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RESEARCH ARTICLE



Development of a farm-specific real-time quantitative RT-PCR assay for the detection and discrimination of wild-type porcine reproductive respiratory syndrome virus and the vaccine strain in a farm under eradication

KINGA FORNYOS¹, ISTVÁN SZABÓ²,
KÁROLY LEBHARDT¹ and ÁDÁM BÁLINT^{3*} 

¹ Eurofins Vet-Controll Ltd., Budapest, Hungary

² National PRRS Eradication Committee, Budapest, Hungary

³ Department of Virology, National Food Chain Safety Office Veterinary Diagnostic Directorate, Tábornok u. 2, H-1143, Budapest, Hungary

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ABSTRACT

Porcine reproductive and respiratory syndrome (PRRS) is one of the most important diseases of swine causing severe economic losses worldwide, therefore intensive efforts are taken to eliminate PRRS virus (PRRSV) from infected herds for complete eradication. The most efficient, fastest but at the same time the most expensive eradication method is depopulation-repopulation. In order to reduce costs, a number of farms prefer to perform their eradication process with continuous production using modified live vaccine (MLV) immunisation. However, the commercial PRRSV RT-PCR kits do not have the capacity to discriminate infected from vaccinated animals. In this paper, we describe a simple discriminatory duplex TaqMan RT-PCR assay based on common forward and reverse primers, as well as two differently labelled MLV- and wild-type PRRSV-specific probes. The discriminatory PCR test we designed is a fast and efficacious method for processing large quantities of samples. The assay is cheap, flexible, easy to apply in different herds using different MLVs, but should be checked, and can be modified based on the sequence data obtained during the permanent monitoring examinations. Owing to its simplicity the test can serve as a significant complementary assay for PRRS control and elimination/eradication.

KEYWORDS

PRRS, eradication, discriminatory, PCR

INTRODUCTION

Porcine reproductive and respiratory syndrome (PRRS) is one of the most important diseases of swine causing severe economic losses worldwide (Holtkamp et al., 2013; Renken et al., 2021). The disease is caused by PRRS virus (PRRSV) that is an enveloped, single-stranded RNA virus of the *Betaarterivirus* genus within the *Arteriviridae* family, a member of the order *Nidovirales* (Brinton et al., 2018). The PRRSV genome is approximately 15 kilobases (kb) in length that contains 11 overlapping open reading frames (ORFs). ORFs 1a and 1b comprise 80% of the genome, and encode non-structural proteins responsible for transcription, replication and immunomodulation. Eight ORFs (2a, 2b, 3–7, and 5a) encode viral structural proteins (Kappes and Faaborg, 2015). Based on their 30–45% genetic distance (Nelsen et al., 1999),

*Corresponding author. Tel.: +36 30 984 51 02.
E-mail: balintad@nebih.gov.hu

PRRSV strains can be classified into two distinct species *Betaarterivirus suid-1* (PRRSV-1) and *Betaarterivirus suid-2* (PRRSV-2) (Brinton et al., 2018).

The clinical signs of the disease range from reduction in the reproductive performance of sows to respiratory diseases in young piglets. The immunosuppressive effect of PRRSV in combination with other pathogens within the herd significantly increases morbidity and mortality (Rossow, 1998).

Specific treatments for PRRS are not available. Control of PRRSV can be achieved by limiting the adverse effects of the virus in various stages of production using vaccines and biosecurity measures, but the most effective method of elimination of PRRSV in infected herds is complete eradication. In the US, the American Association of Swine Veterinarians take on the leading role in the process of PRRSV eradication (Vansickle, 2015). In Europe, four countries are free from PRRSV (Norway, Sweden, Finland and Switzerland), and a local eradication programme started in Denmark (Rathkjen and Dall, 2017) and the Netherlands (Nodelijk et al., 2003), while in Scotland it was extended to national level in 2018 (Scottish Agricultural Organisation Society, 2018).

In Hungary, the prevalence of PRRSV infection and the economic losses led to the launching of a national PRRSV Eradication Programme in 2014 that was based on territorial principles and was accepted by the competent veterinary health committee of the EU. As a result of the implemented program introduced in 2014, the swine population of 13 out of 19 counties of Hungary became officially free from PRRSV by the end of 2020 (Nemes et al., 2019; Szabó et al., 2019, 2020). The programme is expected to be finished in 2022.

