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A lateral flow dipstick combined with reverse transcription recombinase polymerase amplification for rapid and visual detection of the bovine respirovirus 3

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ABSTRACT

Bovine respirovirus 3 also known as Bovine parainfluenza virus type 3 (BPIV3) is one of the most important viral respiratory agents of both young and adult cattle. Rapid diagnosis could contribute greatly in containing epidemics and thus avoid economic losses. However, the lack of robust isothermal visual method poses difficulty. In this study, a novel isothermal assay for detecting BPIV3 was established. The method includes a lateral flow dipstick (LFD) assay combined with reverse transcription recombinase polymerase amplification (RT-RPA). First, the analytical sensitivity and specificity of BPIV3 LFD RT-RPA were tested. The LFD RT-RPA assay has a detection limit of up to 100 copies per reaction in 30 min at 38 °C. Then the performance of LFD RT-RPA was evaluated using 95 clinical samples. Compared to qPCR, the LFD RT-RPA assay showed a clinical sensitivity of 94.74%, a clinical specificity of 96.05% and 0.8734 kappa coefficient. These results have demonstrated the efficiency and effectiveness of the method to be developed into a point of care protocol for the diagnosis of BPIV3.

1. Introduction

Bovine respirovirus 3 also known as Bovine parainfluenza virus type 3 (BPIV3) is a member of the *Paramyxoviridae*, genus *Respirovirus*. BPIV3 is one of the most important viral respiratory agents of both young and adult cattle [1]. While most acute infections are subclinical, they can cause respiratory disease characterized by cough, fever and nasal discharge [2]. In some instances where animals are also subjected to high stress, resulting in severe bronchopneumonia from secondary bacterial infections [3]. A variety of factors, such as environmental temperature, transportation, hygiene, stocking density, co-mingling and host immune status, can contribute to increased susceptibility to secondary bacterial infection and severity of clinical disease [4]. BPIV3 has been associated with the bovine respiratory disease complex (BRDC) [4,5], which is a major health problem of cattle worldwide. Thus, early detection of clinical infection is important as it can facilitate more rapid implementation of rigorous controls, which can result in reduced health care costs and improved cure rates.

The common and widely accepted molecular technique for detecting BPIV3 is reverse transcriptase polymerase chain reaction (RT-PCR) or quantitative RT-PCR (RT-qPCR) [6,7]. However, this method requires thermal cycling equipment and the settings of professional diagnostic laboratory. Moreover, most nucleic acid amplification technologies assays, including many isothermal amplification methods, require power-dependent instrumentation for incubation [8]. With a reaction time of 1–2 h, these can also be fairly time-consuming. Recently, recombinase polymerase amplification (RPA), a novel isothermal technique, has emerged as an ideal molecular technology for rapid and economical diagnostics [9]. With the development of nucleic acid purification technology independent of a laboratory, a lateral flow dipstick (LFD) combined with a RT-RPA assay is especially suitable for on-site diagnosis of clinical specimens. The commercial reverse transcriptase kits (PrimeScript RT Master Mix; TaKaRa, Dalian, China) incubate the samples for 15 min at 37 °C. The RPA tolerates temperatures ranging from 30 to 42 °C without losing reaction efficiency and has been so far already successfully used to construct rapid detection of

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Table 1
Recombinase polymerase amplification (RPA) primers and probes designed in this study.

Name	Sequence (5′-3′)	Genome location	Amplification size (bp)
F1	TTGGTACATCAATACAATCGGGAATAAACAC	260–290	303/421
F2	AATTATATTCCATTATCTTTGACACAACAA	322–351	241/359
F3	TTTATCAATGAATTAGCAAACAAGAGAGAT	370–399	193/311
LF	[FAM]GAGATGTACCTCTGGCAATCCATCCCTGACAAG [dSpacer]AACCCAAAGATAAGAC [C3-spacer]	471–520	92/210
R1	[Biotin]TTACAGTGGTAGATGCTGCTAACAGACTAG	533–562	
R2	[Biotin]CCGATTTGTAATACTTGATAAGACTTCCCT	651–680	

Note: F: forward primer; R: reverse primer; LF: probe; FAM: 6-Carboxyfluorescein; dSpacer: an exonuclease site; C3 Spacers: a polymerase extension blocking site.

many pathogens [8,10–13].

