Journal of Virological Methods 266 (2019) 121–125

Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

Protocols

Application of high-resolution melting curve analysis for identification of Muscovy duck parvovirus and goose parvovirus

Jiawen Dong[a,d,e], Gali Bingga[b], Minhua Sun[a,d,e], Linlin Li[a,d,e], Zhicheng Liu[a,d,e], Chunhong Zhang[a,d,e], Pengju Guo[c], Yu Huang[f], Jianfeng Zhang[a,d,e,*]

[a] Institute of Animal Health, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, China
[b] Vocational and Technical College, Inner Mongolia Agricultural University, Inner Mongolia 014109, China
[c] Guangdong Key Laboratory of Laboratory Animals, Guangdong Laboratory Animals Monitoring Institute, Guangzhou 510633, China
[d] Key Laboratory of Livestock Disease Prevention of Guangdong Province, Guangzhou 510640, China
[e] Scientific Observation and Experiment Station of Veterinary Drugs and Diagnostic Techniques of Guangdong Province, Ministry of Agriculture, Guanzhou, 510640, China
[f] Institute of Animal Husbandry and Veterinary Medicine, Fujian Academy of Agricultural Sciences, Fuzhou, 350013, China

ARTICLE INFO

Keywords:
Muscovy duck parvovirus
Goose parvovirus
VP3 gene
HRM

ABSTRACT

This study reports the findings of a high-resolution melting (HRM) curve analysis combined with PCR technique (PCR-HRM) to differentiate between Muscovy duck parvovirus (MDPV) and goose parvovirus (GPV). A degenerate primer set was designed based on the VP3 gene of MDPV and GPV. The PCR HRM assay was able to discriminate between MDPVs and GPVs by differences in melting curve shapes and melting temperatures. A total of forty-five clinical samples, passaged in the allantoic cavity of Muscovy duck eggs, were detected by the PCR-HRM assay. Among the 12 positive samples, two were identified as MDPV and two as GPV with high genotype confidence percentage (GCP) values. Seven positive samples had low GCP values for the melting curve analysis and were identified as co-infection samples. One of the 12 positive samples, designed GDNX strain, was identified as a variant strain with a divergent melting curve profile. To assess the capability of PCR-HRM assay to distinguish MDPV and GPV, fifty-two field samples were collected and examined. Seven samples were positive for MDPV and/or GPV. Thus, this developed assay was useful for discrimination of MDPVs and GPVs and can also be suitable for detecting co-infection samples.

1. Introduction

Waterfowl parvoviruses, which are highly contagious and lethal to Muscovy ducklings and goslings, can be divided into Muscovy duck parvoviruses (MDPVs) and goose parvoviruses (GPVs) (Glavits et al., 2005). MDPV causes disease in Muscovy ducklings, while GPV causes Derzy's disease in geese and is also pathogenic for Muscovy ducklings (Chang et al., 2000). Both parvoviruses have been associated to epidemics in many Muscovy duck breeding regions, including Hungary, Thailand, Japan, USA, France, and China, leading to a large economic loss (Chen et al., 2015; Wang et al., 2017, 2013; Yu and Li, 2016; Zhu et al., 2014). In China, although the attenuated vaccines against MDPV and GPV have been used for more than 20 years, both parvoviruses continue to affect the waterfowl industry. Many small-scale Muscovy ducks farms in poor breeding conditions experienced sporadic outbreaks of MDPV or GPV infection, or co-infection of MDPV and GPV (Chunhe et al., 2015).

For detection of MDPV and GPV, conventional methods have been developed, such as virus isolation in gosling or duckling embryos (Gough et al., 2005), enzyme-linked immunosorbent assay (ELISA) (Zhang et al., 2010), polymerase chain reaction assay (PCR) (Limn et al., 1996), fluorescent quantitative real-time PCR (qPCR)

Abbreviations: HRM, high-resolution melting; MDPV, Muscovy duck parvovirus; GPV, goose parvovirus; GCP, genotype confidence percentage; ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction assay; qPCR, fluorescent quantitative real-time PCR; RFLP, restriction enzyme fragment length polymorphism analysis; LAMP, loop-mediated isothermal amplification method; MDRV, Muscovy duck reovirus; AIV-H9, Avian influenza virus H9; DTMUV, Duck Tembusu virus; DPV, Duck plague virus; NDV, Newcastle disease virus; EDSV, Egg drop syndrome virus

[*] Corresponding author at: Institute of Animal Health, Guangdong Academy of Agricultural Sciences, Baisigang, Wushan Road, Tianhe District, Guangzhou 510640, China.