Successful eradication in areas with high pig density requires a complex approach, assessment and design (Alvarez et al., 2016). Among the eradication methods, the most efficient, fastest but at the same time the most expensive is depopulation-repopulation (Nathues et al., 2018), and this method leads to a long-term loss of production. In contrast, the significantly less expensive load, close, homogenise (LCH) method contributes to stabilising the PRRSV status of the breeding herd by vaccination before the introduction of new PRRSV-negative animals (Murtaugh and Genzow, 2011; Linhares et al., 2015). However, a number of farms intend to perform their eradication process with continuous production without herd closure. Here, homogenous PRRS herd immunity is achieved and maintained using vaccination (Linhares et al., 2015), but strict biosecurity measures are required in order to block virus transmission within the herd (McCaw, 1995). For breeding herd stabilisation, mostly live attenuated vaccines (MLV) are used in Europe. These include Porcilis PRRS (MSD, DV strain), Amervac/Uni-strain (Hipra, VP-046 BIS strain), ReproCyc PRRS EU/PRRS FLEX EU (Boehringer Ingelheim, 94881 strain) and Suvaxyn PRRS MLV (Zoetis, 96V198 strain). Further data on the application of the above vaccines in the Hungarian PRRS eradication programme have been published recently (Bálint et al., 2021).

MLVs induce a strong immune response (Balka et al., 2016; Berton et al., 2017; Nathues et al., 2018), and MLV vaccination results in the improvement of production data (Cano et al., 2016). However, MLVs have several safety and efficacy shortcomings, like spreading of MLVs within a herd, reversion to virulence, recombination with wild-type strains and ineffective protection against heterologous strains (Nan et al., 2017; Kvisgaard et al., 2020). Furthermore, differentiation of infected from vaccinated animals can be achieved only by molecular biological methods.

According to the PRRS classification of swine herds (Holtkamp et al., 2011), for breeding herd stability, 30 piglet sera have to be tested by PCR (based on 10% prevalence and 95% confidence, representing one piglet/litter) four times at 30-day intervals.

PRRSV is extremely diverse in Europe (Balka et al., 2018) and in Hungary (Szabó et al., 2020); therefore, it is very important to regularly update the commercial PRRSV RT-PCR kits. Furthermore, these kits do not have discriminatory capacity, they cannot differentiate the herd-specific wild virus from the applied MLV strain (Toplak et al., 2012). Therefore, subsequent classical PCR and sequencing are required for discrimination. These additional tests are expensive, and significantly prolong the duration of the exact diagnostics.

The aim of the present study was to develop a cheap, fast, sensitive and robust discriminatory RT-PCR method, which is able to discriminate between the wild-type herd-specific and the applied MLV strain, and can be included in the diagnostic regime of the herd under eradication.

MATERIALS AND METHODS

Properties of the investigated herd

A farrow-to-finish farm of 1,400 sows got infected with PRRSV in 2003. Since then, immunisation has been continuously performed using live and/or inactivated (KV) PRRS vaccines based on different vaccination practices (mass vaccination of the whole herd, later 6/60 programme with MLV and application of KV at the 90th day of gestation). From 2017, only the breeding animals in the herd were vaccinated.

Viruses and RNA extraction

Of the live vaccines, only the Porcilis MLV[®] was used. The herd was subjected to regular (weekly) laboratory monitoring to detect the spread of PRRS virus within the herd. Several vaccine and wild-type viruses were identified from serum and organ samples, and their ORF5 genes were sequenced. The ORF5 of the MLVs were identical with the original Porcilis PRRS MLV (GenBank acc. no.: AY743931; data not shown). In contrast, three minor genetic variants of the wild-type PRRSV were identified, showing 92.6%, 93.9% and 96.6% nucleotide identity, respectively (Fig. 1).

The RNA was extracted using the QIAmp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The RNA was stored at –80 °C until used.