In this study, we established a LFD RT-RPA assay for detection of BPIV3 using oligonucleotides targeting a conserved region of *HN* gene [6,14]. Furthermore, we evaluated the detection sensitivity, specificity and performance of LFD RT-RPA assay when compared with the RT-PCR or qPCR methods to assess its performance and reliability as a potential point-of-care field test.

2. Materials and methods

2.1. Viruses and clinical specimens

Bovine respirovirus 3 (Bovine parainfluenza virus type 3, BPIV3)/BN-1 strain, Bovine alphaherpesvirus 1 (infectious bovine rhinotracheitis virus, IBRV)/BarthaNu/67 strain, Pestivirus A (bovine viral diarrhea virus 1, BVDV1)/NADL strain were preserved in our laboratory, and the viruses were cultured following previously published procedure in our laboratory [11]. Other bovine virus strains used in this study were provided by full-length cloned cDNA: entire genome sequence of bovine orthopneumovirus (bovine respiratory syncytial virus, BRSV)/A51908strain, bovine fever ephemerovirus (bovine ephemeral fever virus, BEFV)/(Accession number: JX234571.1) [15], Betacoronavirus 1 (bovine coronavirus, BcoV)/ENT strain (Accession number: NC_003045.1), respectively. The cDNA of Indiana vesiculovirus (vesicular stomatitis virus, VSV) was kindly provided by Wenqi He (Jilin University, China).

A total of 95 clinical specimens (62 nasal swabs and 33 tissue specimens) were collected between January 2017 and February 2018 from 62 cattle from 17 different dairy farms suspected to be infected with bovine respiratory disease (BRD). The 17 farms were located in seventeen distinct geographic regions [16,17] of Shandong province, China. A respiratory disease score was assigned based on rectal temperature, the character of nasal discharge, eye or ear appearance, and presence of a cough based on the method by Lago et al. (2006) [18]. Cattle with a score of 6 or higher had at least two clinical signs of respiratory disease and were thus considered sick, and the 62 nasal swab samples taken from all 17 dairy farms in which case suggests after BRD. 33 fresh lung samples were sampled from postmortem calf with BRD.

2.2. Isolation of DNA/RNA, cDNA synthesis

DNA of IBRV and RNA of BVDV, BPIV3 was extracted following **a** previously published procedure in our laboratory [12,19]. The DNA/RNA was eluted in 50 μL of nuclease-free water. The extracted RNA was used as template for cDNA synthesis using reverse transcription with random primers according to the instructions of the PrimeScript RT Master Mix (TaKaRa, Dalian, China). All templates were stored at −70 °C until further needed.

2.3. Generation of RNA molecular standard

The BPIV3 *HN* gene (1719 bp) was cloned into pGEM-T Easy vector (Promega, USA). RNA molecular standard was prepared as previously described [20] with some modifications. In brief, the recombinant plasmid was linearized by *Nde* I (Thermo Fisher Scientific, USA), and purified using the MiniBEST DNA Fragment Purification Kit (TaKaRa, Dalian, China). A total of 1 μg of linearized product was used for in vitro transcription (T7 RiboMAX Large Scale RNA Production System, Promega, USA) following the manufacturer's instructions. In vitro transcribed BPIV3 RNA was digested with the supplied RNase-free DNase and purified. The purified RNA was quantified with the Quant-iTTM RiboGreen RNA Assay Kit (Thermo Fisher Scientific, USA) in accordance with the manufacturer's instructions. The copy number of RNA molecules was calculated by the following formula: Amount (copies/μL) = [RNA concentration (g/μL)/(transcript length in nucleotides × 340)] × 6.02 × 10^{23}.

