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Short communication

Comparison of loop-mediated isothermal amplification (LAMP) and cross-priming amplification (CPA) for detection of African swine fever virus

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Abstract

The reliable and rapid diagnosis of infectious animal diseases presents an exceptionally important aspect when considering their control and prevention. The paper describes the comparative evaluation of two rapid isothermal amplification methods for diagnosis of African swine fever (ASF). The robustness of loop-mediated isothermal amplification (LAMP) and the cross-priming amplification (CPA) were compared using samples obtained from ASF confirmed animals. Both assays were evaluated in order to define their diagnostic capabilities in terms of ASF diagnosis and reproducibility of the results. Investigations showed no cross-reactivity for other pig pathogens and no significant differences in the specificity of both assays. The sensitivity of LAMP reached 90%, while that of CPA was 70%. In conclusion, both methods are suitable for implementation in preliminary ASF diagnosis but further improvements are required to enhance their diagnostic sensitivity.

Key words: African swine fever, loop-mediated isothermal amplification, cross-priming amplification, comparison, diagnostic evaluation

Introduction

African swine fever (ASF) is a notifiable fatal viral disease of pigs and wild boars with significant veterinary and economic impact (Sánchez-Vizcaíno et al. 2015). Given the current critical situation according to the increasing ASFV occurrence in Central Europe, and recent emergence in China and Belgium, the disease constantly possesses a serious threat of worldwide spreading (Śmietanka et al. 2016, Zhou et al. 2018). The currently applicable preventive methods include strict sanitary measures, but reliable diagnosis of ASF is the other crucial point (World Organisation for Animal Health (OIE) Terrestrial Manual 2012). Some of OIE-approved diagnostic methods are laborious or require employment of sophisticated equipment that excludes their application by the veterinary practitioners or hunters. An isothermal nucleic acid amplification technology has been developed in order to simplify the assay workflow (Craw and Balachandran 2012). Recently, an alternative of cross-priming amplification (CPA) and loop-mediated isothermal amplifi-

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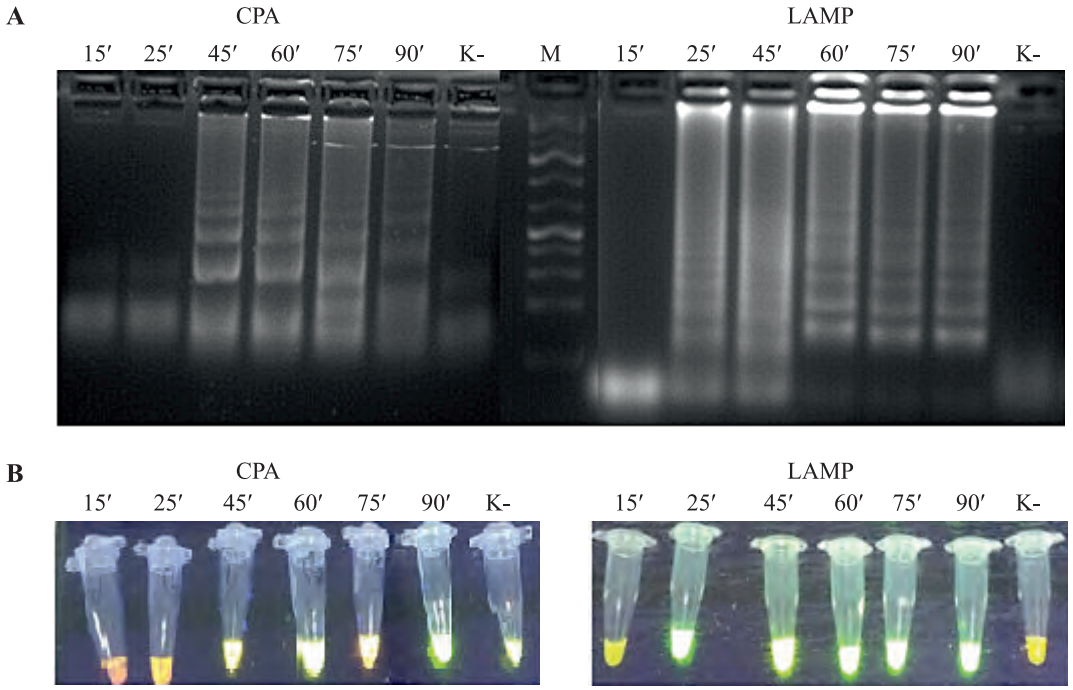

Fig. 1. Visualization of loop-mediated isothermal amplification (LAMP) and cross-priming amplification (CPA) optimization results. (A) gel electrophoresis of LAMP and CPA products on agarose gel. (B) The fluorescence illumination of positive samples under UV light.

cation (LAMP) for rapid ASFV detection has been developed. These techniques combine sensitivity, specificity of classical molecular methods with simplicity and short time required to perform the test in usually 20-60 min (James et al. 2010, Atuhaire et al. 2014, Frączyk et al. 2016). The basic feature of isothermal amplification is application of the thermostable polymerase enzyme and set of 3-6 primers (Atuhaire et al. 2014; Frączyk et al. 2016). The resulted amplicons with different length can be detected by observation of turbidity or color change of positive reaction mixture solutions after addition of fluorescence dye (Notomi et al. 2000, Craw and Balachandran 2012, Bai et al. 2015).

