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Short communication

Reverse transcription loop-mediated isothermal amplification for the detection of highly pathogenic porcine reproductive and respiratory syndrome virus

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Abstract

A reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay targeting the open reading frames 1a of highly pathogenic porcine reproductive and respiratory syndrome virus genome was developed. The 10 reference strains, 1 clinical isolation strain and 122 positive samples were tested. Positive reactions were confirmed for all strains and specimens by reverse transcription loop-mediated isothermal amplification and nested reverse transcription polymerase chain reaction (RT-PCR). The results showed this detection technique is more reliable and convenient for rapid and sensitive diagnosis of highly pathogenic porcine reproductive and respiratory syndrome virus infection.

Keywords:
Porcine reproductive and respiratory syndrome virus (PRRSV)
Highly pathogenic
Detection
Reverse transcription loop-mediated isothermal amplification (RT-LAMP)

Porcine reproductive and respiratory syndrome (PRRS) is a serious swine disease and the causing agent is PRRS virus (PRRSV) which belongs to the member of arteriviruses, a group of small, enveloped, positive-strand RNA virus (Conzelmann et al., 1993). PRRSV was first observed in the United States in 1987 (Keffaber, 1989) and in Europe in 1990 (Wensvoort et al., 1991). To date, PRRS has spread worldwide and caused enormous economic losses each year (Gao et al., 2004). Recently, the unparalleled large-scale outbreaks of a highly pathogenic PRRS, which spread to many provinces in China, have cause severe economic losses for the Chinese swine industry. Autopsies combined with immunological tests showed clearly that multiple organs were infected by the highly pathogenic PRRSV with severe pathological changes observed (Tian et al., 2007; Li et al., 2007; Normile, 2007). The prerequisite for controlling the disease is a rapid and accurate identification of this organism.

Virus isolation of PRRSV is difficult. This is mainly because the cell of choice for virus isolation is the porcine alveolar macrophage, which needs to be harvested from pigs (preferably specific pathogen free (SPF)) under 6–8 weeks of age (Wensvoort et al., 1991; Yoon et al., 1992). Not all laboratories have a ready supply of such pigs available, and continuous cell lines cannot replace fully the alveolar macrophages because these cell lines are generally less susceptible to the virus. In addition, different batches of macrophages are not always equally susceptible to the virus, and results are not obtained rapidly. Although reverse transcription polymerase chain reaction (RT-PCR) is a highly sensitive and specific method (Kono et al., 1996; Laroche and Magar, 1997; Mardassi et al., 1994; Van Woensel et al., 1994), the dependence on special equipment limits its extensive use.

A novel nucleic acid amplification method, loop-mediated isothermal amplification (LAMP), relies on autocycling strand displacement DNA synthesis performed by Bst DNA polymerase (Notomi et al., 2000; Mori et al., 2001; Nagamine et al., 2002; Chen et al., 2008). Furthermore, reverse transcription LAMP (RT-LAMP) method has been applied successfully for the detection of human influenza A virus, severe acute respiratory syndrome coronavirus and Newcastle disease virus (Hong et al., 2004; Pham et al., 2005; Poon et al., 2005). In the present study, RT-LAMP method was developed with the HPBEDV strain for the detection of highly pathogenic PRRSV from blood, semen and lung samples. RNA transcripts corresponding to the open reading frames (ORF) 1a (nucleotides 2710–2946) of highly pathogenic PRRSV genome were generated to use as standards in the sensitivity analysis of the assay, respectively. A series of the five times dilutions spanning 1 to 52 copies/tube was used as template. Briefly, RNA was extracted...
from HPBEDV strain using the QIAamp RNA extraction kit. The purified RNA was resuspended in diethylpyrocarbonate treated water and used in the RT-PCR reaction. The amplified product of ORF 1a was cloned into the pCR-XL-TOPO vector (Invitrogen Inc., Shanghai, China) according to the manufacturer’s directions and sequenced to verify its accuracy. The recombinant plasmid pCR-NSP2 was linearized and gel purified and used as template with a RiboMax T7 In Vitro Transcription System (Promega, Madison, WI) according to the manufacturer’s recommendations. The length of the RNA transcripts was verified by agarose gel analysis, and the RNA of ORF 1a was quantitated using UV spectrophotometry at 260 nm. To test the applicability of this method, 10 reference strains and one clinical strain of highly pathogenic PRRSV (Table 1) were used. Strains were isolated from lung tissues of highly pathogenic PRRSV-affected pigs and homogenised with Dulbecco’s modified Eagles medium (DMEM), freeze–thawed three times and centrifuged at 10,000 × g for 10 min. The supernatant was passed through a 0.22-μm filter and adapted to Marc-145 cell monolayers. The cells were incubated at 37 °C for 5 days and examined for cytopathic effects (CPE) daily. After the appearance of CPE, viral isolates were stored at −70 °C until used. RNA was extracted by using a RNeasy Mini Kit (Qiagen). For further evaluation of RT-LAMP assay with clinical specimens, 122 specimens of blood, semen and lung tissue were obtained from highly pathogenic PRRSV-infected pigs (Table 2). The specimens were frozen at −70 °C until transported and tested.

