



NUCLEIC ACID EXTRACTION AND MOLECULAR DETECTION
OF CLASSICAL SWINE FEVER VIRUS, PORCINE REPRODUCTIVE
AND RESPIRATORY SYNDROME VIRUS, PORCINE EPIDEMIC
DIARRHOEA VIRUS, AND PORCINE CIRCOVIRUS TYPE 2
IN FAECAL SAMPLES

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Summary

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The goal of this study was to compare the efficiency of three co-extraction methods, namely the QIAamp cadof Pathogen Mini Kit (Q), the Viral Gene-spin™ Viral DNA/RNA Extraction Kit (G), and the E.Z.N.A. Universal Pathogen Kit (E), for isolating DNA and RNA from 92 pooled faecal samples. TaqMan tests, which were to detect classical swine fever (CSF), porcine reproductive and respiratory syndrome (PRRS), porcine epidemic diarrhoea (PED), and porcine circovirus type 2 (PCV2) viruses were used to evaluate the extraction efficiency of the three kits. The Q kit performed the best extraction, which indicated the highest average yield, elution volume, and purity in both DNA and RNA. No PCR inhibitors were detected. The Q and G kits were able to yield detectable PEDV RNA products and PCV2 DNA products in four dilutions. The number of the positive samples of the Q and G kits was higher than those of the E kit. No significant differences in the C_q values were detected between the extraction kits. In addition, the TaqMan assays successfully detected these four viruses in field samples and seemed suitable for the use of epidemiological surveillance. Further studies on a large number of positive samples, more available kits for extraction, and other types of samples should be performed.

Key words: extraction kits, classical swine fever virus, porcine circovirus type 2, porcine epidemic diarrhoea virus, porcine reproductive and respiratory syndrome virus

INTRODUCTION

In the pig industry, a number of swine viral diseases are a constant threat. Some diseases can be eliminated or eradicated, while others cause sporadic or epidemic

outbreaks in the affected countries (Kedkovid *et al.*, 2020). Classical swine fever virus (CSFV), porcine reproductive and respiratory virus (PRRSV), porcine epi-

demetic diarrhoea virus (PEDV), and porcine circovirus type 2 (PCV2), which are the important disease causative agents account for huge economic losses in Vietnam. The disease status becomes complicated when a highly pathogenic PRRSV (HP-PRRSV) strain has emerged and re-emerged in Vietnam. The virus had spread to almost all regions of the country by 2010 (Huong Giang *et al.*, 2016) and had undergone rapid evolutionary changes compared with Vietnamese HP-PRRSV isolates before (Do *et al.*, 2016; Li *et al.*, 2024). PED's first outbreak was reported in Vietnam in 2008 and then occurred again in 2013. The disease caused substantial economic costs (Diep *et al.*, 2018; Nguyen *et al.*, 2023). The evidence suggests that PCV2 is endemic in the pig population in Vietnam (Lee *et al.*, 2020; Lai *et al.*, 2024), where 70–75% of the total pig production is on small farms without PCV vaccinations (Lee *et al.*, 2020). Live attenuated vaccines have been widely used on pigs, resulting in a dramatic decrease in CSF incidences in Vietnam. Sporadic cases have frequently occurred across the country (Choe *et al.*, 2020; Izzati *et al.*, 2021; Nguyen *et al.*, 2021).

Routine surveillance of CSF, PRRS, PED, and PCV2 requires a combination of sample collecting and laboratory testing. Nasal swabs, oral fluids, and faecal samples have been used to detect CSFV, PRRSV, PEDV, and PCV2 by molecular techniques (Plut *et al.*, 2020; Turlewicz-Podbielska *et al.*, 2020; Liu *et al.*, 2021; Robert *et al.*, 2024). Among them, faecal collection is the simplest and easiest to handle; it can be done at any time by the animal caretakers or on an as-needed basis (Gerber *et al.*, 2015). Real-time PCR (qPCR) has been widely applied in molecular diagnostics because of its enormous sensitivity and specificity. Diagno-