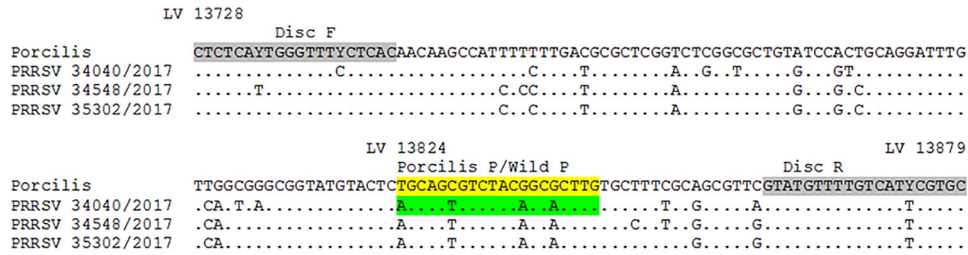


Fig. 1. Positions of the primers and the probe used for the assay. The sequences are shown in sense orientation. The reference sequence is Porcilis MLV. The grey shading indicates consensus forward and reverse primer sequences, while the yellow and green shading refers to Porcilis MLV- and wild-type-specific probes, respectively

Primer and probe design

Sequence alignments of the MLV and vaccine strains identified in the farm were performed by the Molecular Evolutionary Genetics Analysis version 6.0 (MEGA 6.0) software (Tamura et al., 2013). Primers and probes were designed with Primer3 software (Untergasser et al., 2012). The targeted region was the ORF5 gene, a highly variable region of the PRRSV genome. The forward and reverse primers were designed to match both the vaccine and wild-type PRRSV sequences. In contrast, two different TaqMan probes were designed; each matches either the vaccine or the wild-type virus sequences, respectively. The two probes differed by four nucleotides in order to prevent annealing to the non-target sequence. The vaccine-specific probe was labelled with FAM, while the wild-type PRRSV-specific probe contained HEX as fluorophore dye. Sequences of the primers and the probes are shown in Table 1.

Discriminatory TaqMan RT-PCR assay

Reaction parameters (primer, probe concentration and annealing temperature) were optimised in order to achieve low cycle threshold (Ct) values and high fluorescence signal. Reactions were run in a Rotor-Gene Q Real-Time Amplification instrument (RG-6000, Qiagen), with a total reaction volume of 25 µL, under the following conditions: reverse transcription at 50 °C for 30 min, followed by 95 °C for 15 min, and 40 cycles of 95 °C for 15 s, 50 °C for 20 s and 72 °C for 30 s. Fluorescence signal was detected during the annealing step after each cycle on the green and yellow channels.

Table 1. Sequences of the primers and the probe used in the study. The position of primers and probes is indicated in Fig. 1

Name	Orientation	Sequence, 5'–3'
Disc F	Genomic	CTCTCAYTGGGTTTCTCAC
Disc R	Reverse	GCACGRATGACAAAACATAC
Porcilis P	Genomic	FAM-TGCAGCGTCTACGGCGCT TG-BHQ1
Wild P	Genomic	HEX- AGCAGTGTCTACAGCACT TG-BHQ1
ORF5 F	Genomic	TAATACGACTCACTATAGGGA- GTTGCTSCATTTCMTGACAC ^a

^a Nucleotides shown in bold letters indicate the specific T7 promoter sequence added to the forward primer.

Sensitivity and specificity of the assay

The sensitivity of the test was assessed using exact amounts of recombinant RNA prepared from the Porcilis MLV[®] and the farm-specific wild-type virus PRRSV 30040/2017 NEBIH. Briefly: conventional RT-PCR was performed with ORF5 specific primers (Balka et al., 2008), where a specific T7 promoter sequence was added to the 5' end of the forward primer (Table 1). The amplicons were gel purified using the QiaQuick Gel Extraction kit (Qiagen, Hilden, Germany). Purified DNA was transcribed to RNA using MEGAscript[®] T7 Kit (Ambion, Austin, TX, USA). A Nanodrop ND 1000 instrument (Wilmington, USA) was used to determine RNA concentrations of the undiluted samples. Copy number was calculated by Avogadro's formula. Tenfold dilutions were prepared from each sample ranging from a 10¹⁰ to 10⁰ RNA copy number/µl in RNase-free water. The PCR efficiency was determined by using the E = 10(–1/s) – 1 formula (E: efficiency, s: slope). The results of the test of the tenfold RNA dilutions were used to calculate the standard curve. A selection of swine pathogens [porcine circovirus type 2 (PCV2), swine influenza virus (SIV) H1N1 and H3N2, African swine fever virus (ASFV), classical swine fever virus (CSFV), porcine respiratory coronavirus (PRCV), Aujeszky's disease virus (ADV), porcine parvovirus (PPV) and porcine cytomegalovirus (PCMV)] were also tested in order to exclude cross-reactivity of the PRRSV discriminatory RT-PCR assay.

