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Short communication

Diagnostic specificity of a real-time RT-PCR in cattle for foot-and-mouth disease and swine for foot-and-mouth disease and classical swine fever based on non-invasive specimen collection

G.T. Fosgate [a,*], S. Tavornpanich [a,1], D. Hunter [b], R. Pugh [b], J.A. Sterle [c], K.R. Schumann [d], A.J. Eberling [d], T.R. Beckham [d], B.M. Martin [e], N.P. Clarke [f], L.G. Adams [b,f]

[a] *Departments of Veterinary Integrative Biosciences, Texas A&M University, College Station, TX 77843, USA*
[b] *Veterinary Pathobiology, Texas A&M University, College Station, TX 77843, USA*
[c] *Animal Science, Texas A&M University, College Station, TX 77843, USA*
[d] *Foreign Animal Disease Diagnostic Laboratory, National Veterinary Services Laboratories, Veterinary Services, Animal and Plant Health Inspection Service, United States Department of Agriculture, Greenport, NY 11944, USA*
[e] *National Veterinary Services Laboratories, Veterinary Services, Animal and Plant Health Inspection Service, United States Department of Agriculture, Ames, IA 50010, USA*
[f] *National Center for Foreign Animal and Zoonotic Disease Defense, Department of Homeland Security, and College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX 77843, USA*

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Abstract

Foot-and-mouth disease virus (FMDV) and classical swine fever virus (CSFV) are highly contagious and can cause great economic losses when introduced into disease-free regions. Accurate estimates of diagnostic specificity (Sp) are important when considering the implementation of surveillance for these agents. The purpose of this study was to estimate diagnostic Sp of a real-time reverse-transcriptase PCR assay developed for detection of FMDV in cattle and domestic swine and CSFV in domestic swine based on non-invasive specimen collection. One thousand and eighty-eight range beef cattle were sampled from thirteen geographic locations throughout Texas. One thousand and one hundred market hogs and cull sows were sampled. Results for both FMDV and CSFV were considered positive if amplification occurred at or before 40 PCR cycles, inconclusive between 40 and 45 cycles and negative otherwise. Ten cattle had nonspecific PCR amplifications for FMDV, but none were classified as positive and only one as inconclusive. Specificity (95% confidence interval) was estimated as 100% (99.7, 100). There were 19

* Corresponding author. Tel.: +1 979 845 2828; fax: +1 979 847 8981.
E-mail address: gfosgate@cvm.tamu.edu (G.T. Fosgate).
[1] National Institute of Animal Health, Department of Livestock Development, Ministry of Agriculture & Cooperatives, Bangkok 10400, Thailand.

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nonspecific PCR amplifications for FMDV in sampled swine with 1 classified as positive, 6 as inconclusive, and 12 as negative. Specificity (95% confidence interval) was estimated as 99.9% (99.5, 100). There were 21 nonspecific PCR amplifications for CSFV, and 1 was classified as positive. Specificity (95% confidence interval) was estimated as 99.9% (99.5, 100). These assays have high Sp, but nonspecific PCR amplifications can occur.

Keywords: Foot-and-mouth disease; Classical swine fever; RT-PCR; Specificity; Surveillance

1. Introduction

Foot-and-mouth disease virus (FMDV) belongs to the Picornaviridae family within the genus *Aphthovirus* (Lubroth, 2002). The viral genome is comprised of a single molecule of positive-sense single-stranded RNA (Alexandersen et al., 2003), and seven serotypes (O, A, C SAT 1–3, and Asia 1) have been identified (Thomson and Bastos, 2004). Classical swine fever virus (CSFV) belongs to the family Flaviviridae within the genus *Pestivirus* (Kleiboeker, 2002). The CSFV genome is a single molecule of positive-sense single-stranded RNA, and only a single serotype has been recognized (Paton and Greiser-Wilke, 2003).

PCR-based diagnostic assays have been developed to identify FMDV and CSFV (Harding et al., 1994; McGoldrick et al., 1998; Reid et al., 2000; Callahan et al., 2002; Hearps et al., 2002; Aguero et al., 2004; Hoffmann et al., 2005; Oem et al., 2005; Risatti et al., 2005; Depner et al., 2006; Ferris et al., 2006; Ophuis et al., 2006). FMDV can be present in most physiologic fluids and isolated several days prior to development of clinical lesions (Alexandersen et al., 2003; Thomson and Bastos, 2004). Oral cavity swabs can be used to identify the virus in animals prior to development of these lesions. Tonsils are often the first tissue where CSFV can be identified after oral exposure (van Oirschot, 2004), and oral and nasal swabs van be used as specimens for diagnostic detection of the virus in infected swine.

Validation of a diagnostic test is the process of evaluating the effectiveness for a particular use (Jacobson, 1998). A validated assay identifies the presence of a particular analyte (e.g. RNA sequence) that allows predictions to be made concerning the true disease status of the animal. Accuracy is measured as sensitivity (Se) and specificity (Sp), which are the probability of correctly identifying diseased and non-diseased animals, respectively. Field validation of a diagnostic assay is primarily concerned with measuring the effects due to factors that affect the concentration and composition of the analyte in the collected specimen to be tested (host factors). Host factors can be classified as intrinsic (e.g. age, sex, breed, genetic resistance or susceptibility) or acquired (e.g. actively or passively acquired immunity). The strain of the disease agent might also affect the assay through mechanisms associated with the host (tests based on immunity) and the agent itself (tests based on agent detection). Non-host factors in the field, such as contamination or deterioration of the sample, might also affect analyte quality and quantity and subsequently overall diagnostic accuracy.

Accurate estimates of Sp are important when considering surveillance for foreign animal diseases such as FMD and CSF. Sensitivity is important for early recognition of disease to reduce spread, but it must be balanced against required high Sp because false-positive results need complete epidemiologic and diagnostic workups to eliminate the possibility that these viruses were present. The objective of this study was to estimate diagnostic Sp of a real-time reverse-transcriptase PCR assay developed for the identification of FMDV infection in cattle and domestic swine and CSFV in domestic swine based on non-invasive specimen collection.

2. Materials and methods

Animal protocols were approved by the Clinical Research Review Committee at the College of Veterinary Medicine and Biomedical Sciences, Texas A&M University.