2.4. Design of RPA primers and LF probe

By alignment analysis of the *HN* gene with the data from NCBI/GenBank (D84095.1; AF178655.1; NC_002161.1; JQ063064.1; EU277658.1; HQ530153.1; JX969001.1; AB770484.1; LC000638.1), six combinations of candidate primers (3 forward and 2 reverse) and one TwistAmp LF-probe were designed according to RPA operating instructions of TwistDx (TwistDx, Cambridge, UK), and synthesized by Sangon Biotech (Shanghai, China). In summary, the TwistAmp LF Probe constitutes of an oligonucleotide backbone, which includes a 5′-antigenic label FAM group, an internal abasic nucleotide analogue "dSpacer" and a 3′-polymerase extension-blocking group C3-spacer. One amplification primer opposing TwistAmp LF Probe is labeled with Biotin at its 5′ end in order for the dual-labeled amplicon to be detected simultaneously. Oligonucleotide sequences of RPA primers and LF probes used in the study are listed in Table 1 and Fig.S1.

2.5. BPIV3 LFD RT-RPA assays

Reaction of RPA and visualization of RPA amplicons were carried out following previously published procedure in our laboratory [12,13]. The BPIV3 RPA was performed in a 50 μL final reaction volume according to instructions outlined in the TwistAmp info kit (TwistDX, Cambridge, UK). In brief, the rehydration solution contained 1 μL DNA template, 2.1 μL (10 μM) forward primers and reverse primers, respectively, 0.6 μL (10 μM) TwistAmp LF Probe. All test samples were incubated for a prototypical 25 min at 38 °C. The tubes were incubated at 38 °C in an incubator block for 4 min. As recommended, samples were blended top down and bottom up 6–8 times after 4 min incubation, and an additional incubation was continued for 21 min. Then RPA products were purified using MiniBEST DNA fragment purification kit (TaKaRa, Dalian, China). A 2% agarose-gel electrophoresis was performed to determine the size of the products. Visualization of the amplicons was carried out using a LFD tool kit (HybriDetect, Milenia Biotec GmbH, Germany): 2 μL of RPA product and 98 μL HybriDetect assay buffer were blended in a 200 μL centrifuge tube, then the dipstick was put directly into the buffer and maintained at an upright position for 5 min under room temperature.

To determine the optimal reaction condition, the preferred temperature (30 °C, 34 °C, 38 °C, 42 °C, 45 °C and 48 °C) and time (5 min,

10 min, 15 min, 20 min, 25 min and 30 min) for the BPIV3 LFD RT-RPA reaction were also evaluated.

2.6. Analytical of sensitivity and specificity of the assay

To assess the detection limit of BPIV3 genomic copies, the in vitro transcribed RNA was diluted in a 10-fold serial dilution manner to achieve RNA concentrations ranging from 10^7 to 10^0 copies/μL. One μL of each dilution was amplified by RT-RPA reactions within the same sample run to determine the analytical sensitivity of the assay. The analytical specificity of the assay was assessed among other viral pathogens of cattle. DNA of IBRV, RNA of BVDV and BPIV-3 were prepared from cell culture supernatant, and cDNA of BVDV, BPIV-3 was prepared as above mentioned. Clone of full-length genome of BEFV, BRSV, BcoV and VSV were supplied as templates in the LFD RT-RPA reaction. Additionally, 10^4 copies RNA molecular standard per reaction and BPIV3-free samples was used as a positive control and a negative control respectively.

2.7. Clinical specimen preparation

BPIV3-free nasal swab specimens were obtained from healthy calves for negative control. A total of 95 clinical specimens including 62 clinical nasal swabs and 33 lung tissue specimens were collected from suspected dairy cattle cases of BPIV3 infections in Shandong Province, China. Details of clinical specimen were listed in Table 2. The extraction of RNA from clinical specimens and cDNA synthesis was prepared following previously published procedure in our laboratory [12]. The volume of 1 μL of cDNA extracted from each specimen was used as a template in the LFD RT-RPA reactions.