sis of viral infection from stool suspensions by real-time PCR is increasingly replacing diagnosis based on viral isolation and identification in tissue culture. However, faecal samples were among the most difficult clinical samples to process because the potential inhibitors presented in nucleic acid amplification, which reduced diagnostic sensitivity (Acharya *et al.*, 2017). Numerous studies have been conducted to analyse the effectiveness of the commercial kits for the extraction of RNA or DNA (Skrzypek *et al.*, 2020; Tesfamichael *et al.*, 2020; Chaves *et al.*, 2024) or both RNA and DNA (Bodewes *et al.*, 2014; Paskey *et al.*, 2019; Sabatier *et al.*, 2020). However, there is a wide selection of kits for RNA or DNA extraction compared to a much smaller selection of kits for extraction of both DNA and RNA (Lever *et al.*, 2015). Co-extraction of DNA and RNA from the faecal samples will provide a significant advantage in terms of simplifying and reducing time-consuming and labour-intensive detection process, and saving the cost of the diagnosis. Therefore, selecting a co-extraction method for the simultaneous isolation of high-quality and high-yield DNA and RNA from the faecal samples to detect different viruses is a practical and meaningful goal.

In strict epidemiological surveillance, it is extremely important to perform fast, reliable, and cost-effective methods for detecting emerging viruses in field samples. The goal of this study was to compare the efficiency of three commercial DNA/RNA co-extraction kits and to evaluate them in detecting PRRSV, PEDV, CSFV, and PCV2 from the faecal samples for epidemiologic studies. The extraction systems were the QIAamp cadof Pathogen Mini Kit (QIAGEN Inc., Valencia, CA, USA), Viral Gene-spin™ Viral DNA/RNA Extraction Kit (iNtRON Bio-

technology, Inc., Korea), and E.Z.N.A. Universal Pathogen Kit (Omega Bio-tek, Inc., USA). All of the systems employed manual and dual nucleic acid extractions using silica columns. Average yield, elution volume, and purity in both DNA and RNA, as well as the presence of the potential PCR inhibitors after extraction by three kits, were analysed.

MATERIALS AND METHODS

Faecal sample collection

During the period between July 2018 and December 2018, a total of 460 faecal samples were collected from apparently healthy pigs in pig-raising households in six selected provinces of northern Vietnam (YenBai, ThaiNguyen, PhuTho, QuangNinh, HaNoi, ThaiBinh). Each fresh faecal sample was collected from an individual pig by a Sub-Department of Livestock and Animal Health staff under the guidelines of the National Technical Regulation on Animal Diseases—General requirements for sample collection, storage, and shipment (QCVN01-83:2011/BNNPTNT). The samples without preservatives were placed in the sterile sample tubes and were frozen at -20°C until shipped to the laboratory within 24 hours. Five faecal samples were pooled by mixing 1 gram of each sample reaching at a final weight of 5 grams. A small portion of the pooled sample was vortexed for 15 seconds in 0.9% saline with glass beads. Stool suspension was clarified by centrifugation at $2,500\times g$ for 10 min and then frozen at -20°C for further molecular analysis.

Nucleic acid extraction and quantification

The nucleic acids were extracted from the faecal suspensions using the three differ-

ent commercial kits, which were the QIAamp cadzor Pathogen Mini Kit (QIAGEN Inc., Valencia, CA, USA), the Viral Gene-spinTM Viral DNA/RNA Extraction Kit (iNtRON Biotechnology, Inc., Korea), and the E.Z.N.A.[®] Universal Pathogen Kit (Omega Bio-tek, Inc., USA). In this study, the acronym names of three kits above were given by the letters of Q, G, and E, respectively. The starting volumes of the faecal materials and the elution volumes of the DNA and RNA solutions were 200 μL and 150 μL for the kit Q; 150 μL and 60 μL for the kit G and 250 μL and 100 μL for the kit E according to the manufacturer's recommendations. Finally, the DNA and RNA yields were quantified on a NanoDropTM 2000 UV-VIS Spectrophotometer (Thermo Scientific; Model: NanoDrop 2000; Serial No. S691; U.S.A.). The measure of purity in nucleic acid extractions was assessed by the A260/280 and A260/230 purity ratios, which ranged between 1.8 and 2.0 and 2.0 and 2.2 for DNA and RNA, respectively.

