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RESEARCH PAPER

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Comparison of Three Terminal Detection Methods Based on Loop Mediated Isothermal Amplification (LAMP) Assay for Spring Viremia of Carp Virus (SVCV)

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Abstract

Spring viremia of carp virus (SVCV) is a significant cyprinid-pathogenic virus. SVCV detection is usually performed in a laboratory with apparatus. But the virus outbreaks are generally in fishery banks. The study was developed a loop-mediated isothermal amplification (LAMP) assay, and compared to three different terminal detection methods in order to achieve SVCV field-based detection. A set of six specific primers for LAMP were designed based on SVCV glycoprotein (G) gene. The reaction parameters of LAMP were optimized, and three terminal detection methods (SYBR Green I staining, lateral flow dipstick (LFD), and agarose gel electrophoresis) were applied respectively to the detection of LAMP products. The results showed that 8 mM Mg2+, 320 U/mL Bst DNA polymerase, 1.4 mM dNTP, and 1 M Betaine were optimum at 63 ℃ for 40 min. Among the three terminal ways, LFD was preferred for detection of LAMP products by comparing their sensitivity and specificity. The detection limit of LAMP combined LFD (LAMP-LFD) was 860 fg, and no cross-reaction with other aquaculture viruses. Thus, the presented LAMP-LFD was suitable for field-based detection of SVCV with its advantages of speed, simplicity, and disposability. Meanwhile, the study also provides a valuable alternative to immunoassays and PCR-based tests for other virus or bacteria.

Introduction

Spring viremia of carp virus (SVCV), a member of the genus *Vesiculovirus* of the family Rhabdoviridae, is a notable pathogen listed by the world organization for animal health. It causes the highly contagious spring viraemia of carp disease associated with haemorrhagic symptoms in cyprinids, especially common in carp *Cyprinus carpio* (Ahne *et al.*, 2002; Ashraf *et al.*, 2016). Moreover, SVCV also infects other economically important fish species, including sheatfish *Silurus glanis*, rainbow trout *Oncorhynchus mykiss*, tilapia *Sarotherodon niloticus*, and some aquarium water fish, e.g., goldfish *Carassius auratus*, koi *Cyprinus carpio koi* (Ahne *et al.*, 2002). It was firstly detected in the United States in 2002, in both farmed and wild fish populations experiencing mortality events (Goodwin, 2002; Dikkeboom *et al.*, 2004). Later on, SVCV was detected in Europe, Southeast Asia and the Middle East (Stone *et al.*, 2003). SVCV infection is highly lethal in young fish, with mortality rates up to 90% (Baudouy, Danton, & Merle, 1980) and thus causes substantial economic losses to the aquaculture industry.

SVCV is one of the transmissible viruses of farmed fishes that cause considerable economic losses. Therefore, rapid and accurate diagnosis of the virus is vital to prevent the spread of the virus, and to minimize SVCV-associated economic losses. Previously, some conventional serological methods were used to detect SVCV, including virus neutralization test (Cowen & Hitchner, 1975) immunoperoxidase assay (Faisal, & Ahne, 1984), indirect immunofluorescence assay (IFA) and enzyme-linked immunosorbent assay (ELISA) (Way, 1991; Rodak *et al.*, 1993). However, these techniques

are time-consuming. Moreover, IFA and ELISA appear to easy cross-reaction with other rhabdoviruses, leading to possible false-positive diagnoses (Way, 1991). During the last decades, various PCR-based assays have been applied to SVCV detection, e.g., reverse transcription combined with nested PCR (RT-PCR) (Koutna, Vesely, Psikal, & Hulova, 2003) multiplex real-time quantitative RT-PCR (Liu *et al.*, 2008), and one-step TaqMan real-time quantitative (Yue *et al.*, 2008) and improved RT-PCR (Shimahara *et al.*, 2016). Although these assays have clearly improved the specificity and sensitivity for SVCV detection (Kim, 2012), the PCR assays are not widely applied on-site because of apparatus expense, the need for skilled operation and their long detection times. Therefore, the development of a rapid, simple and cost effective method for SVCV field-based detection is urgently needed.