2.8. Real-time qPCR and RT-PCR for amplification of BPIV3

The RT-qPCR assay was performed on a LightCycler 480 II (Roche, Germany) using the Probe qPCR Mix (TaKaRa, Dalin, China) with the primers and probe described by Horwood and Mahony [21]. Briefly, the primer pairs for real-time qPCR used for amplification of BPIV3 were BPIV3-F: 5′-TGTCTTCCACTAGATAGAGGGATAAAATT-3′, BPIV3-R: 5′-GCAATGATAACAATGCCATGGA-3′, and probe: 5′-FAM-ACAGCAAT TGGATCAATAA-MGB-BHQ2-3'. The reaction was prepared as a 20 μL reaction volume containing 1 μL of the cDNA template, 200 nM of each primer, 200 nM of each probe, 2 × Probe qPCR Mix, and sterile DNase-free water. The following thermal cycling parameters: initial denaturation at 95 °C for 30 s, followed by 40 cycles of 94 °C for 5 s, and 60 °C for 75 s followed by a final cooling step at 40 °C for 30 s. The results were analyzed by gene scanning software version 1.5 (Roche, Germany). The assessment of clinical samples was also carried out by RT-PCR assay following previously described by our laboratory [12]. Two methods were operated with the same amount of template.

3. Results and discussion

Firstly, the analytical specificity of RPA primers and LF-probes was performed by agarose-gel electrophoresis consulting recent studies [12]. We've especially noticed that when LF-probe is cut by nfo nuclease at the dSpacer position, the probe will be transformed into a primer for extension [9]. Therefore, the RPA product consists of two kinds: one amplified from the common F and R primers, and the other from the LF-probe and R. Two bands were thus observed in the electrophoresis (Fig. 1A). Experiments have found that primer set F3/LF/R2 yielded highest amplification accuracy, producing products with an expected size of 311/210 base pairs (Fig. 1A). This set also yielded a faster and darker LFD RT-RPA test line than others within 5 min (Fig. 1B). Following reagent instruction (TwistDX), a temperature range of 30–48 °C, and a time range of 5–30 min were tested. Results indicated that the reaction adapts to a wide temperature interval from 30 to 45 °C, with the test band being the brightest in 38 °C (Fig. 1C). Therefore, 38 °C was chosen as the approved reaction temperature for all latter LFD RT-RPA assays. As for time, distinct band could be seen in the test zone position for a range of 10–30 min (Fig. 1D). Based on the results, time of incubation was controlled in 30 min in following LFD RT-RPA testing.

In addition, analytical sensitivity of BPIV3 LFD RT-RPA was performed using the quantity of template (10-fold serial diluted, 10^7 to 10^1 copies per reaction). This result revealed that the minimum virus detection limits of LFD RT-RPA assay were as low as 100 copies per reaction (Fig. 2A and B). Next, the analytical specificity of the LFD RT-RPA was tested among other viral pathogens of cattle with similar clinical signs including BcoV, BRSV, BVDV, IBRV, BEFV and VSV. The results indicated that no cross-reactions of the BcoV, BRSV, BVDV, IBRV, BEFV and VSV were observed on the dipstick within 5 min (Fig. 3A and B). To check the quality of RNA/DNA preparations used for the specificity of the assay, we undertook PCR reaction firstly with positive primer of corresponding template respectively on 2% agarose gel (Fig. 3C). The viral specific primers used in this study were following previously published by our laboratory [12].

Finally, the diagnostic performance of the LFD RT-RPA was compared to the RT-PCR or real-time qRT-PCR method by their respective sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and kappa coefficient. Results showed that LFD RT-RPA has a little higher sensitivity comparing with RT-PCR assay, and the positive rate was 22.11% (21/95) and 15.79% (15/95) (Table 2), respectively. When compared with the real-time qPCR method, the established LFD RT-RPA has similar sensitivity comparing with qPCR assay. The positive rate of LFD RT-RPA and qPCR assays was 22.11% (21/95) and 20.00% (19/95) (Table 3), respectively, and yielded 100% sensitivity, 67.19% specificity and 100% negative predictive value (NPV) and 0.5720 kappa coefficient.