E-mail addresses: hellendongjiawen@163.com (J. Dong), glbg@imau.edu.cn (G. Bingga), smh2002smh@163.com (M. Sun), lingdang1000@163.com (L. Li), rainman136@aliyun.com (Z. Liu), 13660450420@139.com (C. Zhang), guo_pengju@hotmail.com, 1518227522@qq.com (P. Guo), huangyu_815@163.com (Y. Huang), 13668939298@139.com (J. Zhang).

https://doi.org/10.1016/j.jviromet.2018.12.018
Received 26 June 2018; Received in revised form 19 December 2018; Accepted 26 December 2018

(Wozniakowski et al., 2012; Yang et al., 2009), restriction enzyme fragment length polymorphism analysis (RFLP) (Sirivan et al., 1998; Wan et al., 2016), loop-mediated isothermal amplification method (LAMP) (Ji et al., 2010; Yang et al., 2010), and sequence analysis (Chunhe et al., 2015). However, these detection methods have issues, including poor specificity and sensitivity, and difficulty in differentiating MDPV and GPV when co-infection occurs, especially when nucleotide variations are present.

The PCR high resolution melting (HRM) provides a simple and cost-effective alternative to genotyping. The detection method was used to evaluate variations in nucleotides, including polymorphisms, mutations, and drug resistance (Bingga et al., 2014; Ostertag-Hill et al., 2015; Reed et al., 2007). The presented study aimed to develop a PCR-HRM assay that could be able to discriminate between MDPVs and GPVs and also be suitable for detecting co-infection samples.

2. Materials and methods

2.1. Sample collection and DNA extraction

Forty-five samples (heart, liver, intestine and spleen) were collected from Muscovy ducks with symptoms of anorexia, wheezing, watery diarrhea, and locomotory dysfunction at different Muscovy duck farms located in Guangdong and Fujian province, China. Samples were homogenized in sterile phosphate-buffered saline (PBS, pH 7.2) and then centrifugated at 5000 × g for 8 min. The supernatant was filtered through a 0.22-μm filter, and the filtrate was inoculated into allantoic cavities of 10-day-old Muscovy duck eggs and then cultured in an incubator at 37 °C. The allantoic fluid was collected after 4–5 days post-inoculation and frozen at -80 °C prior to DNA extraction. Fifty-two field samples (heart, liver, intestine and spleen) were collected from different Muscovy duck farms located in Guangdong province, China. By using the QIAamp viral DNA mini kit (Qiagen, Hilden, Germany), viral DNA was extracted from allantoic fluid or sample supernatant according to the manufacturer's instructions. In this study, the MDPV-gd14-1 (GenBank Accession No. KR068553) and GPV-gd14-1 (GenBank Accession No. KR068556) strains, isolated in our laboratory, were used as positive controls for the PCR-HRM assays.

2.2. Design of primers

Oligonucleotide primers MGV-P1 (5′-GGAGGAACAGAYAACTATGCRA-3′) and MGV-P2 (5′-TAWATATCYCTGTTCTGCCARACCA-3′), specific for the VP3 gene of MDPV and GPV, were designed using the Primer Premier 5.0 software (PREMIER Biosoft, California, USA) based on the complete sequence of the GPV strain (GenBank Accession No. KY511293) and the sequence of the MDPV strain (GenBank Accession No. JF926698). The expected ampicons are 371 bp. To assess the suitability for HRM analysis, a web-based DNA melting simulation program, uMeltSM (https://www.Dna.utah.edu/umelt/u melt.html), was used to predict the melting curve profile of the amplified fragment.

