Journal of Virological Methods 266 (2019) 34–40

Contents lists available at ScienceDirect

Journal of Virological Methods

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

Rapid and sensitive detection of goose parvovirus and duck-origin novel goose parvovirus by recombinase polymerase amplification combined with a vertical flow visualization strip

Wen-jun Liu[a,b], You-tian Yang[c], Si-min Du[a], Hua-dong Yi[a], Dan-ning Xu[a,b], Nan Cao[a,b], Dan-li Jiang[a,b], Yun-mao Huang[a,b,*], Yun-bo Tian[a,b,*]

[a] Zhongkai University of Agriculture and Engineering, Guangdong, Guangzhou, 510225, China
[b] Guangdong Province Key Laboratory of Waterfowl Healthy Breeding, Guangdong, Guangzhou, 510225, China
[c] Foshan University, Guangdong, Foshan, 528000, China

ARTICLE INFO

Keywords:
Goose parvovirus
Novel goose parvovirus
Recombinase polymerase amplification
Vertical flow visualization strip
Waterfowl

ABSTRACT

Goose parvovirus (GPV) is one of the most serious viral pathogens in goslings. Recently, a new pathogen to the Chinese mainland—duck-origin novel goose parvovirus (N-GPV)—was found to be 90.8–94.6% identical to the nucleotide sequence of GPV, and typically causes growth disorders and high infection rates in meat ducks. The spread of both of these viruses hinders the healthy development of the waterfowl breeding industry. In this study, recombinase polymerase amplification (RPA) was combined with a vertical flow (VF) visualization strip to develop a universal assay for the rapid detection of GPV and N-GPV. A set of specific primers and probes were designed to target the *VP3* gene. Detection was possible at a constant temperature of 37 °C within 5–10 min. The assay successfully detected GPV and N-GPV with high-specificity and did not exhibit cross-reactivity with other waterfowl viruses and bacteria. The analytical sensitivity of the GPV-RPA-VF assay was 2×10^2 copies of GPV plasmid. Validation of the GPV-RPA-VF assay—using 60 samples from the field—confirmed 100% similarity between the results of GPV-RPA-VF and conventional qPCR. The results indicate that the GPV-RPA-VF assay was accurate, sensitive, and specific. This assay can be performed with minimal equipment and training to rapidly detect GPV and N-GPV during the early phase of an outbreak, especially when timely veterinary diagnoses are needed in the field and in rural areas.

1. Introduction

Goose parvovirus (GPV) is a serious disease that affects goslings and Muscovy ducklings. The illness, also known as Derzsy's disease, causes great economic losses in the waterfowl industry (Derzsy, 1967; Derzsy et al., 1970), where the majority of goslings and ducklings infected with GPV die within the first two weeks of life and mortality is nearly 100% (Niu et al., 2016). Infected birds are asymptomatic in most cases, although some show signs of leg weakness (Takehara et al., 1995). Yet another disease affecting waterfowl—short beak and dwarfism syndrome (SBDS), which gives rise to short beaks, protruding tongues, fragile tibias, and pteroids, and growth retardation in commercial ducks—made its first appearance on the Chinese mainland in 2015 (Chen et al., 2015). Phylogenetic analyses have indicated that the causative virus was similar to a mule duck-isolated GPV (strain D146/02) and European GPV isolates. Genomic sequence analyses confirmed that this new pathogen was 90.8–94.6% identical to the nucleotide sequence of GPV. Therefore, it was named duck-origin novel goose parvovirus (N-GPV) (Chen et al., 2015). The outbreak of SBDS disease has been rapid and widespread in different duck-producing areas of China in recent years, including Fujian, Shandong, Anhui, Shanghai, Hebei, Zhejiang, and Guangdong provinces (Chen et al., 2016a,b; Li et al., 2018; Wang et al., 2016; Yu et al., 2016). The morbidity rate ranges from 10 to 50%, thereby resulting in enormous economic losses to the duck industry in China (Xiao et al., 2017).

To reduce the economic losses caused by GPV and N-GPV, it is

Abbreviations: GPV, goose parvovirus; N-GPV, duck-origin novel goose parvovirus; RPA, recombinase polymerase amplification; VF, vertical flow; LF, lateral flow; SBDS, short beak and dwarfism syndrome; bp, base pair; PCR, polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay; T, test; C, control

* Corresponding authors at: Zhongkai University of Agriculture and Engineering, Guangdong, Guangzhou, 510225, China.
E-mail addresses: lwjhero123@126.com (W.-j. Liu), yyte061@163.com (Y.-t. Yang), 1248026471@qq.com (S.-m. Du), 649562017@qq.com (H.-d. Yi), xdanning@126.com (D.-n. Xu), caonan870405@126.com (N. Cao), 305260675@qq.com (D.-l. Jiang), huangyunmao@163.com (Y.-m. Huang), tyunbo@126.com (Y.-b. Tian).

https://doi.org/10.1016/j.jviromet.2019.01.010
Received 28 November 2018; Received in revised form 18 January 2019; Accepted 21 January 2019
Available online 22 January 2019

important to develop rapid and effective methods of detection. Conventional diagnostic techniques for GPV include direct virus isolation, immunofluorescence assay, and electron microscopy, all of which can detect GPV antigens in tissues or identify the pathogen in cell culture (Poonia et al., 2007; Takehara et al., 1994, 1999). In the late 1990s, several polymerase chain reaction (PCR)-based methods were established to detect GPV with more sensitivity and specificity in comparison to conventional methods (Chang et al., 2000; Shien et al., 2008; Yang et al., 2009). Additionally, enzyme-linked immunosorbent assay (ELISA)-based techniques have been shown to be effective in the detection of antibodies against GPV (Wang et al., 2014; Zhang et al., 2010). Recent advances in diagnostic virology have resulted in the development of a novel nucleic acid amplification-based technique (the loop-mediated isothermal amplification [LAMP]) to detect GPV, which can be completed within 60 min while under conditions of constant temperature (Yang et al., 2010, 2017). However, these detection methods are laboratory-based, and require sophisticated techniques performed by trained personnel, and thus their use outside specialized laboratories has been limited.

