Development and preliminary evaluation of a highly sensitive method for detecting African swine fever virus in oral fluids from naturally infected raised pigs in Northern Vietnam

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1 Introduction

Early detection and early slaughter through quarantine are essential to prevent the spread of the African swine fever virus (ASFV). Pooled oral fluids tests have been used for simple pathogen monitoring, but compared to blood tests, the virus concentration in oral fluids is low, resulting in false negative and missing true positive cases. We collected oral fluids from sub-clinical raised pigs in northern Vietnam and attempted a highly sensitive ASFV survey using a newly developed method.

Summary/Keywords

- A method has been developed to detect ASFV in pig oral fluids with up to 100 times greater sensitivity than a reference method.
- The developed method combines innovative pretreatment in less than 60 min with conventional nucleic acid extraction with a column kit followed by real-time PCR or LAMP.
- Applied to preliminary evaluation for ASF diagnosis on raised pigs, the developed method showed higher diagnostic performance than the reference method.
- The developed method can be a useful tool for rapid screening in ASF-eradicated and -endemic areas.

/ African swine fever virus/ concentration/ Early diagnosis/ Oral fluid/ Pig

2 Methods

We have been already developed a new method for concentration and detection of SARS-CoV-2 in pooled human saliva with 100 times higher sensitivity than a reference method (Yamazaki *et al.*, 2024). This developed method, which takes less than one hour to complete, is based on the simple principle of saliva lysis by semi-alkaline protease (SAP), a sputum dissolving agent, and concentration of virions by polyethylene glycol (PEG) and high-speed centrifugation, followed by extraction of nucleic acids by a commercial column kit. In this study, we applied this method for the first time to the detection of trace viruses in animal oral fluids and evaluated the feasibility of this method in the sensitive detection of ASFV using 68 pooled oral fluids collected from raised pigs in Vietnam and Japan.

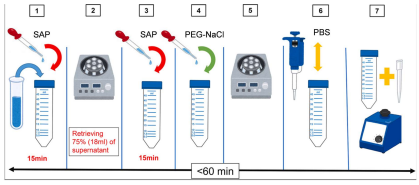

Figure 1. Developed protocol for virion concentration from saliva.

3 Results & Discussion

Table 1. Limit of detections (LODs) of the developed and reference methods for detecting ASFV spiked into ASFV-negative pig oral fluids.

Assays	10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Real-time PCR (Ct)						
Developed method	26.40	30.34	33.25	36.43	No. Ct	No. Ct
Reference method	35.51	No. Ct	No. Ct	No. Ct	No. Ct	No. Ct
Real-time LAMP (Tp)						
Developed method	28:36	32:38	No. Tp	No. Tp	No. Tp	No. Tp
Reference method	No. Tp	No. Tp	No. Tp	ND	ND	ND

Ct, Threshold cycle. Tp, Time of positivity. No. Ct, No threshold cycle values detected using real-time PCR. No. Tp, Time-of-positivity values undetectable using LAMP. ND, Not determined.
*, In a duplicate analysis, using real-time PCR or LAMP assay, all two samples were positive or negative, with no samples showing one positive and one negative.

In a spike test result, the developed method showed up to 100 times greater sensitivity than a reference method(Table 1). For performance evaluation, 68 pooled oral fluid samples were collected. Using real-time PCR, 9/68 (13.2%) were positive by the reference method, while 23/68 (33.8%) were positive by the developed method (Table 2 and Figure 2). Using real-time LAMP, 1/68 (1.5%) were positive by the reference method and 6/68 (8.8%) by the developed method(Table 3 and Figure 3). Therefore, the developed method improved the diagnostic performance of ASFV with oral fluids and enabled early diagnosis before the onset of the disease.

Table 2. Comparison of diagnostic performance using real-time PCR against reference and developed DNA extraction methods.

Developed	Reference	
	Positive (n = 9)	Negative (n = 59)
Positive (n = 23)	9	14
Negative (n = 45)	0	45*

Diagnostic sensitivity, 100% (9/9); Diagnostic specificity, 76.3% (45/59).
*; Containing five Japanese pig oral fluids as negative control samples.

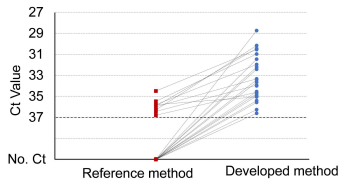

Figure 2. Variation of Ct values in the developed and reference methods for 23 samples that showed positive by real-time PCR detection.

Reference, Real-time PCR with DNA using an extraction column kit or an automated extraction platform.
Developed, Improved DNA extraction combined with real-time PCR.

Table 3. Comparison of diagnostic performance using real-time LAMP against reference and developed DNA extraction methods.

Developed	Reference	
	Positive (n = 1)	Negative (n = 67)
Positive (n = 6)	1	5
Negative (n = 62)	0	62*

Diagnostic sensitivity, 100% (1/1); Diagnostic specificity, 92.5% (62/67).
*; Containing five Japanese pig oral fluids as negative control samples.

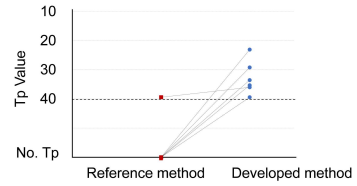

Figure 3. Variation of Tp values in the developed and reference methods for 6 samples that showed positive by real-time LAMP detection.

Reference, Real-time LAMP with DNA using an extraction column kit or an automated extraction platform.
Developed, Improved DNA extraction combined with real-time LAMP.

4 Conclusion

The developed method has the potential to enable simple and highly sensitive diagnosis of ASF, which can contribute to its early diagnosis and early containment by rapid screening in ASF-eradicated and -endemic areas.

5 References & Acknowledgements

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