Materials and Methods

The aim of this study was to evaluate the performance of LAMP in comparison with CPA using 20 ASFV clinical specimens, which were confirmed by the World Organisation for Animal Health (OIE) recommended Universal Probe Library (UPL) real-time PCR as ASFV-positive. The DNA of specimens was extracted from the whole blood, serum and tissue homogenates of wild boars and pigs. A set of 2 controls for cross-species specificity testing was composed by cDNA of classical swine fever virus (CSFV) and porcine reproductive and respiratory syndrome virus (PRRSV). LAMP and CPA were conducted accordingly to the previously described protocols (Atuhaire et al. 2014, Frączyk et al. 2016). The illumination of positive reaction mixtures was confirmed by gel electrophoresis. LAMP and CPA were compared in terms of temperature and incubation time, sensitivity, specificity, reproducibility and performance using field samples. The sensitivity of both methods was tested using seven 10-fold serial dilution of standard ASFV plasmid at concentrations ranging from 7.2×10^{6} to 7.2×10^{-1} copy per μl. The field performance of both tests was verified using 24 specimens, 20 of them originated from ASF cases and outbreaks. The remaining 4 specimens were represented by the positive p72 plasmid control and 3 specificity controls represented by cDNA of CSFV, PRRSV and tissues obtained from uninfected animals.

Results and Discussion

Both LAMP and CPA positive specimens exhibited green illumination and the presence of ladder-like bands pattern after gel electrophoresis (Fig. 1). The fluorescence was not detected in negative specimens. The best effects of LAMP were obtained after incubation at 64°C-66°C for 25 min while for CPA at 56.2°C for 45 min (Fig. 1). The sensitivity of LAMP was determined as 330 copies, while for CPA reached 7.2 copies of standard ASFV plasmid, thus it was equal to the UPL real-time PCR. In case of field performance the LAMP detected ASFV DNA in 18 out of 20 specimens (90%), while the CPA in 14 out of 20 specimens (70 %) (Fig. 2).