The LFD RT-RPA assay has the advantages of rapidness: nucleic acid amplification can be completed within 20–30 min; convenience: operating at a lower temperature (RT: 37 °C; RPA: 38 °C), and economical value: lower dependence on thermal cycling equipment and specialized operations. With the combination of nucleic acid purification technology independent of a laboratory, LFD RT-RPA assay may be suggested as an alternative point-of-care diagnostics in resource limited settings [22,23]. Since for the major part of the reaction, simple heat source system, such as chemical heaters, water bath or even body heat could be employed [22–24]. These partial simulations of conditions in the field have all posed the future potentials of LFD RT-RPA assay as a rapid, efficient and exact field test for diagnosis of BPIV3.

Table 2
Comparative performances of RT-PCR, real-time qPCR and LFD RT-RPA assays for detection of suspected BPIV3 infectious clinical specimens.

Samples	Number of samples	RT-PCR		Real-time qPCR		LFD RT-RPA	
		Positive	Negative	Positive	Negative	Positive	Negative
nasal swabs	62	9	53	11	51	13	49
fresh lungs	33	6	27	8	25	8	25
Total	95	15	80	19	76	21	74

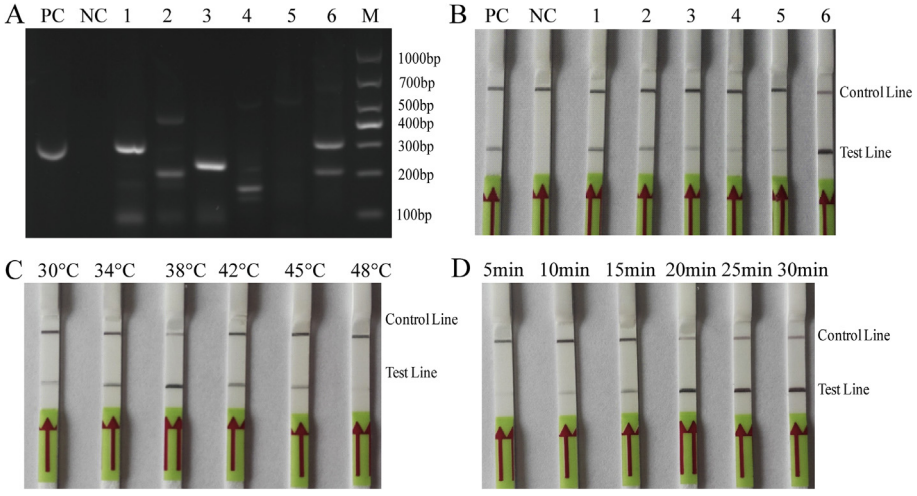

Fig. 1. Optimization of BPIV3 LFD RT-RPA assay. (A) Agarose gel electrophoresis of RPA products generated using designed primers/probes. Lane M: molecular weight standard (DNA Marker 1000). Lane PC: positive control (supplied by Twist Amp nfo kit); Lane NC: negative control (DNase-free water); Lane 1 to 6 was designed primer and probe sets: F1/LF/R1, F1/LF/R2, F2/LF/R1, F2/LF/R2, F3/LF/R1, and F3/LF/R2, respectively. Specifically, Lane 6 was primers/probe set F3/LF/R2, and the expected size of the product was 311/210 bp. (B) Lateral-flow strip end-point analysis of RPA products generated by using six set of primers and probe combination. Lane PC: positive control (supplied by Twist Amp nfo kit); Lane NC: negative control (DNase-free water); Lane 1 to 6: BPIV3 amplicons performed with RPA primers and probe combination, the order was the same as Lane 1 to 6 of (A). (C) Optimization of incubation temperature for BPIV3 LFD RT-RPA assay. The amplification performance of LFD RT-RPA was effectively within the range of 30 °C–45 °C. Samples were tested in triplicate with one reaction displayed in figure for each triplicate. (D) Optimization of incubation reaction time for BPIV3 LFD RT-RPA assay. After 10 min of amplification reaction, the test line was clearly visible on the strip. Samples were tested in triplicate with one reaction displayed in figure for each triplicate.