Recombinase polymerase amplification (RPA) is a next-generation molecular diagnostic technique similar to traditional PCR (Piepenburg et al., 2006). The technology utilizes three main enzymes: i) recombinase proteins to separate the DNA, ii) single-strand DNA binding proteins (SSB) to stabilize the open complex, and iii) polymerases to begin DNA synthesis using two opposing primers and a probe. RPA is an isothermal technique that can amplify target nucleic acid sequences with a high degree of specificity, ranging from trace levels to detectable amounts of product in less than 20 min at a low and constant temperature (37–42 °C) (Wang et al., 2018). RPA amplification products are easily detected by commercial lateral flow (LF) strips or real-time fluorescence (Yin et al., 2017). Although RPA technology has been successfully applied to the detection of viral, bacterial, and parasitic infections (Hou et al., 2017a,b; Lillis et al., 2014; Wang et al., 2017; Xu et al., 2014; Zaghloul and El-Shahat, 2014), there have been no RPA assays developed for the detection of GPV or N-GPV to date.

In the present study, we developed a protocol for the rapid and effective detection of both GPV and N-GPV using RPA combined with vertical flow (VF) visualization strips. RPA primers and LF probes were designed based on the conserved regions of the GPV *VP3* gene. The optimal amplification temperature and time for the experiments were obtained, and the analytical sensitivity and specificity of this method were evaluated using GPV and other waterfowl viruses and bacteria. Finally, the performance of the RPA assay was evaluated using clinical samples for the diagnosis of GPV and N-GPV. This method exhibited high sensitivity and specificity in both experimental and clinical tests.

2. Materials and methods

2.1. Viruses and samples

Live vaccines of GPV (GD strain), duck plague virus (AV1222 strain), and Newcastle disease (Lasota strain) were obtained from Guangdong Winsun Biological Pharmaceutical Co., Ltd (Guangzhou, China). A live vaccine of H9N2-AIV (SS strain) was obtained from South China Agricultural University Biological Pharmaceutical Co., Ltd (Guangzhou, China). Duck tembusu virus (WF100 strain) live vaccine was obtained from Qilu Animal Health Products Co., Ltd (Jinan, China). N-GPV (named QY02) was isolated in Qing-yuan in the Guangdong province of China, while *Escherichia coli* (E. coli.), *Pasteurella multocida* (P.M.), *Riemerella anatipestifer* (R.A.), and *Salmonella* spp. (S.S.) were isolated and maintained in our laboratory. The genomic DNA of Muscovy duck parvovirus (MDPV) (P strain, KU844281.1) was synthesized by Sangon Biotech Company (Shanghai, China).

Table 1
Primers and probe used in this study.

Primer name	Sequence(5'-3')
RPA-F	5'-CTT CAG GGG GTG CCG ATG GAG TGG GTA ATG -3'
Lateral flow reverse primer	5'-(biotin)GGG TCT GAT TCC CCA ATG GTT GTT GAT AAG -3'
Lateral flow probe	5'-(FAM)GGG AAA CAC AGT CAT CAC AAA GAC CAC CA(THF)AAC CTG GGT CCT GCC AAG(SPC3)-3'
G1	5'-ATCCTGTCAACACGGCACC-3'
G2	5'-CACAGAATTTACAGATTTTGAGTTA-3'
P1	5'-CCTAGAGACTGGCAGAGACTTA-3'
P2	5'-GATCCTGCGTTGTGACTTCT-3'

2.2. Extraction of DNA and RNA

Viral DNA was extracted using the TIANamp Genomic DNA Kit (TIANGEN Biotech, Beijing, China) according to the manufacturer's instructions. Bacterial genomic DNA was extracted using the EasyPure Bacteria Genomic DNA Kit (Transgen, Beijing, China). Viral RNA was extracted using the MiniBEST Universal RNA Extraction Kit (TaKaRa Bio Inc., Dalian, China) according to the manufacturer's instructions. Extracted RNA was used as a template for cDNA synthesis using primeScriptTM RT Master Mix (RR036 A; TaKaRa Bio Inc.) according to the manufacturer's instructions. The cDNA templates from all samples were stored at −80 °C until use.

2.3. Recombinant plasmid construction

The genomic DNA of GPV was extracted from GPV vaccines (GB strain) using the TIANamp Genomic DNA Kit (TIANGEN Biotech) according to the manufacturer's instructions. Target sequences containing *VP3* genes from GPV were amplified by PCR using G1 and G2 primers (Table 1). The resulting 1783 base pair (bp) PCR product was cloned into the PMD-18T plasmid according to the manufacturer's instructions using the pMD™18-T Vector Cloning Kit (TaKaRa Biotech). Next, the recombinant plasmid was transformed into DH5α-competent cells, the plasmid DNA was purified using the Plasmid Mini Kit I (Omega Bio-Tek, Georgia, USA), and the concentration of DNA was determined with a ultra-micro spectrophotometer (BioTek Epoch, Vermont, USA). Plasmids were stored at −20 °C until further use.