Four primers of FIP, BIP, F, and B for the RT-LAMP test were designed by targeting the conserved regions of ORF 1a (GenBank access number EU236259) and listed in Table 3. RT-LAMP was also compared with nested RT-PCR for detection in clinical specimens. Positive reactions were confirmed in all of the 122 samples by RT-LAMP and nested RT-PCR. The results indicated that this diagnostic technique was reliable for the detection of highly pathogenic PRRSV in blood, semen and lung tissue samples.Sem en and blood are the preferred samples during the early stage of infection, which may have a higher predictive value of detecting highly pathogenic PRRSV infection during disease surveillance screening. Importantly, the early detection of highly pathogenic PRRSV suggests potential value as a surveillance tool in areas free of the disease and as a screening assay for the early stage of infection.

The test indicated that the detection limit of nested RT-PCR was 25 copies/tube (Fig. 1B) and that of RT-LAMP was 5 copies/tube (Fig. 1C). The sensitivity of RT-LAMP was therefore higher than nested RT-PCR. In addition, compared with nested RT-PCR, RT-LAMP is convenient, rapid, and sensitive. The reaction time of RT-LAMP method is 45 min, which is more rapid than conventional RT-PCR or nested PCR, and the reaction only needs a laboratory water bath. From a practical point of view, RT-LAMP is more suit-

### Table 1

| Strain       | Genotype | Name of strain | GenBank access number |
|--------------|----------|----------------|-----------------------|
| Reference strains |          |                |                       |
| North America |         | CH-1a          | YF032626               |
| North America |         | BJ-4           | AF331831               |
| North America |         | HUB1           | EF075945               |
| North America |         | HuN            | EF175962               |
| North America |         | HUN4           | EF635006               |
| North America |         | HN1            | AY457635               |
| North America |         | JXwn06         | EF641008               |
| North America |         | JX0612         | EF488048               |
| North America |         | JXA1           | EF132445               |
| North America |         | GD             | EU209503               |
| Clinical isolate |      | HPBEDV         | EU236259               |

### Table 2

| Specimen | Strain (positive number) | Positive number of result by |
|----------|--------------------------|-----------------------------|
|          |                         | RT-LAMP                     |
| Blood    | JXA1 (12)                | 12                          |
|          | GD (13)                  | 13                          |
|          | HPBEDV (12)              | 12                          |
| Semen    | JXA1 (13)                | 13                          |
|          | GD (12)                  | 12                          |
|          | HPBEDV (19)              | 19                          |
| Lung     | HPBEDV (41)              | 41                          |

### Table 3

| Method | Primer | Genome position | Sequence |
|--------|--------|-----------------|----------|
| RT-LAMP ORF1a | F | 2710–2719 | 5'-GCTCCGCCGCGAGAAGTCA-3' |
|         | B     | 2928–2946      | 5'-GTCGGCATGCTGTTGGTC-3' |
|         | FIP   | 2796–2814      | 5'-GGATGGTGTCGGAAAATTG |
|         | BIP   | 2761–2779      | CTTATAAGGTCTCGAAGAA-3' |
|         |       | 2855–2873      | 5'-CGTCTGCCAGTGTCCCAA |
|         |       | 2876–2894      | -TTTT+ |
|         |       | 14745–14766    | 5'-TCAAGGCTGTGAGGTC-3' |
| Nested RT-PCR ORF7 | N1F | 15207–15228 | 5'-GACGATCCAGGAGGTTGAGGATC-3' |
|         | N1R   | 14867–14888    | 5'-GCATCAAGCTGTTGGAGTT-3' |
|         | N2F   | 15081–15102    | 5'-CAGTGAATCTTATCTTCTTCT-3' |
|         | N2R   | 14745–14766    | 5'-GACGATCCAGGAGGTTGAGGATC-3' |

* Position is marked according to the sequence of HPBEDV strain (GenBank accession number EU236259).
able as a routine diagnostic tool, especially in clinics in which complicated equipment such as thermal cycling machines and electrophoresis apparatus are not available. In addition, RT-LAMP has a potential for field diagnosis.

In conclusion, RT-LAMP assay is rapid, specific, and sensitive for the detection of highly pathogenic PRRSV in blood, semen and lung tissue samples. This method not only reduced the diagnosis time significantly but also has a potential for wider use.

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