Detection of mixed infections

In herds using MLV vaccination for the control or elimination/eradication of PRRS, mixed infections may occur, since both the vaccine and wild-type PRRSV can be carried and shed by the infected animals for a long time, especially in young animals. In order to evaluate the capacity of the discriminatory RT-PCR test to detect both the Porcilis MLV and the herd-specific wild-type PRRSV, the two dilution series above were cross-titrated with variable copy numbers ranging from 10¹ to 10⁸/µl. In the second step, the same dilution series were mixed with a constant copy number of the other virus containing 10⁴/µl copy number. These mixes were subjected to the discriminatory PCR assay, and sensitivity was compared with those of the assays using a single PRRSV as template.



Field samples

The farm under eradication applied strict monitoring regimes. Between 2017 and 2019, 38,447 samples were assayed by the commercially available virotype PRRSV RT-PCR Kit (Qiagen) on a regular (weekly, monthly) basis representing different age groups. All PCR-positive samples were subjected the discriminatory TaqMan assay in order to confirm or exclude the presence of Porcilis MLV or the herd-specific PRRSV. The results of the discriminatory assay were compared with those of the commercially available test, and were confirmed by sequencing and analysing the subsequent PRRSV status of the examined age groups. The sows, having positive results among their 2–4 weeks old preweaning piglets with discriminatory herd-specific PRRSV, were culled.

RESULTS

Primer and probe design

The middle part of ORF5 was found to be suitable for primer and probe design for the PRRSV discriminatory TaqMan RT-PCR assay. The forward and the reverse primers contained two and one degenerated nucleotides, respectively, to allow the attachment on both the Porcilis MLV and the three minor variants of the herd-specific wild-type PRRSVs. The probes were designed based on a sequence stretch containing four mismatches between the Porcilis MLV and wild-type PRRSV in order to exclude the possibility of cross-reaction. The amplicon was 152 nt long (calculated on the Lelystad virus, accession number: AY588319). The positions of the primers and the probes are shown on an alignment of the Porcilis MLV and the three herd-specific PRRSVs in Fig. 1.

Optimisation of the discriminatory TaqMan RT-PCR assay

During the optimisation of the system, our goal was to achieve low Ct values and the highest fluorescence signal using fixed template level (10^4 copy numbers). In the first step, a

combination of four concentrations (100–600 nM) of both forward and reverse primers with a constant (400 nM) probe concentration was determined. The combination showing the highest fluorescence was tested thereafter at four different TaqMan probe concentrations (100–400 nM). The optimal concentrations proved to be a combination of 600 nM for primers and 400 nM for probes. The annealing temperature was also determined, and 50 °C was used in further experiments.

Sensitivity and specificity of the assay

The sensitivity of the system was determined by using exact copy numbers of recombinant viral RNA obtained from both the Porcilis MLV strain and the farm-specific wild-type virus PRRSV 30040/2017 NEBIH. The detection limit was ten copies of viral RNA in the reaction mixture, and the assay allowed linear detection in the range of 10^1 – 10^7 RNA copies/reaction. The standard curves were obtained with the Porcilis MLV and PRRSV 30040/2017 NEBIH strains, respectively. All reactions were performed in triplicates, and the mean data of cycle threshold (Ct) values were used to design the standard curves. The PCR efficiency (E) was 1, while the correlation efficient (R^2) was 0.99 in both cases (Fig. 2). Comparison of the sensitivity of the assay with that of the commercially available virotype PRRSV RT-PCR Kit (Qiagen) revealed 1–3 Ct differences depending on the template concentration, in favour of the commercial kit.

The specificity of the test was evaluated using the serial standard dilutions of Porcilis MLV and PRRSV 30040/2017 NEBIH strains, respectively. No cross-reactivity was obtained, even with 10^{10} copies/reaction, and the same phenomenon was observed in reactions performed with other (non-PRRSV) viruses (data not shown).