Loop mediated isothermal amplification (LAMP) is a novel nucleic acid amplification assay under isothermal conditions, which was originally developed by Notomi *et al.* (2000). Its amplification efficiency is extremely high by the isothermal reaction because there is no time loss caused by a thermal change. The sensitivity of LAMP was even 100-fold higher than the PCR-based methods according to the literature (Yu, L. P., Hu, Y. H., Zhang, X. H., & Sun, B. G. 2013). Moreover, its specificity was also reported no cross-reaction between related viruses (Cai *et al.*, 2010; Xu, Feng, Guo, Ou, & Wang, 2010Furthermore, due to its isothermal reaction, LAMP does not require complicated and expensive equipment, and can be easily manipulated on site. Currently, LAMP assay has been increasingly developed for detection of bacteria and viruses from aquaculture (Xia *et al.*, 2015; Tsai, Wang, Yoshida, Liaw, & Chen, 2013 Caipang, Kulkarni, Brinchmann, Korsnes, & Kiron, 2010; Zhang *et al.*, 2014). But, up until now, there are only two LAMP research reports about SVCV detection (Shivappa *et al.*, 2008; Liu *et al.*, 2008), which describe the standardized LAMP protocol for detecting SVCV, including from RNA extraction to LAMP product detection. However, there are still some problems that need to be improved. For instance, its downstream terminal detection is usually performed by using agarose gel electrophoresis (AGE) in the laboratory, which is not suitable for detection in fishery banks.

The detection of LAMP products is usually performed by agarose gel electrophoresis, followed by staining. To avoid this process and speed up the total time for LAMP assay, a LAMP combined with chromatographic lateral flow dipstick (LFD) has been reported in the literature, and showed promise because of its simple operation, making special instrumentation unnecessary (Arunrut, Seetangnun, Phromjai, Panphut, & Kiatpathomchai, 2011 Kaewphinit *et al.*, 2012; Chowdry *et al.*, 2014). The LAMP combined with LFD (LAMP-LFD) has also been used in shrimp pathogen detection, such as Taura syndrome virus (TSV) (Kiatpathomchai, Jaroenram, Arunrut, Jitrapakdee, & Flegel, 2008) shrimp hepatopancreatic parvovirus (Nimitphak, Kiatpathomchai, & Flegel, 2008) *Vibrio parahaemolyticus* (Prompamorn *et al.*, 2011), shrimp yellow head virus (YHV) (Khunthong *et al.*, 2013), and *Vibrio harveyi* (Thongkao, Longyant, Silprasit, Sithigorngul, & Chaivisuthangkura, 2015. However, there were few applications in the detection of fish virus.

The aim of study is to develop an optimum LAMP system combined with a rapid field-based test for SVCV. The study will firstly optimize the LAMP system and then adopt three terminal detection methods for LAMP products assay, including SYBR Green I staining, lateral flow dipstick (LFD), and agarose gel electrophoresis, and compare their merits in terms of sensitivity and specificity. The schematic diagram is shown in Figure 1.

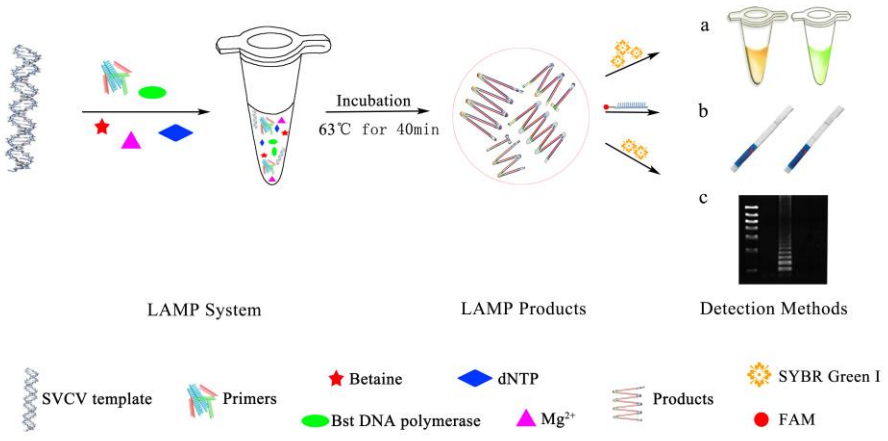


Figure 1. Schematic diagram of three terminal detection methods based on loop mediated isothermal amplification (LAMP) a. SYBR Green I staining; b. LFD; c. Agarose gel electrophoresis.

Materials and Methods

Reagents

Inner primers (FIP, BIP, and FIP 5' labeled with biotin (BIO)), outer primers (F3, B3), loop primers (LF, LB) and a hybrid probe (HP) labeled with fluorescein amidite (FAM) at the 5' end were synthesized by Sangon Biotech Co. Ltd. (Shanghai, China). 8000U/mL Bst 2.0 WarmStart DNA polymerase (Art.No.M0538) was purchased from New England Biolabs (Ipswich, MA, US). 5M Betaine solution (Art.No.B0300-1VL) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). SYBR Green I (a 10000×solution in dimethyl sulfoxide (DMSO), Art.No.SR4110) was from Beijing Solarbio Science & Technology Co. Ltd. (Beijing, China). Lateral flow dipstick (LFD) was obtained from Milenia Biotec GmbH Ltd. (GieBen, Germany). Both of viral genomic extraction kit and primescript™ RT reagent kit were from Takara Bio Inc., Japan. The sterile distilled water was from Beijing Dingguo Chang-sheng Biotechnology Co. Ltd. (Beijing, China).