TaqMan real-time PCR assays

Commercially available qPCR and RT-qPCR test kits from the manufacturer (Primerdesign Ltd., Chandler's Ford, United Kingdom), which were genesig Std real-time PCR detection kit for CSFV, genesig Std real-time PCR detection kit for PRRSV for European and North American strains, genesig Std real-time PCR detection kit for PEDV, and genesig Std real-time PCR detection kit for PCV2, were performed on DNA and RNA extracts. The assays employed TaqMan probes, and the specific gene targets were proprietary and not available to the public. The reaction mixture for each qPCR or RT-qPCR assay was carried out in the total volume of 20 μL consisting of 4 μL of RNase/Dnase-free water, 10 μL of 150

test oasig lyophilised OneStep RT-qPCR MasterMix (Primerdesign, Z-oasig-one-step-150, United Kingdom) or 150 test oasig lyophilised 2×qPCR Master Mix (Primerdesign, Z-oasig-standard-150, United Kingdom), 1 µL of primer/probe mix, and 5 µL of DNA or RNA extracts. The thermal cycling profile for the qPCR amplification was an initial step at 95 °C for 2 min, followed by 50 cycles of 95 °C for 10 s and 60 °C for 60 s. The one-step RT-qPCR amplification protocol was 55 °C for 10 min, 95 °C for 2 min, followed by 50 cycles as the PCR. All reactions were performed on the AriaMx real-time PCR system (Agilent Technologies; Model: AriaMx; Catalog code: G8830A; Serial No. MY15445271; Malaysia). An assay was considered valid if the cycle threshold (Cq) value of the positive control was expected to amplify between 16 and 23, and the negative control was 0. A sample was considered positive if a Cq value was observed ≤ 35. If a sample produced a Cq value > 35, the sample was re-extracted and re-tested.

Analysis of positive samples by Cq values, presence of PCR inhibitors, and sensitive detection of targeted viruses

PCR efficiency depends on the concentration of the target. More copies of the target in the input of the reaction, fewer cycles of amplification are needed (Ruiz-Villalba *et al.*, 2021). The Cq value of an amplification reaction is the fractional number of cycles. The target with the highest efficiency will have the lowest Cq value. To analyse the efficiency of detection of CSFV, PRRSV, PEDV, and PCV2 in extracted faecal samples by three kits, the Cq values were compared.

The faecal samples with a low viral titre may be reported falsely as negative due to the potential PCR inhibition. The test sensitivity could be enhanced by re-

lief of the PCR inhibition, achieved by dilutions of the nucleic acid extract. The potential inhibitory effects may be reduced or totally removed (Schrader *et al.*, 2012; Acharya *et al.*, 2017; Cao *et al.*, 2021). The DNA or RNA extracts derived from faecal samples were tested, either undiluted or diluted, and the qPCR and RT-qPCR results of CSFV, PRRSV, PEDV, and PCV2 detection were compared. If the inhibitors were present, dilution would decrease their concentration in the PCR reaction, resulting in improved amplification. The Cq values of the diluted extracts were lower than those of the undiluted extracts.

The sensitive detection of targeted viruses was carried out by ten-fold serial dilution of nucleic acid extracts from PCV2 and PEDV-positive faecal samples collected from pigs with clinical signs, using nuclease-free water. Positive samples with low PRRSV or CSFV nucleic acid extracts were excluded from further dilution. Each dilution was run in five replicates. The number of positive results for each dilution and Cq values for each replicate were recorded. The highest dilutions that showed three times positive in the qPCR and RT-qPCR, indicated the detection limit of the respective assay.

Statistical analysis

Each experiment was repeated at least three times. Data were expressed as the mean ± S.D. The differences between the means were determined using the two-tailed t-test. Data were analysed using the t-test paired two samples for means with an alpha value of 0.05.

Statistical significance was defined as P values less than 0.05.

