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Rapid detection of *Haemophilus parasuis* using cross-priming amplification and vertical flow visualization

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ABSTRACT

Haemophilus parasuis infection is of considerable economic importance in the swine industry due to high morbidity and mortality in naive swine populations. Accurate detection and identification of the causative agent are difficult, yet necessary, for disease control. In this study, a simple and rapid method of cross-priming amplification (CPA) with a vertical flow (VF) visualization strip was established to detect *H. parasuis*. The reaction can specifically identify 15 serovar reference strains and 57 clinically isolated strains of *H. parasuis*, with a detection limit of 14 CFU. The performance of the CPA-VF assay was evaluated and compared with that of species-specific PCR by testing 62 clinical culture-positive specimens of *H. parasuis*. The entire process, from specimen processing to analysis of the results, can be completed in 2 h without a complicated apparatus. The convenience and speed of the CPA-VF assay in this study make it a suitable choice for epidemiological investigation and point-of-care testing (POCT) for *H. parasuis* infection.

1. Introduction

Glässer's disease of pigs, which is caused by *Haemophilus parasuis*, is characterized by fibrinous polyserositis, pericarditis, peritonitis, polyarthritis and meningitis (Oliveira and Pijoan, 2004; Smart et al., 1989). *H. parasuis* is frequently present in the upper respiratory tract of apparently healthy pigs (Moller et al., 1993). Although the acute infectious process emerges sporadically, there may be a considerable mortality rate of greater than 10% caused by *H. parasuis* infection in the pig industry (Baumann and Bilkei, 2002).

It appears that the pathogenic severity of *H. parasuis* is associated with its serovar. Of the 15 known serovars, highly virulent serovars 1, 5, 10, 12, 13 and 14 usually cause acute and lethal infection in four days. Serovars 2, 4, 8 and 15 are moderately virulent and serovars 3, 6, 7, 9 and 11 have been shown to be avirulent (Kielstein and Rapp-Gabrielson, 1992; Rapp-Gabrielson and Gabrielson, 1992).

Isolation and identification of the causative agent are not easy for many laboratories because *H. parasuis* is a fragile, slow-growing, NAD-dependent organism (Kielstein et al., 2001; Moller et al., 1993). To improve the diagnostic success rate for *H. parasuis*, PCR-based methods have been developed in the last few decades (Angen et al., 2007; Frandoloso et al., 2012; Oliveira et al., 2001; Turni et al., 2010). Although these techniques are useful and sensitive to detect *H. parasuis* in clinical samples, PCR-based methods can be time consuming, rendering such tools impracticable for point-of-care testing (POCT) at the farm level.

Cross-priming amplification (CPA) is a novel amplification technique at isothermal conditions (Fang et al., 2009; Xu et al., 2012). A cross-linked primer target to the objective DNA sequence is necessary to initiate the CPA reaction. The reaction can be divided into three steps: (1) products elongated with the cross primer and displaced with displacement primers, (2) multiple extensions and displacements with detecting probes, and (3) extension with probes specific for hairpin-like structures. The CPA method and vertical flow (VF) nucleic acid detection strip cassette have been utilized to effectively detect multiple pathogens and may have potential value in POCT (Cui et al., 2012; Ke et al., 2013; Kuta et al., 2015; Wang et al., 2016; Wozniakowski et al., 2015; Xu et al., 2015).

In the present study, we established an effective CPA-VF assay using five primers targeted to the putative tyrosine kinase (*wzs5*) gene of *H. parasuis*. This method displayed good specificity in detecting 15 serovar reference strains and 57 clinical isolation strains. Notably, the CPA-VF assay was more sensitive than the conventional species-specific PCR in directly diagnosing clinical culture-positive specimens of *H. parasuis*

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Table 1
Primers used in the CPA-VF assay.

Primer name	Sequences 5′-3′	Genome position[a]
F3	TCGGCATTTATGTTAGGACT	1258–1277
B3	GGCTGTTCATTTGAATATGG	1381–1400
B2	Biotin-CATACGTTGGCAAGCCTA	1355–1372
B1	FITC-ACCTCTATGCAGTAGCGTTTTC	1305–1326
CPF (B2 + F2)	CATACGTTGGCAAGCCTA-TGCCTCAGTTACTGCGATA	F2: 1284–1302

[a] The putative tyrosine kinase (*wzs5*) gene of *H. parasuis* strain Nagasaki (GenBank accession no. KC795349).

and was more convenient and rapid. The CPA-VF assay we offered might be a good choice in POCT of *H. parasuis* infection.