2.3. PCR and HRM curve analysis

The PCR amplification was carried out as follow: a 10 μl total volume consisting of 2 μl of 5 × Q5 Reaction buffer, 0.8 μl of dNTPs (2.5 mM), 0.5 μl of each primer (10 mM), 0.1 μl of Q5 High-Fidelity DNA Polymerase (5 U/μl, New England Biolabs), 0.5 μl of LCGreen (Idaho Technology Inc. Salt Lake City, Utah), 4.6 μl of ddH_2O, and 1 μl of template DNA. Cycling conditions were:98 °C for 30 s, followed by 35 cycles of 98 °C for 10 s, 55 °C for 20 s and 72 °C for 20 s, and a final cycle of 72 °C for 2 min by Rotor-GeneQ™ (Qiagen, Hilden, Germany). Melt curve was generated by heating from 70 °C and 90 °C at ramps of 0.3 °C/s. The melting profiles were analyzed with the Rotor-GeneQ™ software v.2.0.2. All samples were tested in triplicate. The HRM genotype confidence percentage (GCP) values were applied to interpret the melting curves. Tested samples were genotyped with reference samples of MDPV-gd14-1 and GPV-gd14-1 in a normalized HRM graph with equal or greater than 95% GCP for MDPVs and GPVs, respectively.

2.4. Specificity, sensitivity, and reproducibility of the PCR-HRM assay

Primers of MGV-P1/MGV-P2 were tested for specificity using DNA or cDNA of Muscovy duck reovirus (MDRV), Avian influenza virus H9 (AIV H9), Duck Tembusu virus (DTMUV), Duck plague virus (DPV), Newcastle disease virus (NDV), and Egg drop syndrome virus (EDSV) in the PCR-HRM assay. MDPV-gd14-1 and GPV-gd14-1 were used as positive controls.

To determine the analytical sensitivity of the PCR-HRM assay, two plasmids were constructed. The 371bp PCR amplicons of MDPV-gd14-1 and GPV- gd14-1 were gel purified using the QIAquick kit (Qiagen, Hilden, Germany) and cloned into the pMD™18 T vector (Takara, Dalian, China) according to the manufacturer's recommendations. Serial dilutions of two constructed plasmids, PMD-MDPV-gd14-1 and PMD-GPV-gd14-1, were prepared in dH_2O and PCR was carried out in triplicate for each dilution using the MGV-P1/MGV-P2 primers, followed by HRM curve analysis.

To assess the reproducibility of the assay, MDPV-gd14-1 and GPV-gd14-1 samples were tested on five days. To evaluate whether PCR-HRM could detect mixed infection in samples, unequal mixtures of two constructed plasmid of PMD-MDPV-gd14-1 and PMD-GPV-gd14-1 were also mixed and tested at ratios ranging from 1:1 to 1:9 (v/v).

2.5. Evaluation of PCR-HRM assay

To appraise whether PCR-HRM could differentiate between MDPV and GPV, Muscovy duck embryo allantoic fluid inoculated with 45 tissue supernatant described in Section 2.1 were used. The samples were amplified and cloned to the vector according to the method described previously. Five colonies of each sample were randomly selected and analyzed by DNA sequencing.

2.6. Clinical testing

In order to further evaluate the clinical application effect of the PCR-HRM assay, a total of 52 flied samples were detected. All the samples were collected from Guangdong province of China in 2018. The positive samples were confirmed and analyzed by DNA sequencing.

3. Results

3.1. PCR-HRM analysis

To evaluate the capacity of the HRM primers to discriminate MDPV and GPV, two isolated strains of MDPV-gd14-1 and GPV-gd14-1 were tested. The MDPV-gd14-1 strain generated two peaks, with the first peak at 79.8 °C and a major peak at 81.9 °C. While the MDPV-gd14-1 strain produced a major peak at 82.8 °C and a low-temperature shoulder peak at 80.0 °C (Fig. 1A).

3.2. Specificity, sensitivity, and reproducibility of the PCR-HRM assay

The MGV-P1 and MGV-P2 primers were examined for specificity using samples of MDRV, AIV-H9, DTMUV, DPV, NDV, and EDSV in the PCR-HRM assay. Specific amplification curves were obtained without other non-specific curves when DNA/cDNA templates of eight different viruses were subjected to HRM analysis (Fig. 1A).