RESULTS

Nucleic acid yield and purity

DNA/RNA yield, elution volume, and quality extracted using the three extraction procedures, which were the Q, G, and E kits, were compared. The manufacturers recommended volumes for the faecal samples that could be used differently for three procedures. Starting volumes of faecal samples used in this study were 200 μL for the Q kit, 150 μL for the G kit, and 250 μL for the E kit, respectively. Among the three kits, the average yields of the DNA showed no significant difference ($P>0.05$). The average yields of the RNA showed a significant difference ($P<0.05$). In general, the Q kit had the highest average nucleic acid yields, which were 11.91 ± 2.36 ng/ μL for DNA and 10.15 ± 1.92 ng/ μL for RNA, respectively. The extraction with the E kit resulted in the lowest average yield of the DNA and RNA extractions, which were 10.93 ± 3.62 ng/ μL for DNA and 7.99 ± 2.03 ng/ μL for RNA, respectively (Table 1). To add, there was a significant difference between the results. The elution volumes were highest for the Q kit (150 μL) and lowest for the G kit (60 μL) (manufacturer's recommendation). According to the Nanodrop results, the Q and G kits were found to yield almost purity in both DNA and RNA extractions

when the average values of the A 260 nm/A 280 nm ratios were 1.79 ± 0.13 and 1.83 ± 0.16 for DNA and 1.99 ± 0.07 and 2.07 ± 0.17 for RNA, respectively. The A 260 nm/A 280 nm observation ratios of 1.38 ± 0.24 and 2.10 ± 0.15 for the E kit indicated low DNA/RNA purity (Table 1).

Evaluation of dual DNA and RNA extraction methods by qPCR and RT-qPCR

The qualitative analysis of the positive samples' Cq values was presented in Table 2. At the original concentration, the Cq values of the Q kit, which were 26.07 ± 0.12 for CSFV, 25.39 ± 0.17 for PRRSV, 19.55 ± 0.19 for PEDV, and 20.12 ± 0.15 for PCV2, were the lowest. The Cq values of the E kit, which were 27.51 ± 0.19 for CSFV, 26.28 ± 0.18 for PRRSV, 20.49 ± 0.18 for PEDV, and 21.3 ± 0.14 for PCV2, were the highest. No inhibitory effects on the CSFV, PRRSV, PEDV, or PCV2 assays were observed. At the 1:10 dilutions of the RNA and DNA extracts, the Cq values increased when they were compared to the full concentrations (Table 2). Analytical sensitivity for the detection of targeted viruses was determined in a dilution series in which the DNA of the PCV2 extracted sample or the RNA of the PEDV extracted sample was diluted in six dilutions, from 10^{-1} to 10^{-6} . The highest dilutions of DNA extracts that were detected by qPCR assay were

Table 1. Yield and purity of DNA and RNA extracted by three DNA/RNA co-extraction kits

Kit	DNA		RNA	
	Yield (ng/ μL)	Purity (260/280)	Yield (ng/ μL)	Purity (260/280)
Q	11.91 ± 2.36	1.79 ± 0.13	$10.15 \pm 1.92^{\text{a}}$	1.99 ± 0.07
G	11.11 ± 2.24	1.83 ± 0.16	$9 \pm 1.61^{\text{b}}$	2.07 ± 0.17
E	10.93 ± 3.62	1.38 ± 0.24	$7.99 \pm 2.03^{\text{c}}$	2.1 ± 0.15

(a) $P=0.001$ for the RNA yield between the Q kit and the G kit; (b) $P=0.001$ for the RNA yield between the G kit and the E kit; (c) $P=0.001$ for the RNA yield between the Q kit and the E kit.

Table 2. Comparison of three DNA/RNA co-extraction kits based on the average Cq values obtained by real-time PCR

Virus	Cq values					
	Q		G		E	
	Original	1:10	Original	1:10	Original	1:10
CSFV (RNA virus)	26.07±0.12	29.16±0.19	26.79±0.2	29.7±0.21	27.51±0.19	30.3±0.20
PRRSV (RNA virus)	25.39±0.17	28.68±0.15	26.13±0.16	29.23±0.16	26.28±0.18	29.58±0.19
PEDV (RNA virus)	19.55±0.19	22.87±0.20	20.02±0.2	23.15±0.21	20.49±0.18	23.48±0.24
PCV2 (DNA virus)	20.12±0.15	23.36±0.17	20.41±0.16	23.89±0.18	21.3±0.14	24.51±0.15

