three replicates were performed of each dilution. Average values and standard deviations are shown. RNA copy number is indicated on the x-axis, while Ct values on the y-axis.

Mixed infections were detected by combining dilutions of Porcilis MLV® and PRRSV 30040/2017 NÉBIH RNA series. When the two RNAs were present in variable amounts in the sample, we observed that discriminative PCR was able to detect both viruses when the copy number ratio of the minor variant was above 0.1% (Table 1).

Table 1: Potency of the discriminatory PCR to detect mixed infections using different concebtrations of template RNA prepared from the Porcilis MLV® and PRRSV 30040/2017 NEBIH strain.

MLV/WT copy number	$10^8/10^1$	$10^7/10^2$	$10^6/10^3$	$10^5/10^4$	$10^4/10^5$	$10^3/10^6$	$10^2/10^7$	$10^1/10^8$
Ct value	12.9/n.d.	15.8/n.d.	19.1/31.4	22.5/26.2	27.3/22.1	32.3/19.3	n.d./15.8	n.d./13.1
MLV/WT copy number	$10^8/10^4$	$10^7/10^4$	$10^6/10^4$	$10^5/10^4$	$10^4/10^4$	$10^3/10^4$	$10^2/10^4$	$10^1/10^4$
Ct value	12.3/n.d.	15.4/37,4	18.8/31.9	22.4/28.1	25.6/26.3	31.3/25.8	37.8/25.8	n.d./25.8
MLV/WT copy number	$10^4/10^8$	$10^4/10^7$	$10^4/10^6$	$10^4/10^5$	$10^4/10^4$	$10^4/10^3$	$10^4/10^2$	$10^4/10^1$
Ct value	n.d./12.7	36.6/15.1	31.7/18,2	27.2/21.7	25.9/25.8	25.4/29.2	25.4/38.2	25.4/n.d.

Out of 38477 serum samples from the farm under derogation, 3164 samples were positive for PRRSV RT-PCR, which were first tested with ViroReal PRRSV EU/NA Real-Time PCR Kit. These positive samples were subjected to discriminatory RT-PCR testing between 2017 and 2019 (Table 2). Results of discriminative RT-PCR testing were all confirmed by sequencing when a single infection occurred and the Ct value was <32. In mixed infections, if Ct values <1 log 10 titre difference was observed, either MLV or wild-type PRRSV virus was confirmed by

sequencing. In contrast, if the ratio was >2 log 10, only the virus with the higher titre was identified by sequencing.

Table 2: Age group and year distribution of field samples examined by the discriminatory RT-PCR test during the eradication programme of the farm.

| | 2017 | | | 2018 | | | 2019 | | |
Age group	Number of samples	PCR positive[*] (%)	Discriminatory PCR wild-type[#] (%)	Number of samples	PCR positive[*] (%)	Discriminatory PCR wild-type[#] (%)	Number of samples	PCR positive[*] (%)	Discriminatory PCR wild-type[#] (%)
Lactating sow	266	5 (1.9)	0 (0)	52	0 (0)	0 (0)	–	–	–
Replacement gilt	60	3 (5)	3 (5)	118	2 (1.7)	0 (0)	–	–	–
2–4 weeks old suckling piglet	1,975	89 (4.5)	61 (3.1)	8,911	97 (1.1)	78 (0.9)	9,906	313 (3.2)	0 (0)
6 weeks old nursery piglet	211	30 (14.2)	30 (14.2)	2,175	317 (14.6)	80 (3.7)	4,896	354 (7.2)	9 (0.2)
7–9 weeks old nursery piglet	139	24 (17.3)	22 (15.8)	966	163 (16.9)	68 (7)	392	84 (21.4)	0 (0)
10–12 weeks old nursery piglet	106	21 (19.8)	15 (14.2)	1,604	413 (25.7)	98 (6.1)	3,356	651 (19.4)	40 (1.2)
10–24 weeks old fattener	395	129 (32.7)	102 (25.8)	444	51 (11.5)	25 (5.6)	2,475	418 (16.9)	135 (5.5)
Total number of samples	3,152	301 (9.5)	233 (7.4)	1,4270	1,043 (7.3)	349 (2.4)	21,025	1,820 (8.7)	184 (0.9)

[*] testing with virotype PRRS RT-PCR assay.
[#] results with probe 'Wild P'.