To investigate the sensitivity of the HRM assay, two constructed plasmids (PMD- MDPV-gd14-1 and PMD-GPV-gd14-1) were examined. We found that at least 34.5 and 32.9 copies of PMD-MDPV-gd14-1 and PMD-GPV-gd14-1 were required for the PCR-HRM assay, respectively (Fig. 1B and 1C)

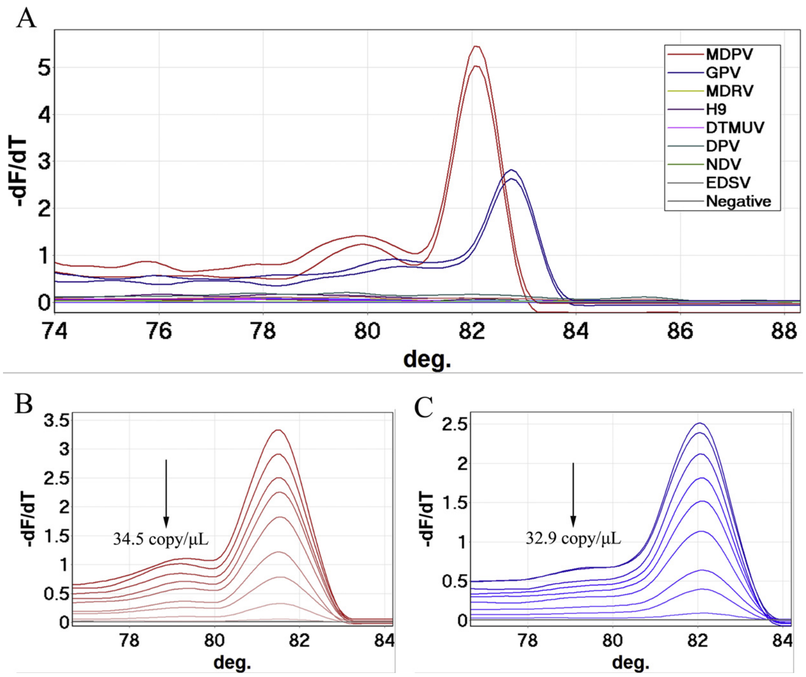

Fig. 1. Specificity and sensitivity tests of PCR-HRM. NOTE: The DNA of MDPV-gd14-1(KR068553)and GPV-gd14-1(KR068556)were used as reference samples of specificity test (A) and the constructed plasmid of PMD-MDPV-gd14-1(B)and PMD-GPV-gd14-1 (C)were used to evaluate the sensitivity tests.

Table 1
Reproducibility tests of PCR-HRM for reference samples.

Name	No. of times tested	Tm1 (Mean ± SD)	Tm2 (Mean ± SD)	GCP (Mean ± SD)a	CV%[b] Intra-	Inter-
MDPV[a]	23	79.38 ± 0.31	81.48 ± 0.37	97.93 ± 1.64	0.4%	0.4%
		79.27 ± 0.10	81.18 ± 0	98.34 ± 1.52	0%	
		79.3 ± 0.10	81.17 ± 0	98.71 ± 1.28	0%	
GPV[a]	19	82.28 ± 0.29		98.07 ± 1.02	0.3%	0.3%
		82.30 ± 0.04		97.56 ± 2.11	0.04%	
		82.29 ± 0.05		99.20 ± 0.44	0.06%	

[a] MDPV-gd14-1 (KR068553) and GPV-gd14-1 (KR068556) were used as reference samples.

[b] CV% values calculated from main melting peak.

To assess the reproducibility of the assay, different DNA templates of reference samples (MDPV-gd14-1 and GPV-gd14-1) were conducted on various days. The HRM curve analysis revealed slight shifts in melting temperatures between PCRs for the same sample (Table 1). However, the relative position and the shape of the conventional and normalized melting curves were consistent. To assess whether the PCR-HRM technique could identify a mixed population of MDPV and GPV strains in the same sample, combinations of the constructed plasmids (PMD-MDPV-gd14-1 and PMD-GPV-gd14-1) with different ratios were tested in separate PCR-HRMs. Following this method, the melting curve profiles of the various ratios were still distinguishable from each other in all of the tested ratios (Fig. 2A)

3.3. Evaluation of PCR-HRM assay

Twelve samples detected by PCR-HRM were positive for MDPV and/or GPV, and the samples were numbered from S1 to S12 (Table 2). When MDPV-gd14-1 and GPV-gd14-1 were selected as the genotype control, sample S1 and S9 were genotyped as the MDPV strain with greater than 95% mean confidence (GCP) and sample S3 and S10 were genotyped as GPV with greater than 95% mean confidence (GCP). All the other samples produced various melt curves and normalized HRM curves to the reference samples, with a mean GCP between 46.51% and 93.54%, and were genotyped as variation strains automatically. It is notable that the S2 was genotyped as a GPV variation strain with a GCP value of 48.40% using GPV-gd14-1 as a genotype control, while when MDPV-gd14-1 was used as a genotype control, the GCP value was just 2.26%. The S2 generated only one peak at 81.2 ± 0.03 °C in conventional melt curve analysis and a distinct melting curve profile compared to MDPV-gd14-1 and GPV-14gd-1 (Fig. 2B and Table 2).