Detection of mixed infections

For the detection of mixed infections, serial dilutions of Porcilis MLV and PRRSV 30040/2017 NEBIH RNA were combined. When variable amounts of the two RNA samples were cross-titrated, it was observed that the discriminatory PCR can detect both viruses if the copy number ratio of the

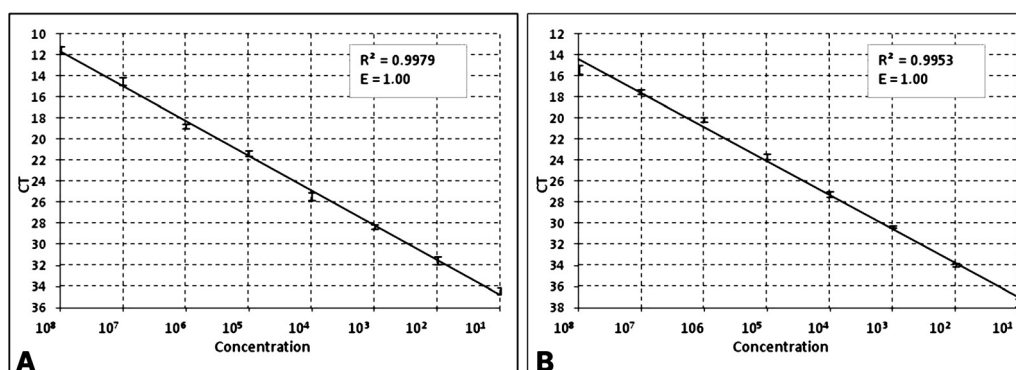


Fig. 2. Standard curves generated using 10-fold dilutions of template RNA prepared from the Porcilis MLV (A) and PRRSV 30040/2017 NEBIH (B) strain. Three replicates were performed of each dilution. Average values and standard deviations are shown. RNA copy number is indicated on the x-axis, while Ct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

Table 2. Potency of the discriminatory PCR to detect mixed infections using different concentrations 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.

minor variant is above 0.1% (Table 2). In the next step, variable amounts of either the Porcilis MLV or PRRSV 30040/2017 NEBIH RNA were mixed with a fixed $10^4/\mu\text{l}$ copy number RNA of their counterparts. Here, the same tendency was observed as above; however, if the copy number of the minor variant strain was at the detection limit (10^1 RNA copies), the 0.1% copy number ratio did not allow the detection of this PRRSV (Table 2).

Field samples

In the farm under eradication, 3,164 out of the 38,447 serum samples proved to be positive by the virotype PRRSV RT-PCR Kit. These samples were subjected to the discriminatory RT-PCR assay between 2017 and 2019 (Table 3). The results of the discriminatory RT-PCR assay were always confirmed by sequencing if a single infection occurred, and the Ct value was <32. In the case of mixed infections, if the Ct values showed <1 \log_{10} titre difference, either the MLV or the wild-type PRRSV virus was detected by sequencing. In contrast, if the ratio was >2 \log_{10} , only the virus with the higher titre was detected by the Sanger method.

During 2017 and 2018, each sow showing positive herd-specific wild-type PRRSV discriminatory PCR result of her 2–4 weeks old piglets, was culled together with the litter. As a result of these measures, herd-specific wild-type PRRSV discriminatory PCR positivity gradually decreased (from 3.1% in 2017 to 0.9% in 2018), and disappeared in suckling piglets by 2019 (Table 3). Subsequent diagnostic monitoring of the PRRS-negative and only MLV-positive animals never revealed the presence of the wild-type virus. The overall wild-type PRRSV positivity in all age groups also decreased from 7.4% (2017) to 0.9% (2019). In contrast, MLV PCR positivity increased from 2.1% (2017) to 7.8% (2019).

DISCUSSION

PRRS control in endemically infected herds is performed mostly by the application of MLVs, since the most effective complete depopulation-repopulation method is not acceptable for a significant segment of pig producers (Holtkamp et al., 2011). In line with the above, in 2017, the World Organization for Animal Health (OIE) introduced a new definition: animals harbouring only PRRSV vaccine strains are not designated as PRRSV infected (OIE, 2017).

Accordingly, the Hungarian PRRSV legislation subsequently introduced the concept and category of vaccinated

free (VF) swine herds, the prerequisite of complete PRRSV-free status (Szabó et al., 2020), in which only the breeding stocks can be immunised without any time limit, and the progeny must be proven free from PRRSV by laboratory methods (ELISA, PCR) in any age group.

Regular virological and serological monitoring is a crucial part of the eradication programme in these farms; however, commercial ELISA and PCR tests cannot differentiate between MLV and the herd-specific wild-type PRRSV. The reason for this is the high genetic diversity of the virus and the fact that the spatial distribution of the different subtypes is very diverse. Currently, discrimination can be performed only by sequencing, which is uneconomical and slow in the case of a large number of samples, and the co-existence of wild-type/vaccine viruses can only be detected to a limited extent. Next-generation sequencing is still rather expensive, and using general protocols in the case of mixed infection, it can detect the minor variant with high confidence if its ratio is above 1% (Song et al., 2021). Therefore, there is a high need for a fast, cheap and robust discriminatory PCR assay that can detect the minor variant in a broader ratio range, and serve as an active element of the diagnostic/monitoring regime of the farms.