The cDNA sequence of glycoprotein (G) gene in SVCV, and thymidine kinase (TK) gene in koi herpesvirus (KHV), and segment S8 sequence in grass carp reovirus (GCRV) was synthesized by Sangon Biotech Co. Ltd. (Shanghai, China), and preserved in *E. coil* (*Escherichia coli*) in glycerol at -20℃.

LAMP Primers and Hybrid Probe Design

Primer sets for LAMP consist of two outer primers (F3 and B3), two inner primers (FIP and BIP), and two loop primers (LF and LB). They were designed according to SVCV glycoprotein (G) gene (GenBank accession No. AY527273) by Primer explorer V5 software (http://primerexplorer.jp/elamp4.0.0/index.html). In addition, a hybrid probe (HP) targeted a conserved region (376-395 bp) in SVCV G gene was also designed for applying to chromatographic lateral flow dipstick (LFD). The locations of primers and HP are shown in Figure 2, and their sequences and secondary structures are shown in Table 1.

Optimization of LAMP Parameters

LAMP reaction parameters, including Mg^{2+}, Bst DNA Polymerase, dNTP, Betaine concentrations, and

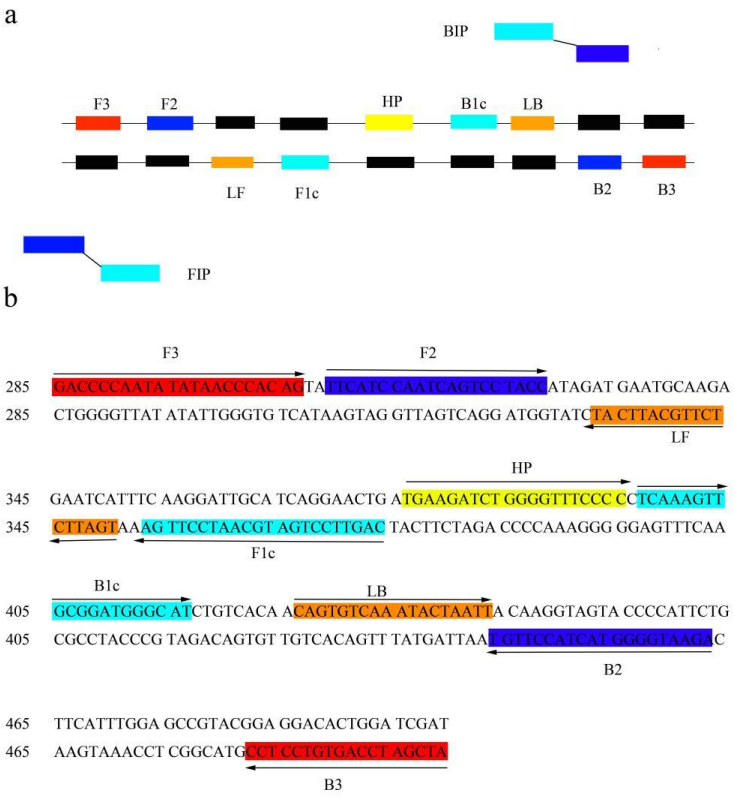
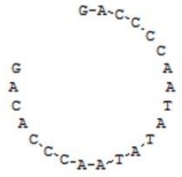
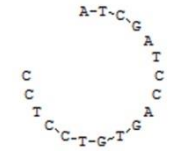
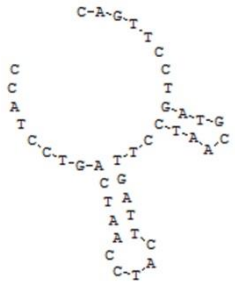
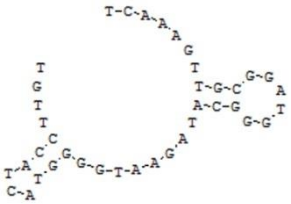
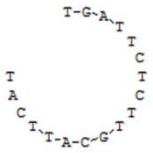
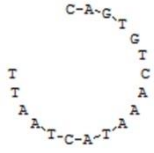
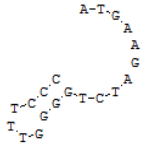


Figure 2. Schematic diagram of primers and probe.

a. Schematic diagram of two outer (F3, B3) primers, two inner (FIP, BIP) and two loop primers (LF, LB) and hybrid probe (HP) for LAMP; b. The positions of primers and HP in nucleotide sequence of SVCV glycoprotein from GenBank (Accession number: AY527273)

reaction temperature and time, were optimized in a 25 µL system. Firstly, Mg^{2+} concentrations were optimized by changing from 2, 4, 6, 8, 10, 12 mM, Bst DNA Polymerase were optimized from 64, 128, 192, 256 and 320 U/mL. dNTP and Betaine concentrations were studied at different concentrations (0, 1, 1.2, 1.4, 1.6, 1.8 mM) and (0, 0.2, 0.4, 0.6, 0.8, 1 M), respectively. Secondly, the reaction temperature was optimized by