The nucleotide sequence obtained from sample S1 and S9 had more than 99.1% identity with the VP3 gene of MDPV (KR068553). Sample S2, S3, and S10 had more than 97.8% identity with the VP3 gene of GPV (KR068556) (Fig. S1). However, other samples, with a confidence percentage below the threshold (95% mean confidence) included multicolored peaks in their sequencing chromatography, which indicated that these samples might contain two or more different viruses simultaneously. The sequence analysis showed that the PCR-HRM products (except S2) with a confidence percentage below the threshold (95% GCP) contain both GPV and MDPV sequences. The sequence analysis of S2 revealed a few nucleotide variations in the region of the VP3 gene compared with GPV (KR068556) (Fig. S1). To confirm the HRM genotyping result of S2, we sequenced its full-length genome, which was designed GDNX and submitted to GenBank under accession No.MH204100 (Table 2).

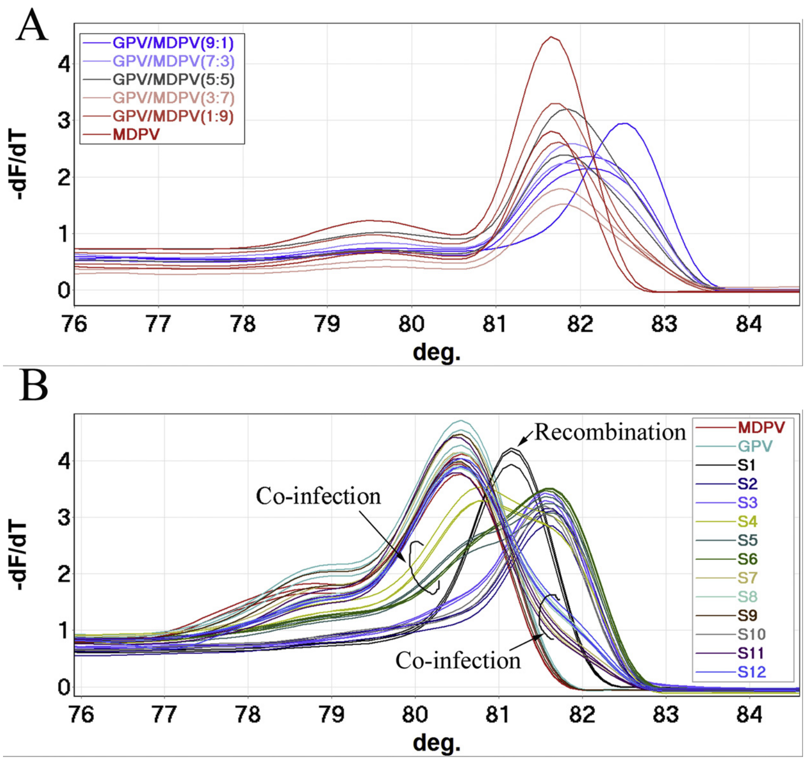

Fig. 2. Conventional melting curve analysis of constructed plasmid mixed at ratios of 1:1 to 1:9 (v/v) (A) and PCR-HRM products from twelve positive samples (B). NOTE: The constructed plasmid of PMD-MDPV-gd14-1and PMD-GPV-gd14-1were used to evaluate the co-infection tests (A). The reference samples of MDPV-gd14-1(KR068553) and GPV-gd14-1 (K R068556) were used as positive controls (B).

3.4. Clinical testing

To assess the capability of PCR-HRM assay to differentiate MDPV and GPV, fifty-two field samples were collected and examined. Seven samples were positive for MDPV and/or GPV. Of the seven positive samples, three were genotyped as MDPV, two were genotyped as GPV. The other two positive samples produced different melt curves to the reference samples were co-infection samples (Fig. S2). DNA sequencing of the field samples confirmed that the developed assay could distinguish MDPV and GPV at 100% accuracy (data not show).