2.4. Design of RPA primers and specific LF probe for GPV

A highly conserved region of the GPV *VP3* gene was screened and selected using GenBank (accession nos. EU582289, AY382886, AY382888, KC996730, KR091960, KR136258, EU088102.1, EU088103.1, HQ891825.1, KU168321.1, KC996729, KT343253, KC171936, and AY512830). Six pairs of primers and LF probes were designed based on the N-GPV sequences (SDLC01, KT343253) according to the guidelines of TwistDX (TwistDx, Ltd, Cambridge, United Kingdom). The specificity and repeatability of several primer pair candidates were tested using the TwistAmp nfo kit for RPA research (TwistDx, Ltd.). The amplified products were purified using a PCR purification kit (Omega) and visualized on a 2% agarose gel to screen for optimal primers. To enable detection by LF strip, the TwistAmp LF probe-oligonucleotide backbone included a 5′-fluorescein FAM, an internal abasic nucleotide analogue (Tetrahydrofuran, THF), and a 3′-polymerase extension-blocking group (SpC3). One amplification primer opposing the TwistAmp LF probe was labeled with biotin at its 5′ end. Details of the primers used in this study are given in Fig. 1 and Table 1. All primers were synthesized and labeled by Sangon Biotech.

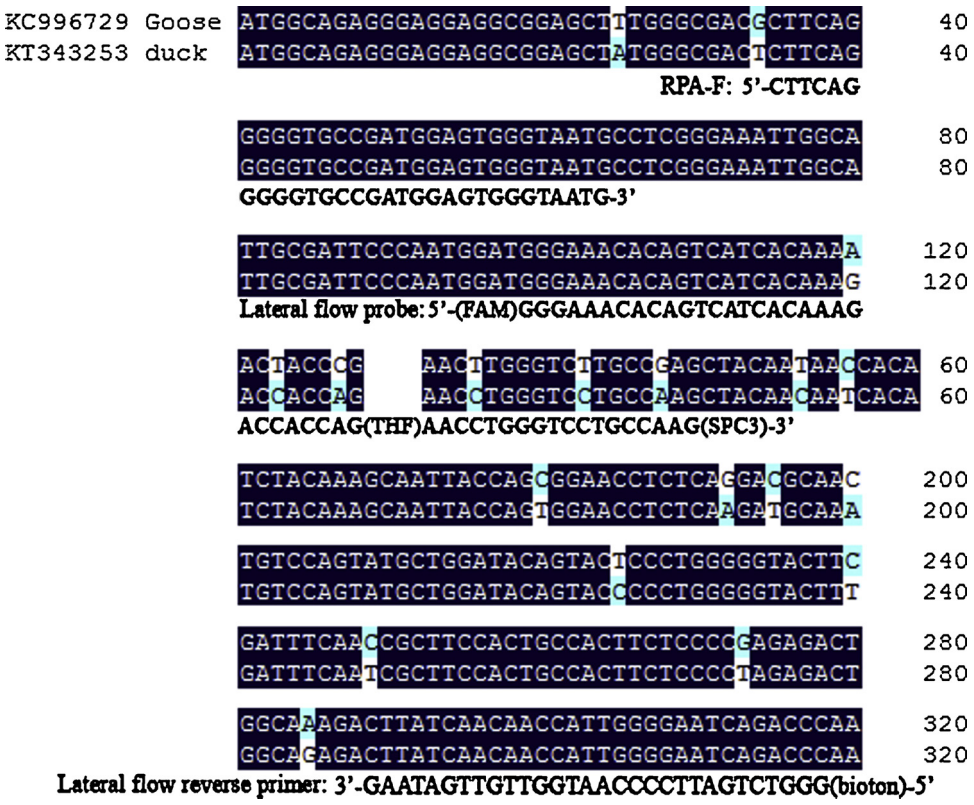

Fig. 1. Partial sequences of the *VP3* gene showing the target region of the RPA primers and probe. KC996729 is the sequence of goose source classical GPV and KT34353 is the sequence of duck source novel GPV (N-GPV).

2.5. RPA reaction and vertical flow (VF) reading

The RPA reaction was performed using the TwistAmp nfo kit (TwistDx, Ltd.) according to the manufacturer's guidelines. Briefly, the amplification mixture contained 29.5 μL of rehydration buffer, 2.1 μL of each primer (10 μM), 0.6 μL of probe (10 μM), 12.2 μL of ddH_20, and 1 μL of DNA template. A total of 47.5 μL of amplification mixture was added into reaction tubes supplied with the freeze-dried pellet, the pellet was resuspended, the reaction was initiated by adding 2.5 μL of magnesium acetate (280 mM), and samples were incubated at 37 °C for 20 min in a metal bath (Bioer, China). Following the incubation, VF visualization strip cassettes were used for the detection of the RPA product (Ustar Biotech, Hangzhou, China). Briefly, A VF nucleic acid detection strip was housed in a sealed plastic device to prevent leakage of amplifications (Chow et al., 2008). Following the incubation, the closed cartridge was inserted into the detection chamber; the handle of the detection chamber was pressed to start the VF assay, the razor blade and plastic pin fixed to the bottom of the detection chamber opened the PCR tube, and the bulb filled with the running buffer from the cartridge. Next, the amplification mixture and running buffer were mixed and added to the DNA detection strip, which coated the anti-FAM antibody and biotin as the test (T) and control (C) lines through a fiberglass paper. Next, the end of the amplified product with FAM- and biotin-labeled primers can combine with the gold nanoparticle-conjugated anti-biotin antibody, while the other end combines with the anti-FAM antibody at the T line. Meanwhile, the biotin-labeled primer amplicon combined with a gold nanoparticle-conjugated anti-biotin antibody and was captured by biotin at the C line. After 5 min of incubation at room temperature, the results were judged by visual observation from the detection window of the chamber. A positive result was indicated by a display of the T line or both the T and C lines, while a negative result displayed only the C line. An invalid score was judged when neither the T nor the C line was visible. The optimal reaction temperature and time of RPA were explored with various temperature settings (30–55 °C) and incubation times (5–25 min).

