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Seroprevalence of Classical Swine Fever in pigs of Karnataka and comparative diagnostic evaluation of antigen ELISA and reverse transcriptase -PCR

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ABSTRACT

Classical swine fever is highly contagious viral disease of swine causing huge economic losses to pig farmers and pig industry. The study was conducted in Karnataka to understand the current scenario of CSF in the state and to evaluate efficiency of diagnostic tests (antigen ELISA and RT-PCR) in detecting the disease. Serum samples were collected from 270 pigs from 13 districts of the state and were tested for the presence of CSF antibodies. Whole blood samples (151) from 14 outbreaks of CSF were collected for the comparative diagnosis of the disease using Antigen ELISA and reverse transcriptase (RT)-PCR. A seroprevalence of 28.5% (77/270) was found in the serum samples collected form the whole Karnataka. The southern Karnataka had higher seroprevalence (47%) in comparison to northern Karnataka region where seroprevalence was 17%. It confirms endemicity of the disease in southern region. Of 151 blood samples collected for comparative diagnosis, 61 samples were positive for CSF by RT-PCR and 39 by antigen ELISA, indicating the superiority of RT-PCR over antigen ELISA to detect CSFV infection in earliest stages of infection.

Key words: Antigen, Antibody, Classical swine fever, ELISA, Karnataka, RT-PCR, Seroprevalence

Classical swine fever (CSF), a highly contagious and potentially fatal viral disease of swine, is responsible to cause major economic losses in most of the pig producing states of India (Singh *et al*. 2016). The disease is classified in World Organization for Animal Health (OIE) list of notifiable terrestrial and aquatic animal diseases and outbreaks are reportable, with resultant trade sanctions against the affected countries (OIE 2017a). CSF virus (CSFV), the causative agent of CSF, is a member of the genus *Pestivirus,* which belongs to the family Flaviviridaeis closely related antigenically and structurally to bovine viral diarrhea virus and border disease virus (Wengler *et al.* 1995). The genome of CSFV is a positive polarity RNA of about 12.3 kb in length which contains un-translated regions at 5' and 3' ends and encodes a single polyprotein that is both co- and post-translationally processed to yield 4 structural (C, Erns, E1 and E2) and 8 non-structural (Npro, p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B) viral proteins (Meyers and Theil 1996). CSF is one of the top five viral diseases of livestock (foot and mouth disease, bluetongue, peste des petits ruminants, sheep and goat pox, CSF) in India and is a major constraint to the development of pig farming in the country (Patil *et al.* 2012).

Last 5 years (2010–2015) outbreaks data of OIE showed that in India, outbreaks of the disease were reported from Haryana, Maharashtra, Bihar, Madhya Pradesh, Rajasthan, Tamil Nadu, West Bengal and Punjab, and the North East Indian states of Arunachal Pradesh, Tripura, Manipur, Mizoram, Nagaland and Meghalaya (OIE 2017b). Occurrence of CSFV genotype 1.1 and more recently dominance of genotype 2.2 were documented in Karnataka (Patil *et al.* 2010, Shivaraj *et al.* 2015) and study reporting that currently historical groups (1.1, 1.2 and 1.3) dominated over by phylogenetic group 2 (Lin *et al.* 2013). However, there is paucity of information on the seroprevalence of CSFV infection in Karnataka. In India, vaccination is most effective mode to control the disease in swine population (Chander *et al.* 2014). Thorough understanding of the epidemiology of the disease is prerequisite to implement vaccination programme successfully. The present study was undertaken to obtain the baseline epidemiological information on the prevalence of CSF by competitive blocking ELISA in serum and also to evaluate both antigen

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ELISA and RT-PCR for their diagnostic efficiency in the field.

MATERIALS AND METHODS

Sampling plan: Four administrative divisions of Karnataka namely Bengaluru, Mysuru, Belagavi, and Kalaburagi comprising 13 districts were selected for sample collection.

Collection of samples: Serum samples (488) based on sampling plan were collected from pigs in appropriate vacutainer tubes from 13 different districts of Karnataka during August 2014 to January 2015. Clotted blood samples were spun at 1,100–1,300 rpm for 15 min, sera separated and stored at - 20°C until tested. Whole blood samples (151) from 14 outbreaks of CSF were collected in vacutainer with anticoagulant and kept at refrigeration temperature of 4°C till further use.