4. Discussion

This study describes a simple method for genotyping MDPVs and GPVs by PCR-HRM. The developed assay is cost-effective and more straightforward than conventional genotyping methods, such as Taqman Probe and sequence analysis. PCR-HRM analysis can be accomplished rapidly using only a generic DNA binding dye. The melting profile of the PCR product is dependent on length, GC content, sequence, and heterozygosity (Herrmann et al., 2006; Reed et al., 2007). It was previously reported that the size and specific nucleotide content of amplicons were the key considerations affecting the discrimination ability of HRM analysis (Dang et al., 2012). The challenge in amplified fragment design is to seek a balance between HRM resolution and sequence coverage. For example, when the amplified fragment is less than 200 bp, there was only one melting peak, which is not suitable for HRM analysis. When the amplified fragment is more than 600 bp, there are multiple melting domains, which complicate the HRM analysis (Steer et al., 2009). Therefore, to maximize the efficiency of HRM analysis, a 371 bp size fragment of the VP3 gene was chosen as an amplification target.

In this study, all the co-infection samples were collected from duck farms located in Fujian province, which had no previous history of using an attenuated vaccine against MDPV or GPV. The reason for the co-infection might be due to the direct contact between infected waterfowl or waterfowl sharing the same waters. In previous reports,

Table 2
GCP (Mean ± SD) values for samples following PCR-HRM analysis.

Sample name	Confidence% (Mean ± SD)[a]	Genotype	Geographical origin	Sample type	GenBank accession no.	Interpretation
MDPV-gd14-1	99.84 ± 0.11	MDPV	Guangdong,China	Allantoic fluid	KR068553	Positive control
GPV-gd14-1	99.79 ± 0.13	GPV	Guangdong,China	Allantoic fluid	KR068556	Positive control
S1	96.01 ± 0.41	MDPV	Guangdong,China	Allantoic fluid	KR068554	MDPV
S2	48.40 ± 0.70	Variation	Guangdong,China	Allantoic fluid	MH204100	Recombination
S3	97.16 ± 0.26	GPV	Fujian,China	Allantoic fluid	KR068557	GPV
S4	67.88 ± 0.21	Variation	Fujian,China	Allantoic fluid		Co-infection
S5	89.07 ± 0.21	Variation	Fujian,China	Allantoic fluid		Co-infection
S6	93.54 ± 0.74	Variation	Fujian,China	Allantoic fluid		Co-infection
S7	66.99 ± 4.29	Variation	Fujian,China	Allantoic fluid		Co-infection
S8	51.55 ± 1.73	Variation	Fujian,China	Allantoic fluid		Co-infection
S9	96.70 ± 0.99	MDPV	Guangdong,China	Allantoic fluid	KR068555	MDPV
S10	98.15 ± 0.22	GPV	Fujian,China	Allantoic fluid	KR068558	GPV
S11	65.89 ± 2.17	Variation	Fujian,China	Allantoic fluid		Co-infection
S12	46.51 ± 4.69	Variation	Fujian,China	Allantoic fluid		Co-infection

[a] MDPV-gd14-1(KR068553) and GPV-gd14-1 (KR068556) were used as genotype controls.

mixed infections of MDPV and GPV have been detected in some Muscovy duck farms in China (Wan et al., 2016). In 2015, Wan reported the occurrence of MDPV and GPV co-infection in a Muscovy duck in Fujian province by performing genomic sequencing of the two viruses (Chunhe et al., 2015).

In the PCR-HRM analysis, the majority of samples were characterized by low GCP values, which were due to the simultaneous presence of the MDPV and GPV strains in the samples. In our study, the S2 was also distinguished with a low GCP value of 48.4%. However, the sequencing result indicated that there was no occurrence of MDPV and GPV co-infection or no more than one virus presented. In previous reports, differences in melting curve profiles were mainly related to the distribution of GC contents throughout the amplicons but not necessarily to nucleotide sequence identities (Steer et al., 2009). In this study, four G or C bases changed into A or T bases in the target region of the S2, while compared to the GPV (Fig. S1). A base change from G/C to A/T is readily determined by the change in Tm made by varying the number of hydrogen bonds (Liew et al., 2004). Thus, it is likely that the presence of nucleotide variations in the amplicon region of S2 was the result of melting temperature changes in the HRM analysis.