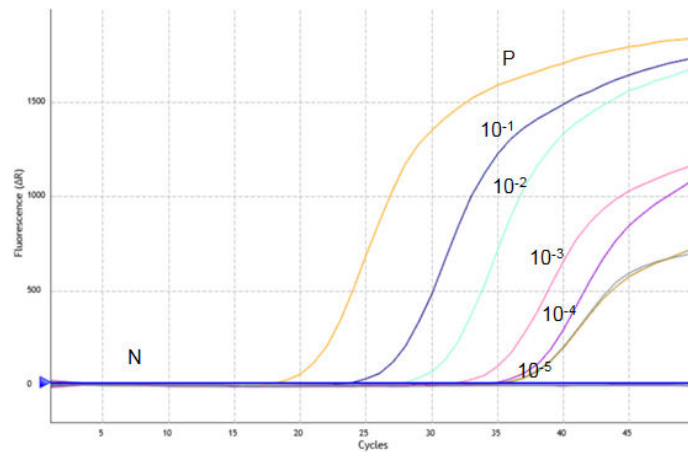


Fig. 1. Amplification of a serial dilution of the DNA extraction by the PCV2 assay. A ten-fold dilution of the faecal extract samples from 10^{-1} to 10^{-6} was prepared.

10^{-4} for the Q (Fig. 1) and G kits and 10^{-3} for the E kit, respectively (Table 3). The highest dilutions of RNA extracts that were detected by RT-qPCR assay were 10^{-4} for the Q (Fig. 2) and G kits and 10^{-3} for the E kit, respectively (Table 3).

The efficiency of qPCR and RT-qPCR to detect CSFV, PRRSV, PEDV, and PCV2 in DNA and RNA extraction from faecal samples was evaluated. As shown in Table 4, CSFV, PRRSV, PEDV, and

PCV2 were detected in 3 (3.26%), 5 (5.43%), 6 (6.51%), and 12 (13.04%) faecal samples by the Q and G kits, respectively. The number of positive samples, which were extracted by the E kit, decreased in 2 samples (CSFV and PCV2). The total positive samples of the E kit were 2 (2.17%) samples of CSFV, 5 (5.43%) samples of PRRSV, 6 (6.51%) samples of PEDV, and 11 (11.96%) samples of PCV2. There were no significant

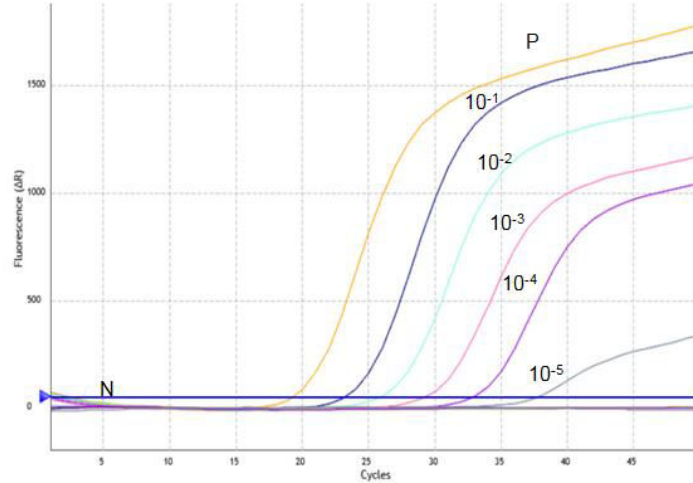


Fig. 2. Amplification of a serial dilution of the DNA extraction by the PED assay. A ten-fold dilution of the faecal extract samples from 10^{-1} to 10^{-6} was prepared.

Table 3. Analytical sensitivity for the detection of targeted viruses in a dilution series