2.6. Specificity of the GPV-RPA-VF assay

The specificity of the GPV-RPA-VF assay was determined by testing a variety of viral and bacterial pathogens of waterfowl, including GPV, MDPV, duck plague virus, duck tembusu virus, Newcastle disease virus, avian influenza virus, and *E. coli*., P.M., R.A., and S.S.

2.7. Comparison of GPV-RPA-VF sensitivity with PCR and quantitative PCR (qPCR) using plasmid copies

The sensitivity of the GPV-RPA-VF assay was determined using ten-fold serial dilutions of plasmids ranging from 2×10^6 copies/μL to $2 \times 10^{\circ}$ copies/μL, where (copies/μL = concentration (ng/μL) × $6.02 \times 10^{23} \times 10^{-9}$/660 × bases). DNase-free water was used as a negative control.

The PCR and qPCR primers P1 and P2 (Table 1), which targeted a 106 bp region of the *VP3* gene, were designed according to a conserved region of the GPV genome. PCR was performed in a 25 μL reaction mixture containing 1 μL DNA, 1 × PCR buffer, 0.2 mM of each dNTP, 0.3 mM of each primer, and 30 U/mL of Taq polymerase (TaKaRa Biotech). The conditions of the PCR were as follows: 95 °C for 3 min; 35 cycles at 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s; and a final extension at 72 °C for 5 min. The products were analyzed on a 2% agarose gel stained with ethidium bromide. Lastly, qPCR was performed in a 20 μL reaction mixture using SYBR® Premix Ex Taq™ II according to the manufacturer's instructions (TaKaRa) on an ABI 7500 Real-Time PCR System (Thermo Fisher Scientific, Foster City, CA, USA) as follows: 95 °C for 2 min, then 40 cycles of 95 °C for 5 s, 55 °C for 30 s, and 72 °C for 30 s.