Table 1 LAMP primer sets and hybrid probe

Primer name	location	Primer sequence(5'-3')	Minimum free energy of structure (kcal/mol)	Figure of structure
F3	285-306	GACCCCAATATATAACCCACAG	0.00	
B3	482-499	ATCGATCCAGTGTCCTCC	0.00	
FIP	309-328+353-374	(BIO)-CAGTTCCTGATGCAATCCTTGATTCATCCAATCAGTCCTACC	-3.12	
BIP	397-416+444-463	TCAAAGTTGCGGATGGGCATAGAATGGGGTACTACCTTGT	-2.33	
LF	333-350	TGATTCTCTTGCATTCAT	0.00	
LB	426-443	CAGTGTCAAATACTAATT	0.00	
HP	376-395	FAM-TGAAGATCTGGGGTTTCCCC		

varying from 61 to 65 ℃. Finally, the reaction time was optimized from 20, 30, 40, 50 to 60 min. The amplification products were analyzed by 1.5% (w/v) agarose gel electrophoresis.

LAMP Reaction System

The LAMP was performed in 25 µL of a mixture containing 1.4 mM dNTP, 0.2 µM F3/B3, 1.6 µM FIP/BIP (or BIO-FIP/BIP), 0.4 µM LF/LB, 320 U/mL Bst DNA Polymerase, 1 M Betaine and 8.6 ng cDNA template in 1×reaction buffer (20 mM Tris-HCl (pH 7.5) buffer with 10 mM $(NH_4)_2SO_4$, 50 mM KCl, 8 mM $MgSO_4$, and 0.1% Tween-20). The reaction mixture without the SVCV template was used as negative controls. The reaction mixture was incubated at 63 ℃ for 40 min. The amplification products were analyzed by SYBR Green I straining, lateral flow dipstick (LFD), and agarose gel electrophoresis (AGE).

Terminal Detection of LAMP Products

SYBR Green I staining: Based on the principle of SYBR Green I dye easily binding to double-stranded DNA, 1 µL SYBR Green I (10×dilution) was used to stain 25 µL LAMP products and produced a color change from orange to green observable with the naked eye.

Lateral flow dipstick (LFD): Based upon the procedure described in references (Kiatpathomchai *et al.*, 2008; Puthawibool, Senapin, Kiatpathomchai, & Flegel, 2009) the principle of LFD was that the BIO-FIP and BIO-labeled LAMP products were combined with nano-gold, and then the gold complexes diffused over the membrane by capillarity. The test line was bond with avidin, which could specifically catch BIO-labeled products. Not-captured gold particles were flowed over the control line and fixed with their specific antibodies. The Schematic diagram of LFD was showed in Figure 3. In the experiment, the inner primer (FIP) labeled with BIO at 5 'end (BIO-FIP) was used in the LAMP system. After the amplified reaction, 20 pmol hybrid probe (HP) labeled Fluorescein amidite (FAM) (FAM-HP) was added to 25 µL of amplified products and incubated for 5 min. Then 10 µL LAMP hybridized product was diluted to 100 µL by using running buffer, and then added into the sample hole of LFD. The red colored lines were observed both in control (C) and test (T) lines on LFD after 2 min for the positive sample. For negative control, only the C line showed the red line.

Agarose gel electrophoresis analysis: 5 µL LAMP product was determined using 1.5% (w/v) agarose gel electrophoresis in Tris-acetate-EDTA buffer, then stained with 1 µL SYBR green I (100×dilution), and visualized on a UV transilluminator.

Specificity of LAMP for SVCV

The specificity of LAMP for SVCV was analyzed using specific gene cDNA of KHV and GCRV as LAMP templates instead of SVCV under optimized conditions. All reagents except template were applied to negative control reaction. The amplified mixtures were analyzed by agarose gel electrophoresis and SYBR Green I staining. The BIO-labeled LAMP products were determined by LFD. Each test was conducted in triplicate.

Sensitivity of LAMP for SVCV

The sensitivity of LAMP for SVCV was evaluated under optimized conditions by using 10-fold serial dilutions of SVCV G gene cDNA template from 10^{-1} to 10^{-7} in sterile distilled water. All reagents except cDNA template were applied to negative control group. The amplified mixtures were analyzed by agarose gel electrophoresis and SYBR Green I staining. The BIO-labeled LAMP products were determined by LFD. Each test was conducted in triplicate.