"A" farm is a farrow-to-wean type, with 2000 sows. The sows were mass vaccinated twice at 1-month intervals with Porcilis MLV® vaccine. After vaccination, the herd was closed. In January 2019, 1/litter rRT-PCR was performed, followed by 3/litter Real-Time PCR and DIVA PCR from February 2019. In testing this colony, we wanted to know if two sow vaccinations would stop the transmission of wild virus to piglets.

Table 3: PCR and ELISA results of Farm "A" using one piglet per litter and 3 piglets per litter for diagnostic investigations.

The results of PRRS test of Farm "A"					
Period	Blood samples from 4-week-old-piglets	PRRSV PCR positive	Wildtype virus positive	Vaccine virus positive	ELISA positive (piglets)
1 /litter PCR	723 (241 litters)	0	0	0	56 (7,75%)
3/litter PCR, DIVA PCR	2508 (836 litters)	60 (20 litter, 2,39%)	3 (1 litter, 0,12%)	57 (19 litters, 2,27%)	87 (3,47%)

" B" farm is a farrow-to-finish herd with 870 sows. Mass vaccination of sows was carried out quarterly with Porcilis MLV® a vaccine. We also found samples in long-immunized flocks that were wild virus positive. In order to be sure that the weaned piglets had not received wild virus, 3 piglets/litter were tested (2% prevalence). The aim was to determine whether there were any wild virus shedding sows left in the flock using the DIVA PCR method.

Table 4: PCR and ELISA results of Farm B using 3 piglets per litter for diagnostic investigations from different age groups.

The results of PRRS test of Farm "B"					
Age group	Number of serum samples	PRRSV PCR positive	Wildtype virus positive	Vaccine virus positive	ELISA positive (piglets)
Weaned piglets 3/litter	3349 (1116 litters)	12 (4 litters, 0,36%)	3 (1 litter, 0,09%)	9 (3 litters, 0,27%)	2241° (7,75%)
35-day-old	1438	17 (1,18%)	1 (0,07%)	16 (1,11%)	848 (58,97%)
67-day-old	1510	79 (5,23%)	14 (0,93%)	65 (4,30%)	617* (43,36%)

° From 3349 serum samples, 2806 were tested with ELISA

* From the 1510 serum samples, 1423 were tested with ELISA

"C" farm is a farrow-to-slaughter type with 1400 sows. Sows were mass vaccinated quarterly with Porcilis MLV vaccine. Also on this colony, three piglets/litter were tested by DIVA PCR for the presence of PRRSV and positives were sequenced. In September 2018, all sows that tested positive for 3-week-old porcine wild-type PRRS wild-type virus were culled. We were able to ensure that the sows were not infecting their piglets at birth and could be reared PRRS wild-type free until they were selected.

Table 5: PCR and ELISA results of Farm C using 3 piglets per litter for diagnostic investigations before and after culling of PRRSV positive farrowed sows.

The results of PRRS test of Farm "C"				
Period	es from 3-wee	PRRSV PCR positive	Wildtype virus positive	Vaccine virus positive
Before culling wild-type PRRSV positive farrowed sows (2018)	8911 (2971 litters)	97 (1,09%)	78 (0,87%)	19 (0,22%)
After culling wild-type PRRSV positive farrowed sows (2019)	8466 (2822 litters)	141 (1,67%)	0 (0%)	141 (1,67%)

"D" farm is a farrow-to-slaughter type with 850 sows. The methodology of the eradication plan is to immunise the breeding herd quarterly, to cease immunisation of the progeny herd and to ensure infection-free rearing through internal disease control methods and laboratory testing. Starting in January 2020, 3 piglets per litter were tested by ELISA, PCR and DIVA PCR laboratory tests and positives were sequenced. The aim was to achieve PRRS-free, vaccinated status (MV) in the colony, with DIVA PCR testing to cull virus positive individuals regardless of age.