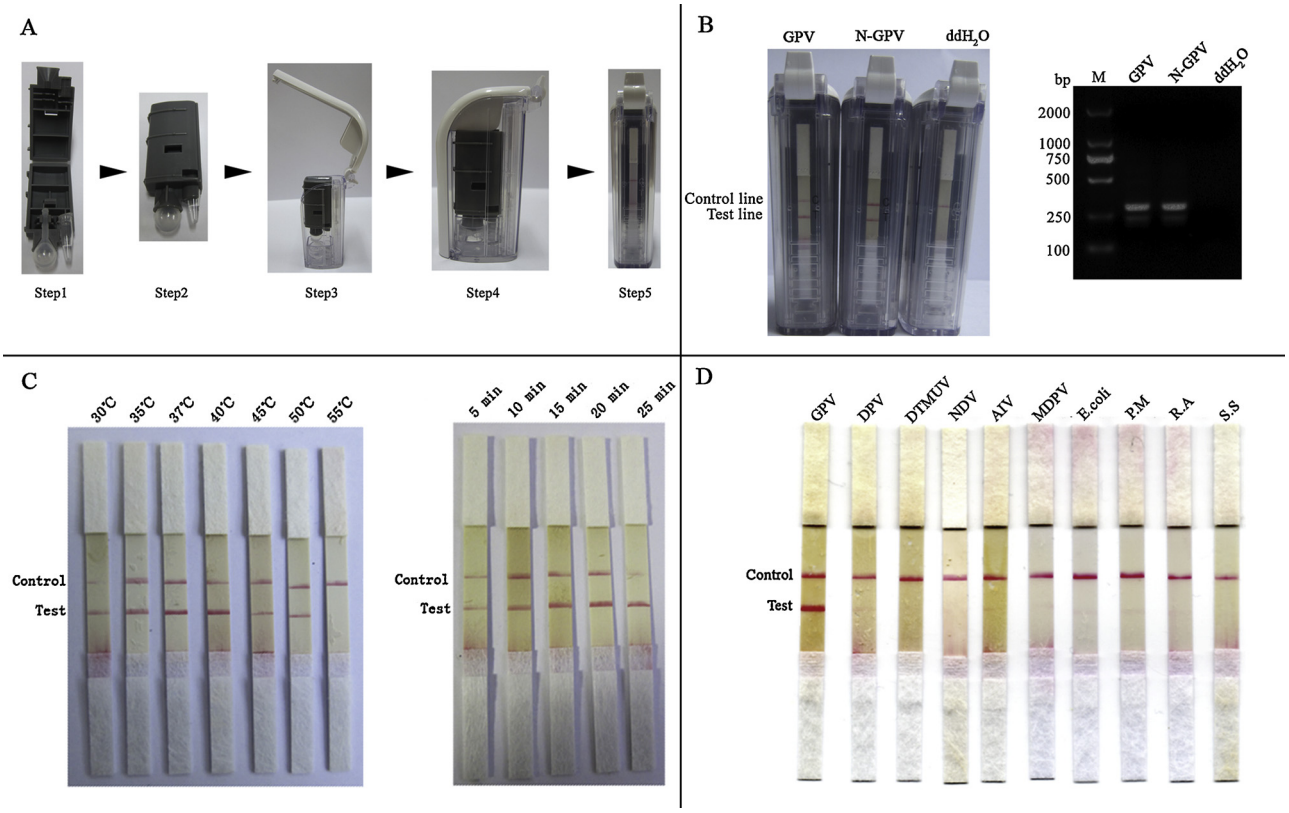

Fig. 2. Establishment of the initial GPV-RPA-VF assay. (A) The procedure for using a VF visualization strip cassette. The reaction tube was pushed transversely into the amplicon cartridge (step 1), the cartridge was closed (step 2), the amplicon cartridge was inserted into the detection chamber (step 3), the handle of the detection chamber was pressed to seal the chamber and to open the buffer bulb and reaction tube (step 4). After 5–10 min, the result can be judged by the naked eye from the detection window of the chamber (step 5). (B) Establishment of the initial GPV-RPA-VF assay, and agarose gel electrophoresis of the RPA products generated. Lane M: molecular weight standard (DNA marker 2000). The expected sizes of the products were 284 bp and 220 bp. (C) The effect of different temperatures and times on the GPV-RPA-VF assay. (D) Specificity of GPV-RPA-VF assay for different waterfowl viruses and bacteria.

2.8. Detection of clinical samples

In order to validate the potential of the GPV-RPA-VF assay for practical applications, a total of 60 clinical blood samples were collected from geese and ducks that were suspected to be infected with GPV or N-GPV from different large-scale farms in Guang-zhou Hai-ou island and Qing-yuan. All blood samples were subjected to three freeze-thaw cycles and were subsequently centrifuged at 8000 rpm at 4 °C for 30 min. Viral DNA was extracted from the supernatants using the TIANamp Genomic DNA Kit (TIANGEN Biotech) according to the manufacturer's instructions. The detection rate of the GPV-RPA-VF assay was compared with that of conventional qPCR methods.

3. Results

3.1. Development of an initial GPV-RPA-VF assay

In the initial GPV-RPA-VF assay, PCR tubes with the amplification mixtures were incubated at 37 °C for 20 min and a VF visualization strip cassette was used to evaluate the results (Fig. 2A). The positive samples with DNA from N-GPV (QY02) and GPV display a red-purple band at both the T and C lines, while the negative control displays only the C band (Fig. 2B). The RPA amplification was purified and visualized on a 2% agarose gel to verify the accuracy of the reaction, and to confirm the amplification of the expected fragments (284 bp and 220 bp) (Fig. 2B). The entire assay was completed in 30 min from the start of the RPA reaction.

3.2. Optimization of the reaction temperature and time for GPV-RPA-VF

The two critical parameters of the RPA reaction, temperature and time, were evaluated to determine the optimal conditions. We found that GPV-RPA-VF exhibited a positive band over a temperature range of 30–50 °C (Fig. 2C) and over a reaction time of 5–25 min using 20 ng of genomic DNA (Fig. 2C). A target band could be detected after as little as 5 min of incubation on the VF visualization strip cassette, and an optimal amplicon was detected after 5 min at 37 °C.

3.3. Evaluation of GPV-RPA-VF specificity

In determining the specificity of GPV-RPA-VF, no cross-reactivity was observed with the cDNA/DNA of the duck plague virus, duck tembusu virus, Newcastle disease virus, avian influenza, MDPV, *E. coli.*, P.M., R.A., or S.S., while only GPV DNA yielded a positive signal at the test line. These results indicate that the primers designed for GPV-RPA-VF reactions were specific to their selected targets (Fig. 2D).

3.4. Analytical sensitivity of GPV-RPA-VF

Sensitivity testing of the RPA assay was carried out using the GPV plasmid from 10-fold serial dilutions ranging from 2×10^6 copies/μL to 2×10^0 copies/μL. We found that the minimum viral detection limits of RPA were as low as 2×10^2 copies/μL (Fig. 3A). The amplified products of the GPV-PRA-VF reaction were also visualized by subsequent agarose gel electrophoresis (Fig. 3B), where a band observed in the lane with the 2×10^2 copies/μL dilution confirmed the limit of detection. In comparison, all templates from the 10-fold serial dilutions were