Phylogenetic analysis and analysis of possible recombination region localization were confirmed that GDNX was a recombination virus (Fig. S3). It was notable that the recombination strain of GDNX was isolated from Muscovy duck in Guangdong province, Southern China, in 2016. However, the SAAS-SHNH was isolated from Muscovy duck in Shanghai, Eastern China, in 2012. The reason for the recombination event is unclear. It might have been due to co-infection with multiple parvovirus strains, or the increasing use of co-vaccination with MDPV and GPV in China, but need further investigation.

One of the important factors of the HRM technique is that the quantity and quality of DNA used in the assay might influence the melting shape. To obtain better reproducible results with HRM, the clinical samples passaged by Muscovy duck eggs were recommended for HRM analysis. However, the clinical samples were also directly detected by the PCR-HRM assay (Fig. S4).

In this study, the uMeltSM DNA melting simulation program was used to confirm whether the PCR-HRM curve analysis can potentially distinguish other sequences that were available in the GenBank database. The resultant melting curve shapes between MDPVs and GPVs were also differentiated from each other (data not shown).

A novel assay was developed to identify waterfowl parvoviruses using a PCR-HRM technique. This assay was useful for discrimination of MDPVs and GPVs from samples and can also be suitable for detecting co-infection samples.

Conflict of interest

None.

Acknowledgements

This work was supported by The National Key Research and Development Program of China (grant numbers: 2016YFD0500800), the Science and Technology Planning Project of Guangdong Province, China (grant numbers: 2014A040401049, 2015B020230004, 2015B020203003, 2016B020212007, 2016B020234006, 2016A020210049, 2017A040403015) and Department of Agricultural Project of Guangdong Province, China (grant number 2017LM2150). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jviromet.2018.12.018.

References

Bingga, G., Liu, Z., Zhang, J., Zhu, Y., Lin, L., Ding, S., Guo, P., 2014. High resolution melting curve analysis as a new tool for rapid identification of canine parvovirus type 2 strains. Mol. Cell. Probes 28, 271–278.

Chang, P.C., Shien, J.H., Wang, M.S., Shieh, H.K., 2000. Phylogenetic analysis of parvoviruses isolated in Taiwan from ducks and geese. Avian Pathol. 29, 45–49.

Chen, H., Dou, Y., Tang, Y., Zhang, Z., Zheng, X., Niu, X., Yang, J., Yu, X., Diao, Y., 2015. Isolation and genomic characterization of a duck-origin GPV-Related parvovirus from Cherry Valley Ducklings in China. PLoS One 10, e0140284.

Chunhe, W., Hongmei, C., Qiuling, F., Guanghua, F., Longfei, C., Shaohua, S., Yu, H., Kaihui, H., 2015. Genomic characterization of goose parvovirus and Muscovy duck parvovirus co-infection in Fujian, China. Kafkas Univ. Vet. Fak. Derg. 26, 923–928.

Dang, X.D., Kelleher, C.T., Howard-Williams, E., Meade, C.V., 2012. Rapid identification of chloroplast haplotypes using High Resolution Melting analysis. Mol. Ecol. Resour. 12, 894–908.

Glavits, R., Zolnai, A., Szabo, E., Ivanics, E., Zarka, P., Mato, T., Palya, V., 2005. Comparative pathological studies on domestic geese (Anser anser domestica) and Muscovy ducks (Cairina moschata) experimentally infected with parvovirus strains of goose and Muscovy duck origin. Acta Vet. Hung. 53, 73–89.

Gough, D., Ceeraz, V., Cox, B., Palya, V., Mato, T., 2005. Isolation and identification of goose parvovirus in the UK. Vet. Rec. 156, 424.

Herrmann, M.G., Durtschi, J.D., Bromley, L.K., Wittwer, C.T., Voelkerding, K.V., 2006. Amplicon DNA melting analysis for mutation scanning and genotyping: cross-platform comparison of instruments and dyes. Clin. Chem. 52, 494–503.

Ji, J., Xie, Q.M., Chen, C.Y., Bai, S.W., Zou, L.S., Zuo, K.J., Cao, Y.C., Xue, C.Y., Ma, J.Y., Bi, Y.Z., 2010. Molecular detection of Muscovy duck parvovirus by loop-mediated isothermal amplification assay. Poult. Sci. 89, 477–483.