Fish Samples Detection

The visceral tissue from carp fish artificially infected SVCV was used to evaluate the performance of LAMP combined respectively with three terminal

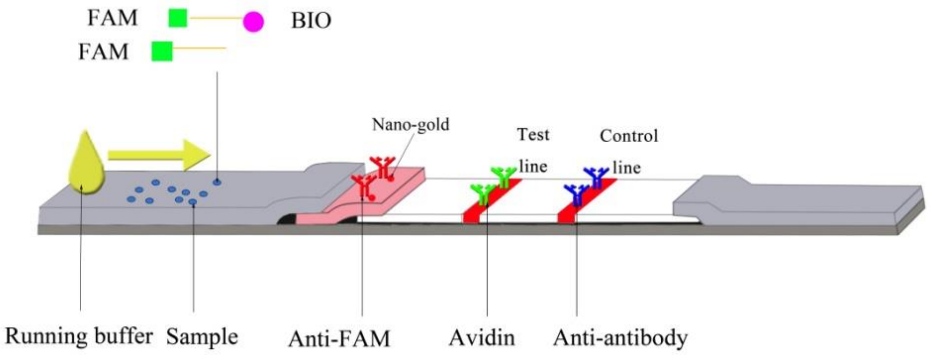
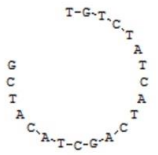
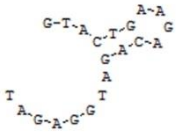
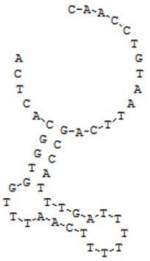
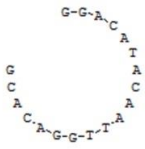
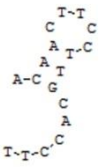
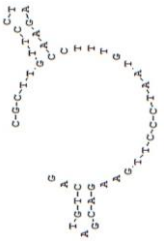
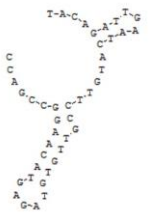


Figure 3. Schematic diagram of LFD detection.

Table 2 Comparison of structural minimum free energy of primers

Primer Report	Primer	Sequence	Minimum free energy of structure (kcal/mol)	Figure of structure
Shivappa et al. (2008)	F3	TGTCTATCATCAGCTACATCG	0.00	
	B3	GTACTGAAGACAGATGGAGAT	-1.72	
	FIP	CCATGATATATTCTGCCCGGATGGTTTTCATTCCTTTTGCTAATTGACTC	-0.84	
	BIP	CAACCTGTAATTCAGCCATTTGATTTTTTTCAATTTGGTGGCACTCA	-4.59	
Liu et al. (2008)	F3	GGACATACAATTGGACACG	0.00	
	B3	ACAACTTCCTTGCACCTT	0.03	
	FIP	CGCTTGTTCCTAGAACCTTTGTAAT-CCCTTGAAGACGATGTCAG	-2.36	
	BIP	TACAGATTGAATCATGTTCCGTTGTG-TAGAGTACAAGGCCGACC	-2.26	

methods. The viral RNA was extracted using viral genomic extraction kit, and then was reversed to cDNA by primescript™ RT reagent kit. Afterwards it was dissolved in 30 µL sterile distilled water, and preserved at -20 ℃. SVCV G gene was amplified by LAMP and detected by SYBR Green I, LFD and AGE, respectively.

Results

LAMP Primers Design

A set of six primers was designed based on the conserved G gene of SVCV. A pair of loop primers can accelerate the LAMP reaction time. Furthermore, the complementary pairs of primers are fewer in the set of primers, and the modulus of the structural minimum free energy of the primers was lower than other two reports, as shown in Table 2.

Optimization of LAMP Parameters

LAMP products were shown at different reaction parameters, as shown in Figure 4(a, b, c and d), the clearest and strongest bands in images of gel electrophoresis were obtained in 8 mM Mg^{2+}, 320 U/mL Bst DNA Polymerase, 1.4 mM dNTP, and 1 M Betaine, which were the best choice for SVCV reaction. Similarly, in Figure 4e and f, the clearest and strongest bands of LAMP products were displayed at 63 ℃ reaction temperature with 40 min incubation. Thus, 63 ℃ was