Dilution	Number positive per test (Cq value)					
	PCV2			PEDV		
	Q	G	E	Q	G	E
10^{-1}	5/5 (23.36±0.17)	5/5 (23.89±0.18)	5/5 (24.51±0.15)	5/5 (22.87±0.20)	5/5 (23.15±0.21)	5/5 (23.48±0.24)
10^{-2}	5/5 (27.12±0.30)	5/5 (27.44±0.33)	5/5 (28.36±0.31)	5/5 (26.22±0.33)	5/5 (26.39±0.37)	5/5 (26.45±0.35)
10^{-3}	5/5 (31.86±0.32)	5/5 (31.98±0.32)	5/5 (32.3±0.36)	5/5 (30.92±0.33)	5/5 (31.06±0.35)	5/5 (31.43±0.34)
10^{-4}	4/5 (34.53±0.36)	4/5 (34.67±0.35)	2/5 (35.53±0.36)	4/5 (34.44±0.39)	4/5 (34.57±0.36)	1/5 (35.25±0.38)
10^{-5}	0/5 (>35)	0/5 (>35)	0/5 (>35)	0/5 (>35)	0/5 (>35)	0/5 (>35)
10^{-6}	0/5 (>35)	0/5 (>35)	0/5 (>35)	0/5 (>35)	0/5 (>35)	0/5 (>35)

differences in the Cq values ([Supplementary Material](#)) of the extracted samples among the three kits ($P>0.05$). In addition, these results indicated that TaqMan qPCR kits could be a useful tool to detect CSFV, PRRSV, PEDV, and PCV2 in faecal samples.

DISCUSSION

During the past three decades, pig production in many developing countries has changed rapidly from smallholders to an intensive industry (Woonwong *et al.*, 2020). The increase in herd size, intensive

Table 4. Detection of CSFV, PRRSV, PEDV, and PCV2 in faecal samples after extraction by three different kits

Number of faecal samples	Virus	Number of positive sample		
		Q	G	E
92	CSFV	3	3	2
92	PRRSV	5	5	5
92	PEDV	6	6	6
92	PCV2	12	12	11

production, and globalisation has led to the emergence and global spread of pathogens in pigs (VanderWaal & Deen, 2018). Viral diseases are still the main threat to the pig industry. Mutated or new viruses continue to emerge and re-emerge, which have caused outbreaks severely affecting the pig industry. The active surveillance components of CSF, PRRS, PED, and PCV2 have great relevance to demonstrating the absence or the presence of these diseases, identifying the emergences or changes in risk factors, and adopting management measures that promote mitigation and minimise the transmission of the viruses.

Vietnam's pig farming sectors mainly comprise smallholder and household/backyard-type farms. In households or backyard farms, many owners do not vaccinate pigs or submit samples to monitor the disease status of the farms if they own just two or three animals. They also don't prefer the local veterinarians or researchers entering the farm to take samples. The reason is that veterinarians or researchers may disturb animals and cause unlucky events that make pigs grow slowly. Faeces remain the primary sample of interest regarding the pig owners more than the other sample types such as swabs or blood. The farmers are able to collect and ship samples to the local veterinarians. Oral fluids are most commonly collected because their technique could provide an

easy and consequent positive impact on the animal welfare approach to sampling collection for nucleic acid and/or antibody-based surveillance (Plut *et al.*, 2020). However, a rope-in-a-bait sampling technique needs to be used in the backyard and scavenging farms. This technique also makes moderate contamination with other body fluids or feces (Dietze *et al.*, 2017). Moreover, all four viruses can be excreted through faeces. Faecal transmission may be an important factor in the spread of CSFV, PRRSV, PEDV, and PCV2 in farms (Blome *et al.*, 2017; López-Lorenzo *et al.*, 2019; Liu *et al.*, 2021; Suda *et al.*, 2021). Faeces are a suitable sample type for field epidemiologic investigations.

Molecular tests are promoted within the trends of diagnostic tools, epidemiological studies, and more importantly, in controlling these diseases due to their potential for rapid delivery of the results, high sample throughput, sensitivity, and specificity (Kumar *et al.*, 2021). However, small viral genomes at low concentrations, inefficient extraction of RNA and DNA, and incomplete removal of PCR inhibitors could reduce the sensitivity and specificity of the molecular assays. Stool samples are considered one of the most difficult specimens for the extraction of nucleic acids due to many unrecognised materials with potential to inhibit the PCR assays (Schrader *et al.*, 2012).