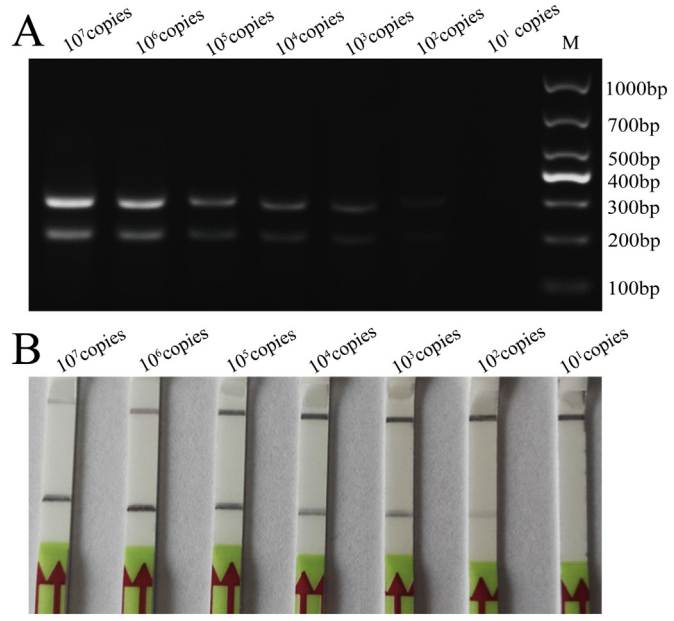

Fig. 2. Testing the BPIV3 LFD RT-RPA assay for analytical sensitivity. (A) Sensitivity of the LFD-RPA assay. Molecular sensitivity test results of LFD RT-RPA using 10-fold serially diluted template of RNA molecular standard. BPIV3 templates of lane 1 to 7 in these reactions ranged from 10^7 to 10^1 copies per reaction, respectively. Samples were tested in triplicate with one reaction displayed in figure for each triplicate. (B) BPIV3 RPA reaction products were detected on a 2% agarose gel. BPIV3 templates of Lane 1 to Lane 7 in these reactions ranged from 10^7 to 10^1 copies per reaction, respectively.

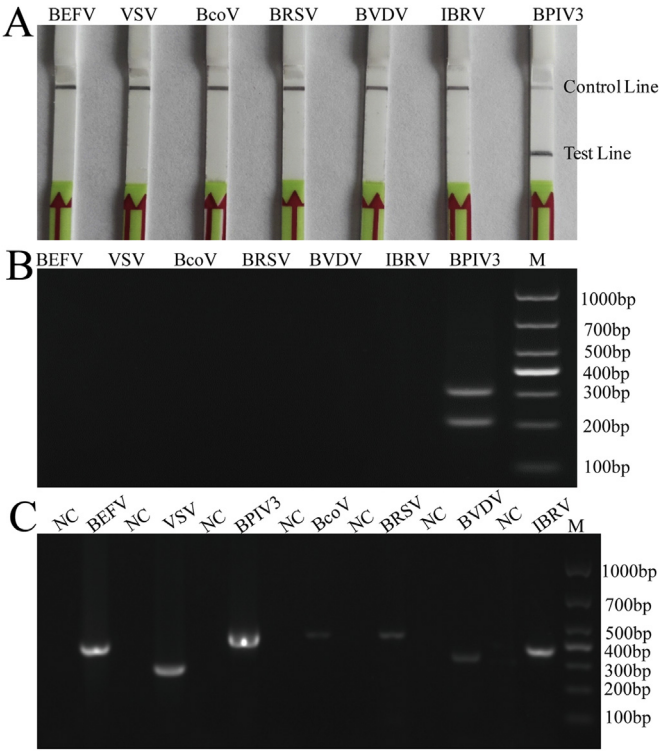

Fig. 3. Testing the BPIV3 LFD RT-RPA assay for analytical specificity. (A) Specificity of the LFD RT-RPA assay. RPA products detected using LFD assay yield visually positive results only when tested using cDNA synthesis from BPIV3; results are visually negative for all other bovine viral pathogens with similar clinical symptoms. Samples were tested in triplicate with one reaction displayed in figure for each triplicate. (B) The results of amplification products of the LFD RT-RPA on 2% agarose gel. Lanes 1 to 6: BEFV, VSV, BcoV, BRSV, BVDV, and IBRV, respectively; Lane 7: positive control of BPIV3. (C) Quality detection of RNA/DNA of BEFV, VSV, BcoV, BRSV, BVDV, IBRV and BPIV3. The RNA/DNA of different virus was prepared for specificity detection by PCR reaction with viral specific primers. The positive amplification results were shown in Lane 2, Lane 4, Lane 6, Lane 8, Lane 10, Lane 12, Lane 14, respectively. Lane 1, Lane 3, Lane 5, Lane 7, Lane 9, Lane 11, Lane 13 were negative controls with DNase-free water as template.