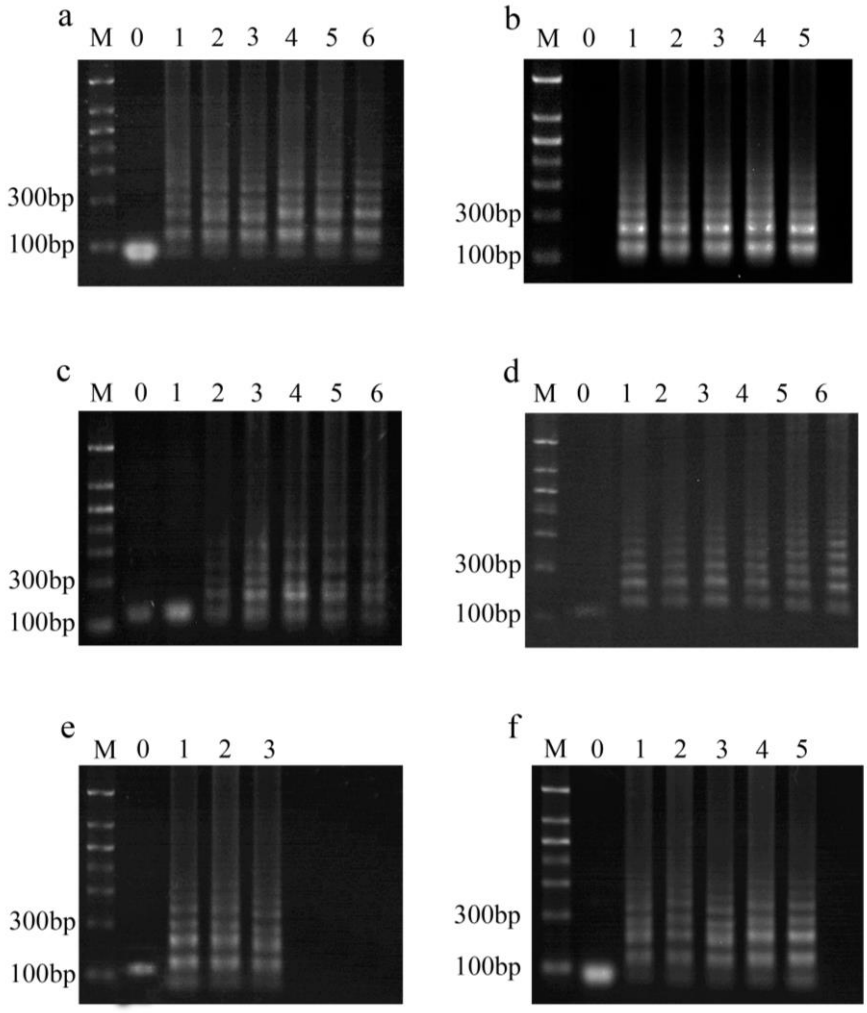


Figure 4. Optimization of LAMP reaction parameters.

Lane M： DNA ladder; lane 0: Negative control; a. Mg^{2+} concentration optimization. lane 1, 2, 3, 4, 5, and 6 LAMP reaction system contained 2, 4, 6, 8, 10 and 12mM Mg^{2+}; respectively. b. Bst DNA Polymerase concentration optimization. lane 1, 2, 3, 4, 5, and 6: LAMP reaction system contained 64, 128, 192, 256 and 320U/mL Bst DNA Polymerase, respectively. c. dNTP concentration optimization. Lane 1, 2, 3, 4, 5, and 6: LAMP reaction system contained 1.0, 1.2, 1.4, 1.6 and 1.8 mM dNTP, respectively. d. Betaine concentration optimization. Lane 1, 2, 3, 4, 5, and 6: LAMP reaction system contained 0.2, 0.4, 0.6, 0.8 and 1M Betaine, respectively. e. LAMP temperature optimization. Lane 1, 2, 3: LAMP reaction was performed at 61, 63, 65℃, respectively. f. LAMP reaction time optimization. Lane 1, 2, 3, 4, and 5: LAMP reaction system was carried out for 20, 30, 40, 50 and 60min, respectively.

the optimal reaction condition, and 40 min was determined reaction time.

Specificity of LAMP Detection

To confirm the specific amplification, KHV and GCRV were adopted, which also easily infect fresh water fish. As shown in Figure 5, the amplified products from KHV and GCRV gene template by SYBR Green I staining, LFD, and AGE were not detected. The LAMP products in positive tubes appeared green after adding SYBR Green I dye, whereas the original orange color of SYBR Green I did not change in KHV, GCRV, and negative control tubes (Figure 5a). With regard to LFD testing, only the SVCV sample displayed a red color in both control and test lines (Figure 5b). Similarly, AGE also showed that only the SVCV sample had amplification products (Figure 5c). Three terminal detection methods indicated that the LAMP assay was excellently specific to SVCV.

Sensitivity of LAMP Detection

To compare the sensitivity of detection, LAMP combined with three terminal detection methods was carried out using the same 10-fold serial dilutions of SVCV cDNA template. This study adopted three terminal detection methods for analyzing LAMP products. As shown in Figure 6, for SYBR Green I straining, its detection limit was 10^{-4} dilution (cDNA template: 860 fg) (Figure 6a). The detection limit of LFD was 10^{-4} dilution