Dual extraction is the process of being able to purify both RNA and DNA from the same biological sample. It is often useful when the sample is in short supply or in a small amount of material and promises to reduce time-consuming, labour-intensive procedures, reagents, and equipment and does not require a large sample size to be split into two sample pools for separating RNA and DNA extraction procedures. Several studies have analysed the effectiveness of the extraction of the Q kit (Eriksson *et al.*, 2017; Klenner *et al.*, 2017). It performed the best among six DNA extraction kits for microbial DNA extraction from the faecal samples of wild Antarctic bird species. The Q kit was proven to yield high-quality DNA from the different faeces of seven different bird species (Eriksson *et al.*, 2017). In a comparison of the four Qiagen extraction kits for viral metagenomics extraction, which included Reovirus (bat mammalian orthoreovirus 342/08), Orthomyxovirus (H1N1 PR8/1934), Orthopoxvirus (vaccinia virus), and Paramyxovirus (Sendai virus) from the cell culture supernatant and the allantoic fluid of infected eggs, the Q kit had the highest percentage of reads only for the Orthopoxvirus. However, the Q kit was the only one tested that did not require a heating block (Klenner *et al.*, 2017). In addition, the Q was used in a variety of samples, including pig faeces, pig oral secretions, bioaerosols, and worker nasal wash samples, to detect adenovirus (ADV), coronavirus (CoV), encephalomyocarditis virus (EMCV), enterovirus (EV), influenza A-D (IAV, IBV, ICV, and IDV), porcine rotaviruses A and C (RVA and RVC), and PCV2 (Borkenhagen *et al.*, 2018). The G kit has been used to extract viral RNA and DNA from the allantoic fluid of infected eggs or the duck embryo liver homogenate samples

for detecting Duck hepatitis A virus type 1 (DHAV-1). The sensitivity for detection of DHV-1 in the supernatant of duckling liver homogenate was 10 ELD₅₀ (Kim *et al.*, 2007). No studies have published the use of the E kit for extracting viral RNA and DNA.

Currently, single, dual or multiplex PCR assays for CSFV, PRRSV, PEDV, and PCV2 have been established (Yang *et al.*, 2017; Wang *et al.*, 2019; Luo *et al.*, 2020; Nishi *et al.*, 2022; Wu *et al.*, 2022). Traditional PCR assays are less sensitive than real-time PCR in detecting the presence of low-concentration viruses in the early stages of infection (Luo *et al.*, 2020). Therefore, the virus infection cannot be identified in time and begins to spread rapidly among pigs, causing serious harm to the pig farms, especially where the density of the pig population was high. In this study, four TaqMan real-time PCR assays were used to detect CSFV, PRRSV, PEDV, and PCV2 in the faecal samples from apparently healthy pigs that had not been vaccinated. Three assays could detect CSF, PRRSV, and PEDV at the same time and in the same reaction system under the same amplification conditions. All assays successfully detected these four viruses in field samples and seemed fit for the purpose of surveillance. The sensitivity of these assays depended on viral nucleic acid extraction kits that were demonstrated by diluted faecal sample results. Other studies demonstrated that the sensitivity of real-time PCR assays depended on the sample types. PRRSV and PCV2 positive results were more often obtained from the oral fluid samples than from the faecal samples of the same animals (Plut *et al.*, 2020). CSFV replication in tonsils and lymphoid nodes could be detected in oral fluids at ≥ 5 days post-infection (Henaodiaz *et al.*, 2020).

The limitations of this study were the low numbers of positive CSFV, PRRSV, PEDV, and PCV2 samples, and only the faecal sample type tested herein. While three dual DNA/RNA extraction kits have been evaluated in this study, it could be interesting to test other commercially available viral nucleic acid extraction kits.

CONCLUSION

In this study, the three dual extraction kits for the purification of CSFV, PRRSV, PEDV, and PCV2 from faecal samples were evaluated. The extracted nucleic acid yield, elution volume, purity, and the Cq values were compared. The results showed that all three kits were efficient for the extraction of both DNA and RNA viruses, and the Q kit had the best performance among them. TaqMan real-time PCR assays were successful to detect CSFV, PRRSV, PEDV, and PCV2 in the field samples. The findings will be very useful for the laboratories in the selection of extraction and detection methods for epidemiological surveillance to detect four viruses. Further studies on a large number of positive CSFV, PRRSV, PEDV, and PCV2 samples as well as on other types of samples, which were serum, oral fluid, and tissue should be performed.

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