MOLECULAR DETECTION OF CLASSICAL SWINE FEVER VIRUS FROM OUTBREAKS IN DIFFERENT FARMS IN THRISSUR DISTRICT

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ABSTRACT

The carcasses of pigs belonging to different age groups were brought from three farms in Thrissur district to the Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Mannuthy for post mortem examination. Classical swine fever (CSF) was suspected based on history, clinical symptoms narrated by the owners and lesions noted on post mortem examination. In all the cases, owners reported heavy morality among the piglets. The post mortem lesions such as haemorrhages in lymph nodes, diffuse white focal areas in kidneys, haemorrhagic gastritis, diphtheritic membrane formation in intestine, degenerated and necrotic areas on liver and pulmonary emphysema with marbled appearance of lungs of varying degrees were noticed. In the fourth case, owner reported a heavy mortality and production loss among pigs in his farm and brought a live piglet of about two-month-old to the Department of Veterinary Microbiology for disease investigation. Erythematous patches was observed on the skin of affected piglet which is indicative of CSF. The piglet showed enlarged lymph nodes, pneumonic changes in lungs, hepatosis and cardiac hypertrophy on post-mortem inspection. All four cases were diagnosed as CSF by a polymerase chain reaction (PCR) targeting the E2gene of CSF virus which revealed an amplicon size of 308 bp.

Keywords: Classical swine fever, RT-PCR, Thrissur

INTRODUCTION

Classical swine fever (CSF) or Hog cholera is a highly contagious disease of swine. The disease is endemic in Asia, Central and South America and parts of Europe and Africa. The infectious agent responsible for the disease is a virus, CSF virus of the genus *Pestivirus* in the family *Flaviviridae*. Severity of disease is related to the strain of virus isolate, age of pig and immune status of the herd. According to Floegel *et al*. (2003), clinical picture of CSF is variable, depending on the age of the affected animals and viral virulence. Acute

disease is still the prevalent form in younger animals, with subacute and chronic forms often observed in older animals.

Diagnosis is done usually by observing clinical signs and post-mortem lesions, but it can be confusing. Harkness (1985) stated that the disease takes several forms apart from the dramatic symptoms described. Moreover, gross lesions observed at necropsy are diverse and often not pathognomonic (Carbrey *et al*., 1966; Dahle and Liess, 1992). Rapid and precise detection of CSFV is critical for disease containment. Current diagnostic methods, including detection of viral antigens in tonsils by using fluorescent antibodies (Ressang and de Boer, 1968) or antigen capture enzyme-linked immunosorbent assays (Clavijo *et al*., 1998, Shannon *et al*., 1993) requires centralized laboratory facilities and submission of clinical specimens that might delay disease diagnosis, thus affecting the efficiency of emergency disease management measures. Konig *et al*. (2011) stated that rapid and accurate diagnosis is of utmost importance in the control of epizootic disease such as classical swine fever. To address this need, detection of genomic RNA by reverse transcription-PCR (Meyer *et al*., 1996; Harding *et al*., 1994; Wirtz *et al*., 1993) offers a rapid and sensitive diagnostic test.

MATERIALS AND METHODS

Tissues of lymph nodes, spleen, portion of kidney and lungs were processed as per Rinsha *et al*. (2016) and RNA was extracted by following Trizol method. Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) was carried out using specific primers as per Kumar *et al*. (2015) and the procedure was conducted with minor modifications. The following primers were used in the reaction

F-5'-ATATATGCTCAAGGGCGAGT-3'

R-5'-ACAGCAGTAGTATCCATTTCTTTA-3'

The first strand cDNA was synthesized using Revert Aid First Strand cDNA Synthesis Kit according to the manufacturer's recommendations (Thermo Scientific). The RNA extracted from the CSF vaccine (Lapinised CSF vaccine, Institute of Animal Health & Veterinary Biologicals, Palode) was served as the positive control.