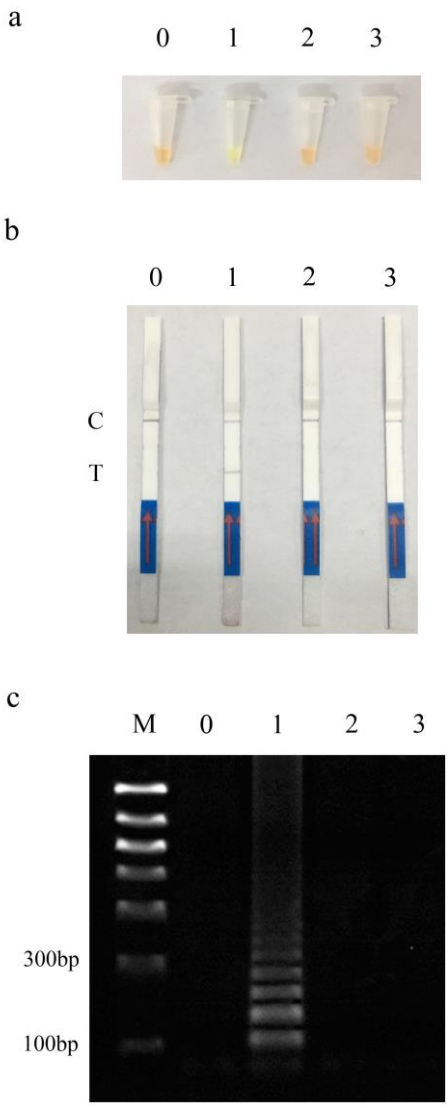


Figure 5. Specificity of SVCV-LAMP based on three terminal detection methods
Lane 0: Negative control; Lane M ： DNA ladder; lane 1: LAMP reaction with SVCV as template; lane 2: LAMP reaction with KHV as template; lane 3: LAMP reaction with GCRV template.

(cDNA template: 860 fg) (Figure 6b). In Fig.5c, the detection limit of AGE was 10^{-5} dilution (cDNA template: 86 fg) (Figure 6c). The sensitivity of AGE was better than that of SYBR Green I straining and LFD.

Fish Samples Verification

The actual samples through SVCV artificial infection were used to verify three terminal detection

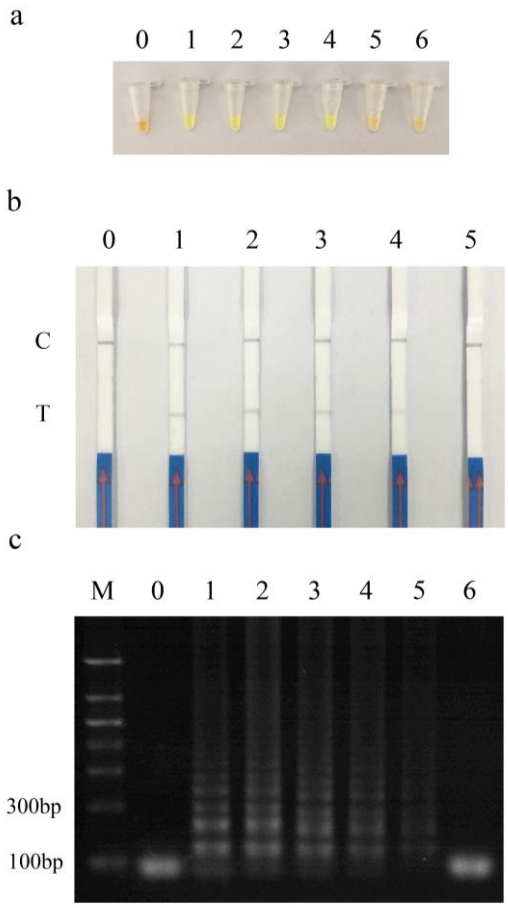


Figure 6. Sensitivity of SVCV-LAMP based on three terminal detection methods
Lane 0: Negative control; Lane M ： DNA ladder; lane 1, 2, 3, 4, 5, 6: LAMP reaction carried out using 10-fold dilutions of cDNA template (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}), respectively.

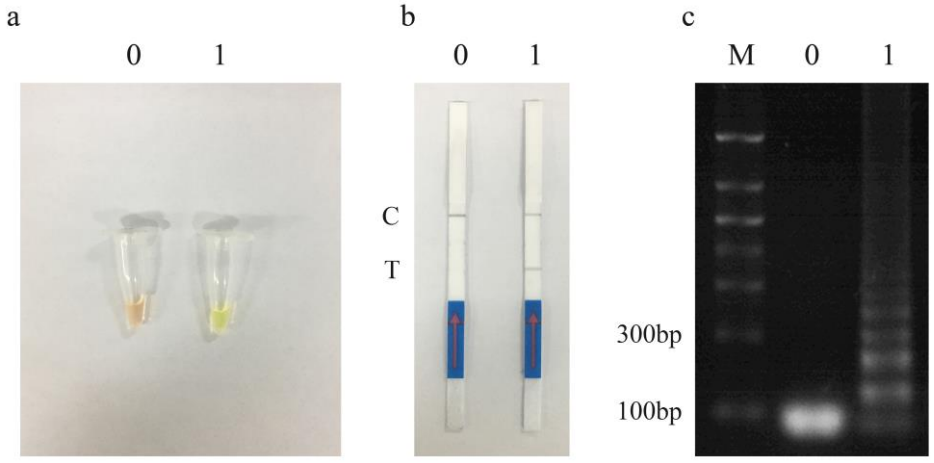


Figure 7. Fish samples detection
Lane 0: Negative control; lane 1: Actual sample; Lane M ： DNA ladder.