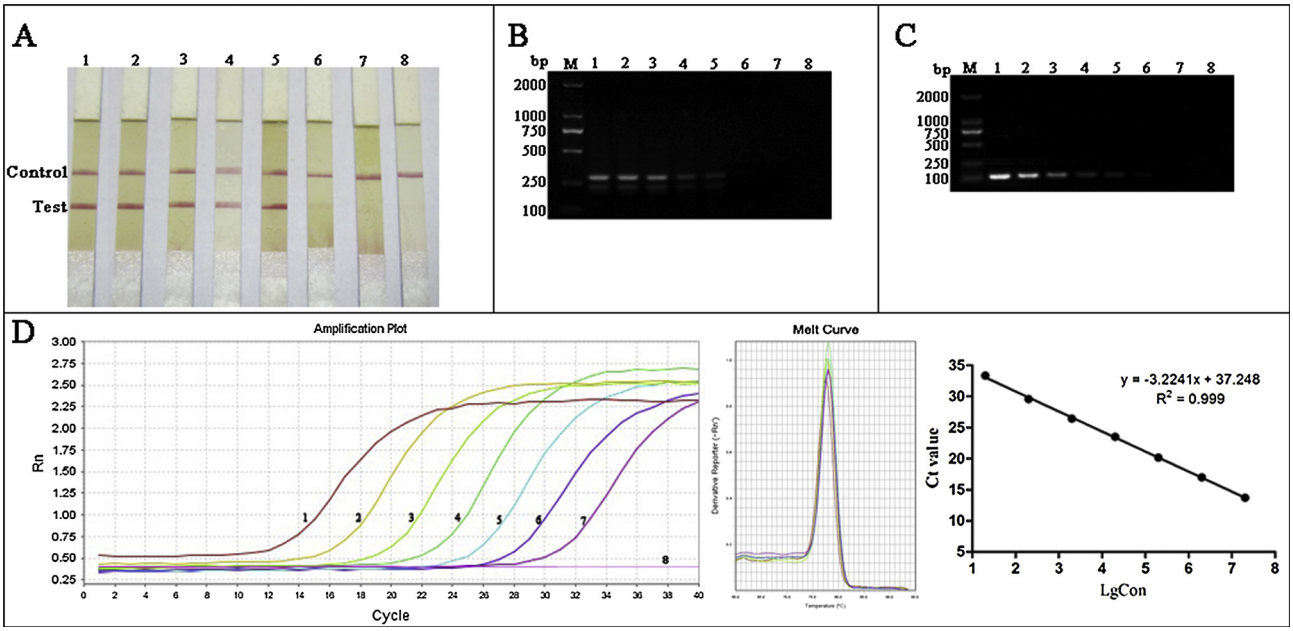

Fig. 3. Sensitivity of RPA for the detection of GPV. Ten-fold serial dilutions of GPV plasmids (2×10^6 copies/μL to 2×10^0 copies/μL) were tested by GPV-RPA-VF (A) and by agarose gel electrophoresis (B), and were compared to PCR (C) and to qPCR (D). Lines 1–7: Ten-fold serial dilutions from 2×10^6 copies/μL to 2×10^0 copies/μL; 8: No template (control/water); M: molecular weight standard (DNA marker 2000). The slope of standard cure is −3.2241, Y-intercept was 37.248, efficiency of 1.04, and the R^2 was 0.999.

detected by conventional PCR and qPCR. Our results show that the minimum virus detection limits were 2×10^2 and 2×10^0 copies/μL via PCR and qPCR, respectively (Fig. 3C and 3D). Therefore, the sensitivity of the GPV-RPA-VF assay was as sensitive as a conventional PCR assay.

3.5. Performance of GPV-RPA-VF assay on clinical samples

A total of 60 blood samples collected from the field were simultaneously detected by qPCR and RPA-VF assays. As a result, the RPA-VF assay and qPCR results were in agreement: 2 and 6 samples that were positive for N-GPV and GPV, respectively, were detected by both methods (Table 2).

4. Discussion

Waterfowl are economically important animals with a long commercial history in China. China currently has the largest waterfowl population in the world, with a production of 2.862 billion ducks and 459 million geese annually according to 2015statistics from the Food and Agriculture Organization of the United Nations (FAO). However, infectious diseases have been a hindrance to the waterfowl breeding industry, and often result in huge losses (Prosser et al., 2016). GPV is one of the most serious viral pathogens in goslings, while N-GPV—a newly discovered pathogen in China—mainly causes growth disorders in ducks and has a high infection rate in meat ducks (Li et al., 2017). Rapid and reliable detection methods are the most effective way of controlling these diseases, and although several detection methods have been established, a method for rapid testing to diagnose both GPV and N-GPV in the field is lacking.

Of the numerous detection methods, RPA is a powerful, innovative, isothermal, nucleic acid test, which has proven helpful in low-resource settings (James and Macdonald, 2015). RPA has more advantages than the diagnostic methods currently in practice for the detection of GPV, which are based on LAMP technology. First, the RPA reaction only utilizes two primers, one probe, and three binding sites, while the LAMP assay requires at least four primers and six binding sites (Notomi et al., 2000). Fewer binding sites should make detection methods more streamlined. Second, although LAMP is also an isothermal amplification technique, the optimum reaction temperatures range from 60 to 65 °C, and LAMP requires 30 to 60 min for amplification. In contrast, the RPA-VF assay can be performed in 5–20 min and can easily take place in a water bath or by using body heat. Furthermore, RPA reagents are provided in a lyophilized pellet and are stable for long periods of time (even when stored for 3 weeks at 45 °C), which allows for long-distance transportation without the need for cold transport and storage (Lillis et al., 2016). Finally, the RPA-VF assay, with its high sensitivity and specificity, does not require specific equipment and can be readily used in field conditions lacking laboratory equipment.

However, RPA detection methods still have some limitations. The most important step of RPA is the primer and probe design, and thus far, there is no primer design software available for RPA assays. It is also worth mentioning that primer and probe sets for RPA are much more stringent than for PCR, as RPA requires longer primers (30–35 bases) and probes (46–52 bases), thereby leading to the increased possibility of the formation of secondary structures. Lastly, downstream primers could easily react with probes, which may yield false positive results.

Table 2
Clinical application of GPV-RPA-VF assay.