4. Conclusions

A LFD RT-RPA assay has been developed for the rapid detection of BPIV3 and by comparison with a previously published qPCR its performance **was** shown to be comparable. The LFD RT-RPA could be potential applicability in the field where simple and rapid diagnosis of BPIV3 is required, especially in the resource-limited settings.

Ethics approval and consent to participate

Experimental protocols for obtaining cattle clinical samples used in this study were carried out in strict accordance with the Chinese Regulations of Laboratory Animals (Ministry of Science and Technology of People's Republic of China, 20110108), and the animal study

Table 3
Sensitivity, specificity, predictive value, and kappa value of LFD RT-RPA and RT-PCR or real-time qPCR methods for diagnosing BPIV3 infection.

		RT-PCR			Real-time qPCR		
		P	N	T	P	N	T
LFD RT-RPA	P	15	6	21	18	3	21
	N	0	74	74	1	73	74
	T	15	80	95	19	76	95
		Se:100%	Sp:92.50%	K:0.7957	Se:94.74%	Sp:96.05%	K:0.8734
		PPV:71.43%	NPV: 100%		PPV:85.71%	NPV:98.65%	

Note: P, Positive; N, Negative; T, Total; Se, Sensitivity; Sp: Specificity; K: Kappa value; PPV: Positive predictive value; NPV: negative predictive value.

proposal was approved by Shandong Normal University Animal Care and Use Committee (approval No. 20160901).

Conflicts of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors' contributions

HBH and XXZ conceived and designed the experiments. ZGM and WHM performed the experiments. ZGM, WHM, and HPL analyzed the data. ZGM and HBH wrote the paper. All the authors read and approved the final manuscript.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.mcp.2018.08.004.