2. Materials and methods

2.1. Bacterial strains

15 reference strains provided by Dr. Lu (Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, China) were used to evaluate the specificity of the CPA-VF assay. 57 strains isolated from clinical specimens infected with *H. parasuis* were identified by the species-specific PCR (Angen et al., 2007). The serotypes of these strains were identified using a recently reported multiplex PCR method (Howell et al., 2015). Our laboratory strains *Pasteurella multocida* (Serovars A and B), *Actinobacillus pleuropneumoniae* (Serovar 1) and *Streptococcus suis* (Serovars 2 and 9) were used as "controls" (one strain per serovar) to evaluate the specificity of the CPA-VF assay.

2.2. Clinical samples

Lung, spleen and liver tissues of pigs suspected to be infected with *H. parasuis* were sent from different farms to our institute for detection. For bacterial culture, dry swabs taken from the tissue samples were inoculated onto tryptic soy agar (TSA) plates supplemented with 5% bovine serum and 10 mg/mL NAD. The plates were incubated overnight at 37 °C. All suspect colonies of *H. parasuis* were identified using the species-specific PCR as described previously (Angen et al., 2007). 62 culture-positive tissues were utilized to extract bacterial DNA. 1 μL DNA was sampled and detected using species-specific PCR and the CPA-VF method.

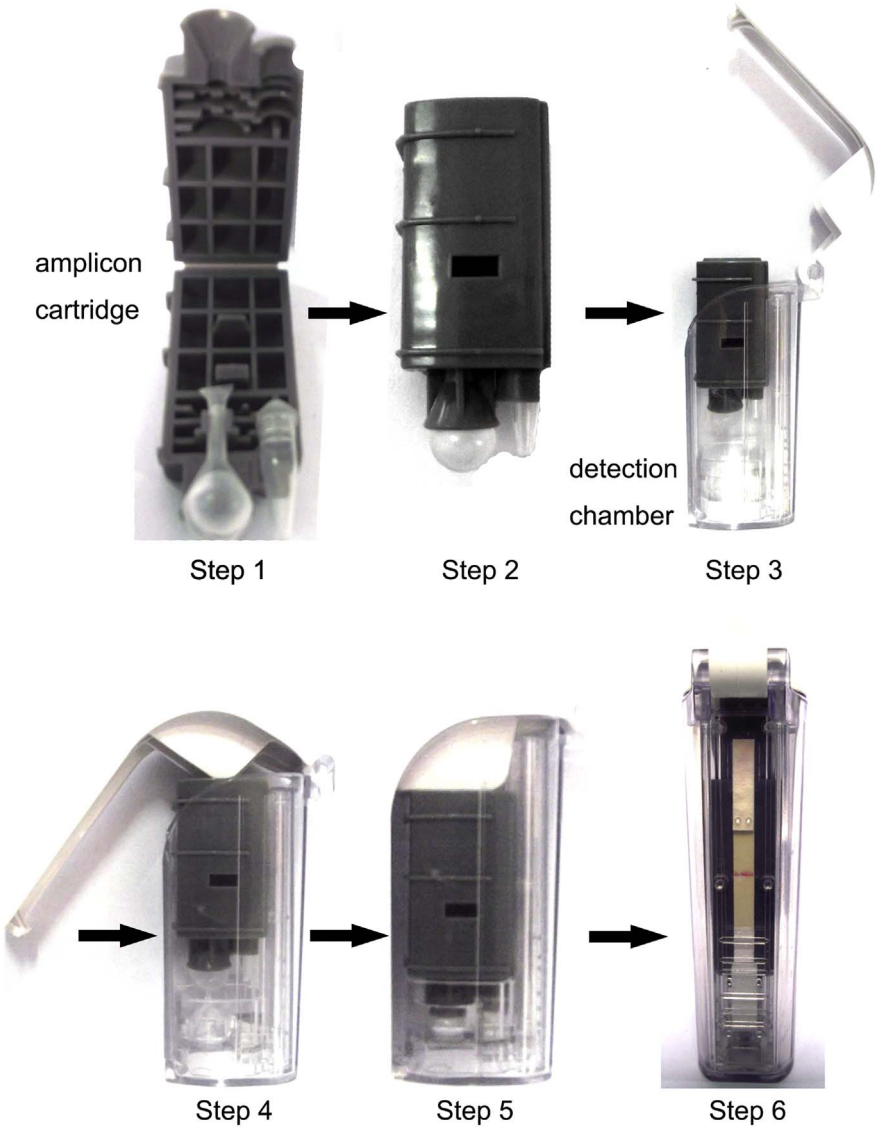

Fig. 1. The procedure of manipulating a VF visualization strip cassette. The reaction tube is immobilized in the cartridge (step 1), the cartridge is closed (step 2), the cartridge is inserted into the detection chamber (step 3), the handle of the detection chamber is pressed (step 4), the chamber is sealed, the running buffer bulb and the reaction tube are punctured simultaneously (step 5), and the results was judged visually through the detection window of the chamber after 5 min (step 6).