methods based on LAMP. The negative cDNA was from not infected carp tissue RNA. As shown in Figure 7a, for SYBR Green I straining, the positive tube was showed green fluorescence, and the negative was orange. As for LFD method (Figure 7b), the test line was presented red as positive one, while none was showed in test line in negative dipstick. In Figure 7c, only the positive lane in AGE was showed ladder pattern. The detecting coincidence rate was 100% for three terminal methods.

Discussion

There are various virus testing methods described in OIE manual, e. g., cell culture, ELISA, immunofluorescence, PCR and so on. Considered their experiment cycle and cost, LAMP method should possess more potential practical significance, which also confirmed by the study.

The primers in the study were designed based on the conserved G gene of SVCV which agreed with the gene sequence in Shivappa's report (2008), and was different from Liu's research (2008). Here, the complementary pairs of primers are fewer, which allowed the primers to be more easily integrated into the target DNA and reduced the reaction temperature. Furthermore, based on the principle of loop primers increasing the starting point of DNA synthesis, and initiating further DNA amplification (Nagamine, Hase, & Notomi, 2002) a pair of loop primers designed in the study also accelerated the LAMP reaction speed. Consequently, due to primer design and optimization, the reaction temperature and time have been improved in the study compared with Shivappa's report (2008).

In the LAMP reaction parameters, Bst DNA polymerase was a critical factor for amplification efficiency. Mg^{2+} can affect primer annealing and DNA polymerase activity. Betaine can reduce base stacking and stimulate the overall rate of reaction and increase target selectivity. Therefore, the parameters must be optimized in the study. A better system of SVCV-LAMP was developed in a shorter time (40 min) at 63 ℃ through optimization than Shivappa *et al.* reported reaction condition (60 min, 65 ℃).

In three different terminal detection methods, gel electrophoresis with UV source was the common method, but it is not well suited for field-based detection even if its sensitivity is higher than other two methods. The fluorescent dye (SYBR Green I) staining can substitute for gel electrophoresis to judge LAMP products by visual inspection even if its detection sensitivity was lower an order of magnitude. However, the SYBR Green I staining was required a 10 times higher dosage than AGE, so the phenomenon of false positives easily appeared. Here, LFD detection avoided the toxicity of fluorescent dye and the possibility of false positives. Meanwhile, it also directly and rapidly showed results with the same sensitivity as SYBR Green I. LFD should be preferred for terminal analysis of LAMP products.

The LAMP-LFD method has already been used in the detection of bacteria and virus since 2008. The application of LAMP-LFD in aquatic pathogen was mainly on the detection of shrimp virus (Jaroenram, Kiatpathomchai, & Flegel ,2009; Kiatpathomchai *et al.* 2008; Puthawibool *et al.*, 2009; Khunthong *et al.*, 2013). According to literature, the LAMP-LFD for detecting TSV needed 70 min and its detection limit was 10 fg (Kiatpathomchai *et al.*, 2008). For infectious myonecrosis virus (IMNV), its total time of LAMP-LFD assay required 75 min and the detection limit was 10 pg (Puthawibool *et al.*, 2009). With regard to *Mycobaterium*, its detection time of LAMP-LFD reached to 100 min and its detection limit was 5 pg (Kaewphinit *et al.*, 2012). Later, Khunthong *et al.* (2013) reported the detection time of LAMP-LFD for YHV was 55 min and the detection limit was 0.1 pg. Here, the developed method needed 50min for detecting SVCV, which was shorter than other reports. As for the sensitivity, the present result was 0.86 pg which also has certain advantages in the field of rapid detection. Thus, the LAMP-LFD method in the study should be recommended as a routine detection of SVCV in fish industries, especially where expensive diagnostic instruments are not available.

In conclusion, LAMP assay is simply and easily performed under isothermal conditions as long as the appropriate primers have been prepared. The LFD terminal detection avoids the dependence on electrophoresis and gel imaging system, and as well as toxic fluorescent dye and its possibility of false positives. Thus, with LAMP advantages stated above, and combining with LFD chromatographic visualization, the LAMP-LFD is more suitable for SVCV detection for field-based detection in fishery banks. Meanwhile, the LAMP-LFD will also provide a valuable alternative to immunoassays and PCR-based tests for other virus or bacteria.

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