Liew, M., Pryor, R., Palais, R., Meadows, C., Erali, M., Lyon, E., Wittwer, C., 2004. Genotyping of single-nucleotide polymorphisms by high-resolution melting of small amplicons. Clin. Chem. 50, 1156–1164.

Limn, C.K., Yamada, T., Nakamura, M., Takehara, K., 1996. Detection of Goose parvovirus genome by polymerase chain reaction: distribution of Goose parvovirus in Muscovy ducklings. Virus Res. 42, 167–172.

Ostertag-Hill, C., Fang, L., Izume, S., Lee, M., Reed, A., Jin, L., 2015. Differentiation of BHV-1 isolates from vaccine virus by high-resolution melting analysis. Virus Res. 198, 1–8.

Reed, G.H., Kent, J.O., Wittwer, C.T., 2007. High-resolution DNA melting analysis for simple and efficient molecular diagnostics. Pharmacogenomics 8, 597–608.

Sirivan, P., Obayashi, M., Nakamura, M., Tantaswasdi, U., Takehara, K., 1998. Detection of goose and Muscovy duck parvoviruses using polymerase chain reaction-restriction enzyme fragment length polymorphism analysis. Avian Dis. 42, 133–139.

Steer, P.A., Kirkpatrick, N.C., O'Rourke, D., Noormohammadi, A.H., 2009. Classification of fowl adenovirus serotypes by use of high-resolution melting-curve analysis of the hexon gene region. J. Clin. Microbiol. 47, 311–321.

Wan, C.H., Chen, H.M., Fu, Q.L., Shi, S.H., Fu, G.H., Cheng, L.F., Chen, C.T., Huang, Y., Hu, K.H., 2016. Development of a restriction length polymorphism combined with direct PCR technique to differentiate goose and Muscovy duck parvoviruses. J. Vet. Med. Sci. 78, 855–858.

Wang, S., Cheng, X.X., Chen, S.Y., Zhu, X.L., Chen, S.L., Lin, F.Q., Li, Z.L., 2013. Genetic characterization of a potentially novel goose parvovirus circulating in Muscovy duck flocks in Fujian Province, China. J. Vet. Med. Sci. 75, 1127–1130.

Wang, J., Ling, J., Wang, Z., Huang, Y., Zhu, J., Zhu, G., 2017. Molecular characterization of a novel Muscovy duck parvovirus isolate: evidence of recombination between classical MDPV and goose parvovirus strains. BMC Vet. Res. 13, 327.

Wozniakowski, G., Samorek-Salamonowicz, E., Kozdrun, W., 2012. Quantitative analysis of waterfowl parvoviruses in geese and Muscovy ducks by real-time polymerase chain reaction: correlation between age, clinical symptoms and DNA copy number of waterfowl parvoviruses. BMC Vet. Res. 8, 29.

Yang, J.L., Cheng, A.C., Wang, M.S., Pan, K.C., Li, M., Guo, Y.F., Li, C.F., Zhu, D.K., Chen, X.Y., 2009. Development of a fluorescent quantitative real-time polymerase chain reaction assay for the detection of Goose parvovirus in vivo. Virol. J. 6, 142.

Yang, J., Yang, R., Cheng, A., Wang, M., Fu, L., Yang, S., Zhang, S., Yang, L., Xu, Z., 2010. A simple and rapid method for detection of Goose Parvovirus in the field by loop-mediated isothermal amplification. Virol. J. 7, 14.

Yu, T.F., Li, M., 2016. Identification of recombination among VP1 gene of Muscovy duck parvovirus from the Mainland of China. Vet. Microbiol. 195, 78–80.

Zhang, Y., Li, Y., Liu, M., Zhang, D., Guo, D., Liu, C., Zhi, H., Wang, X., Li, G., Li, N., Liu, S., Xiang, W., Tong, G., 2010. Development and evaluation of a VP3-ELISA for the detection of goose and Muscovy duck parvovirus antibodies. J. Virol. Methods 163, 405–409.

Zhu, Y., Zhou, Z., Huang, Y., Yu, R., Dong, S., Li, Z., Zhang, Y., 2014. Identification of a recombinant Muscovy Duck parvovirus (MDPV) in Shanghai, China. Vet. Microbiol. 174, 560–564.