Detection methods	clinical samples	Positive	Negative	Positive rate (%)	Conincidence rate (%)
RPA-VF	Duck blood	2	28	6.67	100
	Goose blood	6	24	20	
qPCR	Duck blood	2	28	6.67	
	Goose blood	6	24	20	

In the present study, a rapid RPA-VF assay was developed as a rapid and sensitive alternative to detect GPV and N-GPV infections in waterfowl. This is the first study to report on the development of an RPA-VF protocol for the detection of GPV or N-GPV. According to previous nucleic acid-based methods for the detection of GPV, primers were designed based on conserved regions of the *VP3* gene. In the present study, the primers were designed in such a way that they were able to accurately and simultaneously detect both GPV and N-GPV. The RPA developed here exhibited a limit of detection of 2×10^2 copies/μL, which was determined to have the same detection limit as conventional PCR. A specificity study confirmed that there was no cross-reactivity with the duck plague virus, duck tembusu virus, Newcastle disease virus, avian influenza, MDPV, *E. coli.*, P.M., R.A., or S.S. Furthermore, the clinical sensitivity of GPV-RPA-VF and PCR assays were consistent, based on 60 samples collected in the field. Finally, GPV-RPA-VF detection can be performed in the field, completed in 10 min using only body heat, the results can be directly observed on the VF using the naked eye, and are easily interpreted by people with minimal training.

5. Conclusions

In conclusion, the GPV-RPA-VF protocol described in the present study represents a rapid, highly sensitive, specific, and reliable diagnostic method for the detection of both GPV and N-GPV. In comparison to PCR and other assays, the RPA-VF assay does not require special equipment, is very easy to perform, and detection can be completed in 10 min at 37 °C. This protocol provides an important diagnostic tool for the detection of both GPV and N-GPV infections, and can be used in both laboratory and field settings.

Ethics approval and consent to participate

All the experiments were carried out according to the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by Medical Experimental Animal Center of Guangdong Province (Permit Number: 12-179).

Competing interests

The authors declare that they have no competing interests.

Acknowledgments

This work was supported by the National Key Technologies R & D Program of China (Nos. 2016YFD0500510); Science and Technology Planning Project of Guangdong Province (Nos. 2017A020208069), Guangzhou Science and Technology Plan (Nos. 201804010412), Natural Science Foundation of Guangdong Province (Nos. 2018A030313630), Guangdong Province University Youth Talent Innovation Project (Nos. 20161076).

