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Short Communication

Development and application of loop-mediated isothermal amplification assays based on ITS-1 for rapid detection of *Toxoplasma gondii* in pork

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ABSTRACT

The loop-mediated isothermal amplification (LAMP) assay is a novel method that rapidly amplifies DNA with high specificity and sensitivity under isothermal conditions. In this study, we established a LAMP assay with six primers targeting a highly conserved region of *Toxoplasma gondii* ITS-1 sequence. The amplification protocol completes within 30 min under isothermal condition in a 65 °C water bath while specificity tests confirmed no cross-reactivity with DNA templates of *Neospora caninum*, *Eimeria tenella*, *Cryptosporidium parvum*, *Listeria monocytogenes* and *Streptococcus suis*. The detection limit of the LAMP assay was 0.9 fg *T. gondii* genomic DNA, a sensitivity that was 10-fold higher than that of a conventional PCR assay. Both LAMP assay and conventional PCR were applied to detect *T. gondii* genomic DNA in 118 diaphragm samples obtained from pig farms in Zhejiang Province, China. Our results showed that the LAMP assay is more sensitive than conventional PCR (13.56% and 9.32%). The LAMP assay established in this study provides a simple, specific, sensitive and rapid method of *T. gondii* genomic DNA detection, hence is expected to plays an important role in the monitoring of *T. gondii* contamination in various food products.

1. I d c

Toxoplasma gondii is an agent of toxoplasmosis, a lethal threat to immunocompromised individuals (Dubey, 2004). Among several transmission routes, peroral infectious is the major means caused by the intake of uncooked, infected meat (Zhang et al., 2009). As the main meat source of China, pork possesses an important source of *T. gondii* infection in humans (Tao et al., 2011). The prevalence of *T. gondii* infection in fatting pigs has been reported to vary from 24.5% in central (Tao et al., 2011) to 58.1% in southern China (Zhou et al., 2010), while that in small farms in Zhejiang Province can reach as high as 71.4% (Yu et al., 2011). Therefore, it is of crucial importance to devise means to guarantee pork quality for both consumers safety and farms profits.

Serological and molecular tests are conventional methods to detect *T. gondii* infections. For example, enzyme-linked immunosorbent assay (ELISA) has been widely used to detect toxoplasmosis among humans and domestic animals (Yu et al., 2011; Dubey et al., 2012). However, ELISA may fail to detect IgG or IgM during the active phase period of *T. gondii*

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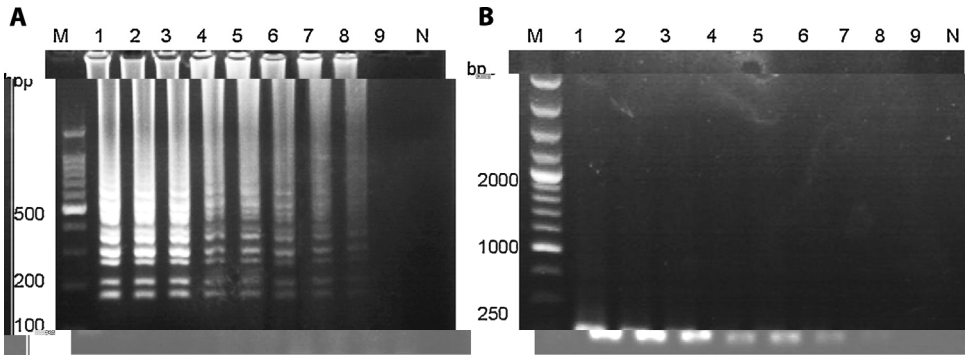
Tab e 1
Nucleotide sequence of LAMP and conventional PCR primers for ITS-1 of *Toxoplasma gondii* designed in this study.

Molecular assay	Target region	Sequence (5′–3′)	Length of primers	Amplification size
LAMP	ITS-1 (X75429.1) 6349–6547	F3: CTGAAGAAAGCCTCGCAGAA	20 bp	199

water was included as negative control in each test. Purified genomic DNA of *T. gondii* tachyzoites was 10-fold serially diluted with deionized water



F . 2. Comparative sensitivities by LAMP (A) and conventional PCR (B) for the specific detection of *Toxoplasma gondii* tachyzoites DNA based on ITS-1 amplification. Lane M, DNA ladder marker; lanes 1–8 represents 9 ng, 900 pg, 90 pg, 9 pg, 900 fg, 90 fg, 9 fg, 0.9 fg, 0.09 fg of *Toxoplasma gondii* DNA, respectively; lane N, negative control.

that LAMP method exhibits higher sensitivity than conventional PCR where the latter may give rise to false negative results.

Our preliminary LAMP results pointed out the prevalence of toxoplasmosis in pig farms in Jinhua, Zhejiang Province, China. A large-scale epidemiological investigation should be followed up to control toxoplasmosis and ensure public health.

In conclusion, a rapid, simple, sensitive and specific LAMP method based on the ITS-1 sequence of *T. gondii* genomic DNA detection was established and optimized. Our current results show that this LAMP assay can be used as a reliable and portable diagnostic tool of *T. gondii* to enable inspection and control of infected livestock. Therefore, this assay will facilitate clinical and epidemiological investigations and play an important role in guaranteeing meat quality and safety.

Ac ed e e

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