References

[1] J.A. Ellis, Bovine parainfluenza-3 virus, Vet. Clin. N. Am. Food Anim. Pract. 26 (2010) 575–593.
[2] J.D. Neill, J.F. Ridpath, B.T. Valayudhan, Identification and genome characterization of genotype B and genotype C bovine parainfluenza type 3 viruses isolated in the United States, BMC Vet. Res. 11 (2015) 112.
[3] E.J. Haanes, P. Guimond, R. Wardley, The bovine parainfluenza virus type-3 (BPIV-3) hemagglutinin/neuraminidase glycoprotein expressed in baculovirus protects calves against experimental BPIV-3 challenge, Vaccine 15 (1997) 730–738.
[4] S. Elankumaran, Bovine parainfluenza virus 3, in: M. Munir (Ed.), Mononegaviruses of Veterinary Importance: Volume 1: Pathobiology and Molecular Diagnosis, CABI Publishing, Wallingford, 2013, pp. 117–140.
[5] S.J. Moore, M.A. O'Dea, N. Perkins, A.J. O'Hara, Estimation of nasal shedding and seroprevalence of organisms known to be associated with bovine respiratory disease in Australian live export cattle, J. Vet. Diagn. Invest. 27 (2015) 6–17.
[6] F.R. Spilki, Bovine parainfluenza virus 3, in: M. Munir (Ed.), Mononegaviruses of Veterinary Importance: Volume 2: Molecular Epidemiology and Control, CABI Publishing, Wallingford, 2013, pp. 98–105.
[7] R.A. Vaucher, A.B. Simonetti, P.M. Roehe, RT-PCR for detection of bovine parainfluenza virus type 3 (bPIV3), Acta Sci. Vet. 36 (2008) 215–220.
[8] A. James, J. Macdonald, Recombinase polymerase amplification: emergence as a critical molecular technology for rapid, low-resource diagnostics, Expert Rev. Mol. Diagn. 15 (2015) 1475–1489.
[9] O. Piepenburg, C.H. Williams, D.L. Stemple, N.A. Armes, DNA detection using recombination proteins, PLoS Biol. 4 (2006) e204.
[10] R.K. Daher, G. Stewart, M. Boissinot, M.G. Bergeron, Recombinase polymerase amplification for diagnostic applications, Clin. Chem. 62 (2016) 947–958.
[11] P. Hou, H. Wang, G. Zhao, C. He, H. He, Rapid detection of infectious bovine Rhinotracheitis virus using recombinase polymerase amplification assays, BMC Vet. Res. 13 (2017) 386.
[12] P. Hou, G. Zhao, H. Wang, C. He, Y. Huan, H. He, Development of a recombinase polymerase amplification combined with lateral-flow dipstick assay for detection of bovine ephemeral fever virus, Mol. Cell. Probes 38 (2018) 31–37.
[13] G. Zhao, H. Wang, P. Hou, C. He, H. He, Rapid visual detection of Mycobacterium avium subsp. paratuberculosis by recombinase polymerase amplification combined with a lateral flow dipstick, J. Vet. Sci. 19 (2018) 242–250.
[14] A.E. Vecherov, P.K. Aianot, A.M. Timina, V.V. Lisitsin, Detection and differentiation of the bovine parainfluenza-3 virus strains studied by amplification and sequencing of the HN gene, Vopr. Virusol. 48 (2003) 46–49 (in Russian).
[15] C.Q. He, Y.X. Liu, H.M. Wang, P.L. Hou, H.B. He, N.Z. Ding, New genetic mechanism, origin and population dynamic of bovine ephemeral fever virus, Vet. Microbiol. 182 (2016) 50–56.
[16] S. Zheng, J. Shi, X. Wu, Z. Peng, C. Xin, L. Zhang, et al., Presence of Torque teno sus virus 1 and 2 in porcine circovirus 3-positive pigs, Transbound. Emerg. Dis. 65 (2018) 327–330.
[17] S. Zheng, X. Wu, L. Zhang, C. Xin, Y. Liu, J. Shi, et al., The occurrence of porcine circovirus 3 without clinical infection signs in Shandong Province, Transbound. Emerg. Dis. 64 (2017) 1337–1341.
[18] A. Lago, S.M. McGuirk, T.B. Bennett, N.B. Cook, K.V. Nordlund, Calf respiratory disease and pen microenvironments in naturally ventilated calf barns in winter, J. Dairy Sci. 89 (2006) 4014–4025.
[19] P. Hou, G. Zhao, C. He, H. Wang, H. He, Biopanning of polypeptides binding to bovine ephemeral fever virus G1 protein from phage display peptide library, BMC Vet. Res. 14 (2018) 3.
[20] J.C. Wang, W.Z. Yuan, Q.A. Han, J.F. Wang, L.B. Liu, Reverse transcription recombinase polymerase amplification assay for the rapid detection of type 2 porcine reproductive and respiratory syndrome virus, J. Virol. Methods 243 (2017) 55–60.
[21] P.F. Horwood, T.J. Mahony, Multiplex real-time RT-PCR detection of three viruses associated with the bovine respiratory disease complex, J. Virol. Methods 171 (2011) 360–363.
[22] Z.A. Crannell, B. Rohrman, R. Richards-Kortum, Equipment-free incubation of recombinase polymerase amplification reactions using body heat, PLoS One 9 (2014) e112146.
[23] L. Lillis, D. Lehman, M.C. Singhal, J. Cantera, J. Singleton, P. Labarre, et al., Non-instrumented incubation of a recombinase polymerase amplification assay for the rapid and sensitive detection of proviral HIV-1 DNA, PLoS One 9 (2014) e108189.
[24] Y. Yang, X. Qin, W. Zhang, Y. Li, Z. Zhang, Rapid and specific detection of porcine parvovirus by isothermal recombinase polymerase amplification assays, Mol. Cell. Probes 30 (2016) 300–305.