In this paper, we describe a farm-specific simple duplex discriminatory TaqMan RT-PCR assay based on common forward and reverse primers, as well as two differently labelled MLV- and wild-type PRRSV-specific probes. This assay could effectively help the eradication process of a given farrow-to-finish pig farm. Preceding the discriminatory PCR design, thorough monitoring of PRRSVs (wild-type and MLV) on the farm had been applied, and during the study period, weekly sequencing of the available clinical samples was carried out in order to detect any changes in the ORF5 region of the circulating PRRSV strains.

The sensitivity of the assay is comparable to those of the commercially available PRRSV RT-PCR kits using synthetic RNA samples. This fact was further confirmed by diagnostic monitoring of the herd at regular intervals from 2017 to 2019, processing 38,447 samples. The specificity of the assay also fulfils the needs of farm diagnostics, since no other swine pathogens were detected, and no cross-reactivity between the wild-type and MLV strains was observed.

The assay was able to detect both the MLV and the wild-type farm-specific PRRSV strains with high confidence even if the ratio of the minor variant is around 0.1%. Detecting both viruses within this ratio range is acceptable for field diagnostics, since it is very unlikely that the farm-specific



Table 3. Age group and year distribution of field samples examined by the discriminatory RT-PCR test in the course of the eradication programme of the farm

Age group	2017			2018			2019		
	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’.



wild-type PRRSV is overgrown by the attenuated, therefore slower replicating MLV strains (Martínez-Lobo et al., 2013) leading to a ratio lower than 0.1%. However, since the discriminatory RT-PCR assay was applied only in farms using Porcilis MLV, further data are needed to assess its application in farms using other MLVs.

After introduction of the discriminatory assay together with the thorough vaccination regime and control measures, the number of wild-type PRRSV-positive animals significantly decreased in all age groups, and the virus disappeared from suckling piglets. As a consequence, no further wild-type PRRSV variants were identified in the herd.

The discriminatory PCR method provides several opportunities compared with the general PRRSV PCR assays.

1. It helps evaluate whether the wild-type virus persists, and if so, in which age group(s) in herds using long-term MLV vaccination. Based on these results, a herd-tailored programme can be developed for the control or eradication of PRRS.
2. Several pig producers believe that the applied MLV is not efficacious, and the only solution for better results is changing for the product of a different vaccine company, which may lead to recombination with serious consequences (Kvisgaard et al., 2020). The discriminatory PCR helps understand whether the vaccine itself, or the vaccination regime and the inner biosecurity measures have to be changed and improved. Detection of recombinant PRRSV strains is a challenge for the discriminatory PCR, but in case of general PCR positivity and discriminatory PCR negativity, subsequent ORF5 sequencing can prove recombination events in this genomic region.
3. PRRSV is a major causative agent of the porcine respiratory disease complex (PRDC). Applying the discriminatory PCR in the breeding herd, wild-type PRRSV-free offspring can be produced, i.e., PRRSV-negative fatteners can be developed, contributing to more economical mass production.
4. In epidemiological situations when wild-type PRRSV infections occur in the close proximity of a PRRSV-free herd, performing the discriminatory PCR enables the application of MLVs together with strict diagnostic monitoring. It also helps restoring PRRS-free status with the gradual cessation of vaccination.
5. The assay is a fast (can be completed within 1.5–2.5 h) and efficacious method for processing large quantities of samples. Together with the commercially available PRRSV detection kits it enables the general detection and discrimination of PRRSV within 24 h. Farms under eradication, due to their technology (keeping breeding animals, farrowing, weaning, fattening), have a strict timeline, therefore it is essential to obtain quick and relevant results between each technological step.
6. The discriminatory PCR system can be applied as a control tool in any swine herd in Europe using different vaccines and having different wild-type PRRSV, since its components can easily be modified according to the available sequence data.

In summary, the presented discriminatory TaqMan RT-PCR assay is cheap, flexible, easy to apply in different herds using different MLVs, and can be modified based on the sequence data obtained during the permanent monitoring examinations. Based on its simplicity the test can be applied even in basic diagnostic laboratories, and can serve as a significant complementary assay for PRRS control and elimination/eradication.

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