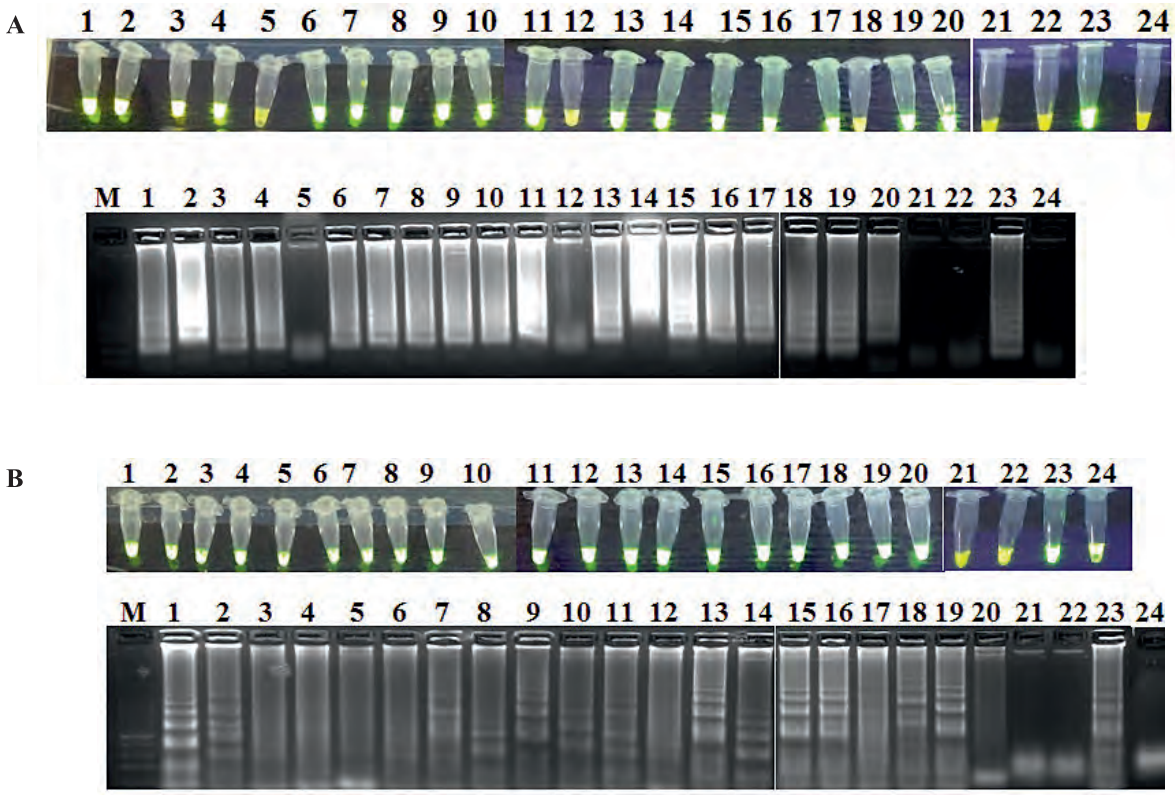

Fig. 2. Visualization of LAMP (A) and CPA (B) results using field African swine fever virus (ASFV) samples under UV light and gel electrophoresis.

The conducted comparison of LAMP and CPA showed that LAMP assay has a higher rate of positive detection specimens than CPA assay in spite of its lower sensitivity. Referring to the failure in diagnostics of some clinical samples it is also important to emphasize that opposite to sophisticated real-time PCR system LAMP or CPA applications are intended to be used on-site as the first line diagnostic tool. Additionally, the described isothermal tests would be used in primary stages of infection, when diagnostic value of material is the most appropriate for viral DNA detection. The previously conducted studies concerning the performance of LAMP revealed its fair effectiveness using both fresh samples as well as samples stored for various periods of time at -20°C (Oura *et al.* 2013). False negative results may be a result of undesirable, spontaneous hybridization of multiple primers to non-target matrices or formation of secondary primer structures (Xu *et al.* 2012). Summarizing features of the isothermal methodology, both CPA and LAMP overcome many of the limitations observed in routinely used diagnostic methods such as the necessity of sophisticated laboratory equipment, well trained staff or a high cost of exploitation. Further modifications of isothermal tests are needed to make them more perfect diagnostic tools (Li and Macdonald 2015).

References

Atuhaire DK, Afayoa M, Ochwo S, Katiti D, Mwiine FN, Nanteza A, Mugasa CM, Matovu, E, Okuni JB, Olaho-Mukani W, Ojok L (**2014**) Comparative detection of African swine fever virus by loop-mediated isothermal amplification assay and polymerase chain reaction in domestic pigs in Uganda. African J Microbiol Res 8: 2322-2328.

Bai Z, Xie H, You Q, Pickerill S, Zhang Y, Li T, Geng J, Hu L, Shan H, Di B (**2015**) Isothermal cross-priming amplification implementation study. Lett Appl Microbiol 60: 205-209.

Craw P, Balachandran W (**2012**) Isothermal nucleic acid amplification technologies for point-of-care diagnostics: a critical review. Lab Chip 12: 2469-2486.

Frączyk M, Woźniakowski G, Kowalczyk A, Niemczuk K, Pejsak Z (**2016**) Development of cross-priming amplification for direct detection of the African Swine Fever Virus, in pig and wild boar blood and sera samples. Lett Appl Microbiol 62: 386-391.

James HE, Ebert K, McGonigle R, Reid SM, Boonham N, Tomlinson JA, Hutchings GH, Denyer M, Oura CAL, Dukes JP, King DP (**2010**) Detection of African swine fever virus by loop-mediated isothermal amplification. J Virol Methods 164: 68-74.

Li J, Macdonald J (**2015**) Advances in isothermal amplification: novel strategies inspired by biological processes. Biosens Bioelectron 64: 196-211.

Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, Hase T (**2000**) Loop-mediated

isothermal amplification of DNA. Nucleic Acids Res 28: E63.

OIE Terrestrial Manual (**2012**) Aetiology Epidemiology Diagnosis Prevention and Control References. World Organisation for Animal Health (OIE) Terr Man pp 1-5.

Oura CAL, Edwards L, Batten CA (**2013**) Virological diagnosis of African swine fever--comparative study of available tests. Virus Res 173: 150-158.

Sánchez-Vizcaíno JM, Mur L, Gomez-Villamandos JC, Carrasco L (**2015**) An update on the epidemiology and pathology of African swine fever. J Comp Pathol 152: 9-21.

Śmietanka K, Woźniakowski G, Kozak E, Niemczuk K, Fraçzyk M, Bocian Ł, Kowalczyk A, Pejsak Z (**2016**) African swine fever epidemic, Poland, 2014-2015. Emerg Infect Dis 22: 1201-1207.

Xu G, Hu L, Zhong H, Wang H, Yusa SI, Weiss TC, Romaniuk PJ, Pickerill S, You Q (**2012**) Cross priming amplification: mechanism and optimization for isothermal DNA amplification. Sci Rep 2: 246.

Zhou X, Li N, Luo Y, Liu Y, Miao F, Chen T, Zhang S, Cao P, Li X, Tian K, Qiu H, Hu R (**2018**) Emergence of African Swine Fever in China, 2018. Transbound Emerg Dis doi:10.1111/tbed.12989.