Table 6: PRRS laboratory analyses of serum samples origined of "D" farm from January 2020-August 2022

	Preweaned piglets (3 piglets/litter)						Nursery pigs						Slaughtered pigs					
YEAR	2020		2021		2022		2020		2021		2022		2020		2021		2022	
ELISA																		
total	4761		5049		3456		1930		3058		1977		1632		5277		3721	
negative	2913	61%	2763	55%	2532	73%	1803	93%	3027	99%	1977	100%	515	32%	4902	93%	3377	91%
positive	1848		2286		924		127		31		0		1117		375		344	
PCR																		
total	4761		5049		3456		1930		3058		1977		1632		5277		3721	
negative	4389	92%	5013	99%	3456	100%	1639	85%	3025	99%	1977	100%	1395	85%	5157	98%	3654	98%
positive	372		36		0		291		33		0		237		120		67	
DIVA PCR																		
total	372		36		0		291		33		0		237		120		67	
negative	9		0		0		6		6		0		0		0		0	
vaccina virus	357	96%	36	100%	0		279	96%	27	82%	0		237	100%	120	100%	67	
resident virus	6	1,6%	0		0		6	2,1%	0		0		0		0		0	

Discussion

Determination of the ORF5 and ORF7 sequences of PRRSV is essential to control and prevent the spread of PRRSV, as it allows the assessment of the risk of infection and a detailed analysis of the mechanism of PRRSV transmission. By visualizing the similarity of sequences and visualizing epidemiological relationships, the possibility for virological (molecular biological) analysis of epidemiological processes is significantly improved for practitioners (Szabó et al., 2020). Based on these results, Porcilis seems to maintain its stability, while variability was significantly higher for Unistrain and Reprocyc strains.

Unfortunately, commercially available ELISA and PCR tests cannot distinguish between MLV and farm-specific wild-type PRRSV. This is due to the high genetic variability and the fact that the spatial distribution of the virus, including the spatial distribution of the different subtypes, is highly variable. At present, discrimination can only be detected by sequencing, which is slow to perform in large numbers of samples and limited in the presence of wild-type and vaccine co-infections. Next generation sequencing is still rather expensive and, when using standard protocols, can detect minor variants in mixed infection with high confidence when the proportion is above 1% (Song et al., 2021). Therefore, there is a great need for a rapid, cheap and robust discriminative PCR test capable of detecting minor variant and can serve as an active element in farm diagnostic/monitoring systems. The method we have developed is a simple duplex discriminative TaqMan RT-PCR assay, which includes common forward and reverse primers and two different probes capable of detecting MLV and wild-type PRRS farm-specific virus. This test method can be an effective aid in the process of decontamination of a pig farm. In our work, we applied the DIVA RT-PCR method to all PRRSV positive samples from the colonies involved in the depopulation. The assay was able to detect both MLV and wild-type virus, and we were able to detect the farm-specific PRRSV strains with high reliability even when the proportion of minor virus strains was around 0.1%.

As a result of our study, we have demonstrated that the sampling method of litter numbers declared PRRSV-free is meaningful, as significantly more PRRSV positive litters were identified when three selected piglets/litters were tested. This means that if too few samples are tested, the status of the selected piglets may be misinterpreted. Using this sampling method and laboratory methods that rapidly detect the presence of wild-type virulent virus in the herd during the rearing period (DIVA PCR (Fornyos et al., 2022)), the presence of virus is significantly reduced by culling PCR positive litters and their sows. Finally, the above mentioned procedures were implemented in a farrow-to-slaughter type farm and successfully inhibited wild-type PRRSV infection in different age groups. Our results showed that the

application of these measures, together with strict biosecurity measures, allowed us to achieve PRRS-free status in the farrow-to-slaughter herds within two years using the DIVA RT-PCR method.

New scientific results

1) We were the first to obtain information on the genetic stability of vaccines used in PRRS vaccination in Hungary.

2) We were the first to develop a fast (1.5-2.5 hours), robust, cheap and efficient farm-specific DIVA Real-Time PCR method that allowed the processing of large sample volumes.

   a) The use of the DIVA PCR method has enabled farms to take prompt action before taking a technological step, reducing their economic losses by providing rapid, relevant results.

3) We were the first to perform discriminative RT-PCR measurements on a large number of samples in Hungary.

4) We were the first to use the DIVA PCR method in the National PRRS Eradication Program, which has greatly contributed to its success in the MLV vaccine-using herds.

5) DIVA PCR can be used in PRRSV positive farms to detect wild virus infection in each age group.

6) DIVA PCR is preventive for infected colonies to detect PRRSV.

   a) Discriminatory PCR helps to understand whether it is the vaccine or vaccination system itself and the herd's internal biosecurity measures that need to be changed or improved.

   b) In epidemiological situations where wild-type PRRSV infections occur in the herd in close proximity to PRRSV-free herds, the use of discriminative RT-PCR allows the use of MLVs in combination with strict diagnostic surveillance.

   c) It also helps to restore and maintain PRRS-free status and to phase out vaccination.

Related publication list of author

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