The following protocol was standardised for the synthesis of cDNA. i.e. viral RNA (8 µL), random hexamer primer (1 µL) and Nuclease free water (added up to 12 µL), and incubated at 90ºC for 5 minutes, followed by addition of 4 µL of 5X Reaction buffer, 1 µL of Ribolock RNase Inhibitor (20 U/µL), 2 µL of 10mM dNTP Mix and 1 µL of Revert Aid M-MuLV RT (200 U/µL). The reaction mixture was incubated at 25ºC for 5 minutes. The reverse transcription was carried out at 42ºC for 60 min in the thermal cycler. This was followed by heat inactivation of M-MuLV RT at 70ºC for 5 min. The strand of cDNA thus synthesized was used as template for PCR.

A total volume of 25 µL in a 200 µL PCR tube was carried out. The combination that gave the best result for amplification was selected and further PCR was carried out in Eppendorf, Thermal cycler (table 2).

The details of reaction mix are given in the table 1.

Table 1. Optimized concentrations of PCR reagents for classical swine fever amplification

Components	Volume (µL)
Template (cDNA)	1
10 X PCR buffer	6.25
Forward primer (10 pM/µL)	1.0
Reverse primer (10 pM/µL)	1.0
Sterile Nuclease free water	6.25

Table 2. PCR conditions for amplification of classical swine fever

Steps		Temperature	Time
Initial denaturation		94ºC	4 min.
35 cycles	Denaturation	94ºC	1 min.
	Annealing	54.4ºC	1 min.
	Extension	72ºC	1 min.
Final extension		72ºC	10 min.
Hold		4ºC	10 min.

After PCR, the products were electrophoresed in one percent agarose gel in Tris-Acetate-EDTA (TAE) buffer (1X) and visualised under gel documentation system (Bio-Rad).

RESULTS AND DISCUSSION

Various techniques like virus isolation, immunofluorescent assays (IFA), enzyme linked immunoassays (ELISA) were employed for the detection of CSF virus (Depner *et al*., 1995). But, these techniques are time consuming and cumbersome, which lead to an urge to use molecular techniques which would be more sensitive and less time consuming. RT-PCR is considered to be the best technique for the timely diagnosis of CSF, which offers results with high sensitivity (Le Dimna *et al*., 2008, Rinsha *et al*., 2016). In the present case study, post-mortem examination of the pigs revealed lesions suggestive of CSF. The cases were further confirmed by a PCR assay targeting the E2 gene of the virus with an amplicon size of 308 bp (Fig. 1). The RT-PCR assay targeting the E2 gene of the

virus offers a rapid and sensitive diagnosis for the detection of CSF in cases where the post mortem lesions are not specific or the animals are presented with non-specific symptoms (Kumar *et al*., 2015 and Ravishankar *et al*., 2007).

SUMMARY

The present communication deals with the molecular detection of CSF using RT-PCR from outbreaks of the disease in different farms in Thrissur district. The technique provides a rapid and sensitive method for the timely diagnosis of the disease, thereby appropriate strategies can be formulated for the control.

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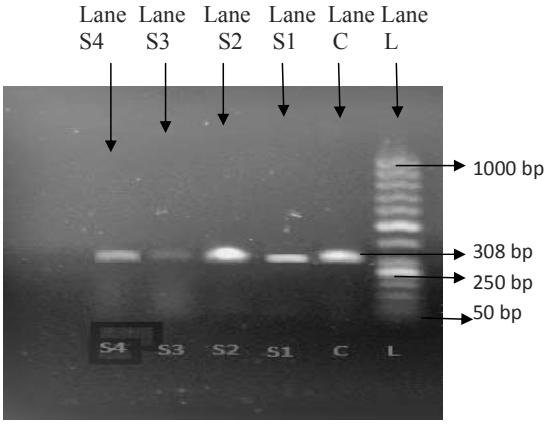

Fig. 1. Polymerase Chain Reaction targeting the E2gene of classical swine fever virus
Lane L: 50 bp ladder; Lane C: Positive control; Lane S1, S2, S3, S4: Positive samples

REFERENCES

Carbrey, E.A., Stewart, W.C. and Young, S.H. 1966. The changing picture of hog cholera: case studies. *J. Am. Vet. Med. Assoc*. **149**:1724.