References

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.
Chen, H., Dou, Y., Tang, Y., Zheng, X., Niu, X., Yang, J., Yu, X., Diao, Y., 2016a. Experimental reproduction of beak atrophy and dwarfism syndrome by infection in cherry valley ducklings with a novel goose parvovirus-related parvovirus. Vet. Microbiol. 183, 16–20.
Chen, H., Tang, Y., Dou, Y., Zheng, X., Diao, Y., 2016b. Evidence for vertical transmission of novel duck-origin goose parvovirus-related parvovirus. Transbound. Emerg. Dis. 63, 243–247.
Chow, W.H., McCloskey, C., Tong, Y., Hu, L., You, Q., Kelly, C.P., Kong, H., Tang, Y.W., Tang, W., 2008. Application of isothermal helicase-dependent amplification with a disposable detection device in a simple sensitive stool test for toxigenic Clostridium difficile. J. Mol. Diagn. 10, 452–458.
Derzsy, D., 1967. A viral disease of goslings. I. Epidemiological, clinical, pathological and aetiological studies. Acta Vet. Acad. Sci. Hung. 17, 443.
Derzsy, D., Drén, C., Szed, M., Surján, J., Tóth, B., Iró, E., 1970. Viral disease of goslings. 3. Isolation, properties and antigenic pattern of the virus strains. Acta Vet. Acad. Sci. Hung. 20, 419.
Hou, P., Wang, H., Zhao, G., He, C., He, H., 2017a. Rapid detection of infectious bovine Rhinotracheitis virus using recombinase polymerase amplification assays. BMC Vet. Res. 13, 386.
Hou, P., Zhao, G., Wang, H., He, C., Huan, Y., He, H., 2017b. Development of a recombinase polymerase amplification combined with lateral-flow dipstick assay for detection of bovine ephemeral fever virus. Mol. Cell. Probes 38, 31–37.
James, A., Macdonald, J., 2015. Recombinase polymerase amplification: emergence as a critical molecular technology for rapid, low-resource diagnostics. Expert Rev. Mol. Diagn. 15, 1475–1489.
Li, P., Lin, S., Zhang, R., Chen, J., Sun, D., Lan, J., Song, S., Xie, Z., Jiang, S., 2017. Isolation and characterization of novel goose parvovirus-related virus reveal the evolution of waterfowl parvovirus. Transbound. Emerg. Dis. 65, e284–e295.
Li, P., Li, J., Zhang, R., Chen, J., Wang, W., Lan, J., Xie, Z., Jiang, S., 2018. Duck "beak atrophy and dwarfism syndrome" disease complex: interplay of novel goose parvovirus-related virus and duck circovirus? Transbound. Emerg. Dis. 65, 345–351.
Lillis, L., Lehman, D., Singhal, M.C., Cantera, J., Singleton, J., Labarre, P., Toyama, A., Piepenburg, O., Parker, M., Wood, R., Overbaugh, J., Boyle, D.S., 2014. Non-instrumented incubation of a recombinase polymerase amplification assay for the rapid and sensitive detection of proviral HIV-1 DNA. PLoS One 9, e108189.
Lillis, L., Siverson, J., Lee, A., Cantera, J., Parker, M., Piepenburg, O., Lehman, D.A., Boyle, D.S., 2016. Factors influencing Recombinase polymerase amplification (RPA) assay outcomes at point of care. Mol. Cell. Probes 30, 74–78.
Niu, X., Chen, H., Yang, J., Yu, X., Ti, J., Wang, A., Diao, Y., 2016. Development of a TaqMan-based real-time PCR assay for the detection of Novel GPV. J. Virol. Methods 237, 32–37.
Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., 2000. Loop-mediated isothermal amplification of DNA. Nucleic Acids Res. 12, E63.
Piepenburg, O., Williams, C.H., Stemple, D.L., Armes, N.A., 2006. DNA detection using recombination proteins. PLoS Biol. 4, e204.
Poonia, B., Dunn, P.A., Lu, H., Jarosinski, K.W., Schat, K.A., 2007. Isolation and molecular characterization of a new Muscovy duck parvovirus from Muscovy ducks in the USA. Avian Pathol. 35, 435–441.
Prosser, D.J., Palm, E.C., Takekawa, J.Y., Zhao, D., Xiao, X., Li, P., Liu, Y., Newman, S.H., 2016. Movement analysis of free-grazing domestic ducks in Poyang Lake, China: a disease connection. Int. J. Geogr. Int. Sci. 30, 869–880.
Shien, J.H., Wang, Y.S., Chen, C.H., Shieh, H.K., Hu, C.C., Chang, P.C., 2008. Identification of sequence changes in live attenuated goose parvovirus vaccine strains developed in Asia and Europe. Avian Pathol. 37, 499–505.
Takehara, K., Hyakutake, K., Imamura, T., Mutoh, K., Yoshimura, M., 1994. Isolation, identification, and plaque titration of parvovirus from Muscovy ducks in Japan. Avian Dis. 38, 810–815.
Takehara, K., Nishio, T., Hayashi, Y., Kanda, J., Sasaki, M., Abe, N., Hiraizumi, M., Saito, S., Yamada, T., Haritani, M., Et, A., 1995. An outbreak of goose parvovirus infection in Japan. J. Vet. Med. Sci. 57, 777–779.
Takehara, K., Nakata, T., Takizawa, K., Limn, C.K., Mutoh, K., Nakamura, M., 1999. Expression of goose parvovirus VP1 capsid protein by a baculovirus expression system and establishment of fluorescent antibody test to diagnose goose parvovirus infection. Arch. Virol. 144, 1639–1645.
Wang, Q., Ju, H., Li, Y., Jing, Z., Guo, L., Zhao, Y., Ma, B., Gao, M., Zhang, W., Wang, J., 2014. Development and evaluation of a competitive ELISA using a monoclonal antibody for antibody detection after goose parvovirus virus-like particles (VLPs) and vaccine immunization in goose sera. J. Virol. Methods 209, 69–75.
Wang, S., Cheng, X.X., Chen, S.L., Xiao, S.F., Chen, S.Y., Lin, F.Q., Wu, N.Y., Yu, F.S., Zhu, X.L., Wang, J.X., Cheng, Y.Q., 2016. Identification of a novel goose parvovirus (GPV) recombinant associated with short beak and dwarfism syndrome in Mainland China, 2015. Infect. Genet. Evol. 41, 289–291.
Wang, J., Wang, J., Li, R., Liu, L., Yuan, W., 2017. Rapid and sensitive detection of canine distemper virus by real-time reverse transcription recombinase polymerase amplification. BMC Vet. Res. 13, 241.
Wang, J., Wang, J., Li, R., Shi, R., Liu, L., Yuan, W., 2018. Evaluation of an incubation instrument-free reverse transcription recombinase polymerase amplification assay for rapid and point-of-need detection of canine distemper virus. J. Virol. Methods 260, 56–61.
Xiao, S., Chen, S., Cheng, X., Lin, F., Wang, S., Zhu, X., Yu, B., Huang, M., Wang, J., Wu, N., Zheng, M., Chen, S., Yu, F., 2017. The newly emerging duck-origin goose parvovirus in China exhibits a wide range of pathogenicity to main domesticated waterfowl. Vet. Microbiol. 203, 252–256.
Xu, C., Li, L., Jin, W., Wan, Y., 2014. Recombinase polymerase amplification (RPA) of CaMV-35S promoter and nos terminator for rapid detection of genetically modified crops. Int. J. Mol. Sci. 15, 18197–18205.
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. 21, 7–14.
Yang, J., Chen, H., Wang, Z., Yu, X., Niu, X., Tang, Y., Diao, Y., 2017. Development of a quantitative loop-mediated isothermal amplification assay for the rapid detection of novel goose parvovirus. Front. Microbiol. 8, 2472.
Yin, F., Liu, J., Liu, A., Li, Y., Luo, J., Guan, G., Yin, H., 2017. Rapid diagnosis of Theileria

annulata by recombinase polymerase amplification combined with a lateral flow strip (LF-RPA) in epidemic regions. Vet. Parasitol. 237, 125–129.
Yu, K., Ma, X., Sheng, Z., Qi, L., Liu, C., Wang, D., Huang, B., Li, F., Song, M., 2016. Identification of goose-origin parvovirus as a cause of newly emerging beak atrophy and dwarfism syndrome in ducklings. J. Clin. Microbiol. 54, 1999–2007.
Zaghloul, H., El-Shahat, M., 2014. Recombinase polymerase amplification as a promising tool in hepatitis C virus diagnosis. World J. Hepatol. 6, 916–922.
Zhang, Y., Zhang, D., Li, N., Li, G., Li, Y., Liu, S., Liu, M., Liu, C., Guo, D., Zhi, H., Wang, X., 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.