Clavijo, A., Zhou, E.M., Vydelingum, S. and Heckert. R. 1998. Development and evaluation of a novel antigen capture assay for the detection of classical swine fever virus antigens. *Vet. Microbiol*. **60**: 155-168.

Dahle, J. and Liess, B.A. 1992. A review on classical swine fever infections in pigs: epizootiology, clinical disease, and pathology. *Comp. Immunol. Microbiol. Infect. Dis.* **15**: 203–211.

Depner, K.R., Muller, A., Gruber, A., Rodriguez, A., Bickhardt, K. and Liess, B. 1995. Classical swine fever in wild boar (*Sus scrofa*) experimental infections and viral persistence. *Dtsch. Tierarztl. Wochenschr*. **102**(10): 381-384.

Floegel, G., Bunzenthak, C., Fischer, S., Moennig, V. and Kaaden, O.R. 2003. Virulence of recent and former classical swine fever virus isolates evaluated by their clinical and pathological signs. *J. Zoonoses Public Hlth*. **50**: 214-220.

Harding, M., Lutze-Wallace, C., Prud'Homme, I., Zhong, X. and Rola. J. 1994. Reverse transcriptase-PCR assay for detection of hog cholera virus. *J. Clin. Microbiol*. **32**: 2600-2602.

Harkness, J.W. 1985. Classical swine fever and its diagnosis: A current view. *Vet. Rec.* **116**(11): 288-293.

Konig, P., Blome, S., Gabriel, C., Reimann, I. and Beer, M. 2011. Innocuousness and safety of classical swine fever marker vaccine candidate CP7_E2alf in non-target and target species. *Vaccine,* **30**: 5-8.

Kumar, P., Upmanyu, V. and Dhar, P. 2015. Biological and molecular characterization of classical swine fever challenge virus from India. *Vet. World*. **8**(3): 330-335.

Le Dimna, M., Vrancken, R., Koenen, F., Bougeard, S., Mesplede, A., Hutet, E., Kuntz- Simon, G. and Le Potier, M.F. 2008. Validation of two commercial realtime RT-PCR kits for rapid and specific diagnosis of classical swine fever virus. *J. Virol. Methods*. **147**(1): 136-142.

Meyer, G., Thiel, H.J. and Rumenapf, T. 1996. Classical swine fever virus: recovery of infectious viruses from cDNA constructs and generation of recombinant cytopathogenic defective interfering particles. *J. Virol.* **70**: 1588-1595.

Ravishankar, C., Priya, P.M., Mini, M., Rameshkumar, P., Selvan, P.S., Jayesh, V., Sunil, K.S., Sharmadha, M.K., Sreekumaran, T. and Jayaprakasan, V. 2007. First confirmed occurrence of classical swine fever in Kerala state, India. *J. Swine Hlth. Prod*. **15**: 156-159.

Ressang, A.A., and de Boer, G.F. 1968. A comparison between the cell culture, frozen tissue section, impression and mucosal smear techniques for fluorescent antibody in the diagnosis of hog cholera. Neth. *J. Vet. Sci*. **1**:72.

Rinsha, B., Surya, S., Mini, M. and Devi, S.S. 2016. Molecular characterisation of classical swine fever virus from an outbreak of disease among pigs in a private farm in Thrissur district. In: *Proceeding of 8th Kerala Veterinary Science Congress*; 12th to 13th November, 2016, Pattikkad. pp. 84-86.

Shannon, A.D., Morrissy, C., Mackintosh, S. and Westbury, H.A. 1993. Detection of hog cholera virus antigens in experimentally infected pigs using an antigen-capture ELISA. *Vet. Microbiol*. **34**: 233-248.

Wirtz, B., Tratchin, J.D., Muller, H.K. and Mitchell. D.B. 1993. Detection of hog cholera virus and differentiation from other pestiviruses by polymerase chain reaction. *J. Clin. Microbiol.* **31**:1148-1154.

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