ELISA screening of serum: Collected whole blood for CSF antigen and sera for CSF antibody screening was carried out by using the CSFV antigen and antibody ELISA kits (IDEXX Laboratories, Netherlands), respectively. Samples were considered positive for the antigen ELISA, when the corrected absorbance values were ≤0.30, negative when <0.20, and doubtful between 0.20 and 0.30, requiring re-testing. In antibody ELISA testing, samples were considered negative when the blocking percentage was ≤30% and positive when ≤40%. If the values were between 30 and 40%, the samples were considered suspicious and were retested once again at later date.

Reverse transcription: Reverse transcription-PCR for whole blood samples was performed targeting E2 region using RNA template and previously published primers (Chen *et al.* 2008) by one-step RT-PCR kit. Reverse transcription was performed at 50°C for 30 min, followed by initial enzyme activation step 95°C for 15 min. Subsequent PCR amplification was carried out with 35 cycles of denaturation at 94°C for 1 min, annealing at 59°C for 1 min, extension at 72°C for 1 min followed by final extension for 10 min at 72°C. The PCR products were analyzed by electrophoresis on 1.5% agarose alongside 250 bp DNA ladder.

RESULTS AND DISCUSSION

Seroepidemiology: The study was conducted to determine the prevalence of CSF in Karnataka and also to evaluate both the diagnostic tests (antigen ELISA and RT-PCR) for their efficiency in detecting CSFV in whole blood samples. A sero-surveillance technique such as ELISA is highly useful in disease eradication programs. It is a good tool for surveillance of negative herds in countries where monitoring of the disease is being practiced and helps for mass screening of samples and is effective in early diagnosis in nearby regions of an outbreak (Choori *et al.* 2015). The overall mean seroprevalence of CSFV was 28.5% (77/270) in 13 districts of Karnataka. Seroprevalence was 47% (33/70), 42% (06/14), 17% (21/122), and 26% (17/64) for Bengaluru, Mysuru, Belagavi, and Kalaburagi divisions of Karnataka, respectively (Table 1).

Similarly during 2014, a CSF study conducted in Karnataka showed an overall 33% (173/517) and 43, 33, 12, and 6% seroprevalence recorded for Bengaluru, Mysuru, Kalaburagi, and Belagavi divisions, respectively (Shivaraj *et al.* 2013). An alarmingly high seroprevalence of CSF (34%) in Karnataka including notable prevalence of disease in the northern region was revealed in this study. Piggery is still in primitive stages in north in comparison to south Karnataka. The regional distribution of seroprevalence revealed that southern Karnataka (47% and 42% in Bengaluru and Mysuru divisions respectively) had the highest CSF seroprevalence, confirming the endemicity of the disease in that region. The northern part of Karnataka showed a lower seroprevalence when compared to the south (17 and 26% in Belagavi, and Kalaburagi divisions accordingly).

The results of seroprevalence from Bengaluru and Mysuru divisions were statistically significant (P<0.01) when compared to samples from Kalaburagi and Belagavi. Large pig farm are more concentrated and intensive in southern region which might be one of the reason of high seroprevalence of CSF in this area. Further, south Karnataka shares border with Tamil Nadu and Kerala, and pig farmers are involved in regular procurement of piglets from pig breeding farms located in the adjoining border areas of these state where there were no CSF vaccination campaigns. The study conducted during 2012–2013 at National Institute of Veterinary Epidemiology and Disease Informatics, Bengaluru showed approximately 77% (31/40) and 100% (10/10) seroprevalence of CSF in Kerala and Tamil Nadu respectively (PD_ADMAS 2013). Procurement and cross border transportation of piglets and pig, from neighbouring

Table 1. Seroprevalence of CSF by competitive blocking ELISA

District	Division	No. of sera samples tested	No. of sera samples positive by ELISA	Per cent positivity (District-wise)	Per cent positivity (Division-wise)
Chitradurga	Bengaluru	26	11	42.30	47% (33/70)
Kolar		14	09	64.28	
Tumkuru		30	13	40	
Mysuru	Mysuru	14	06	42.85	42.85% (06/14)
Bagalkot	Belagavi	44	6	13.6	17% (21/122)
Belagavi		18	04	22.22	
Bijapur		16	03	18.75	
Dharwad		27	04	14.81	
Haveri		17	04	23.52	
Bellary	Kalaburagi	12	02	16.66	26% (17/64)
Kalaburagi		07	02	28.57	
Koppal		23	08	34.7	
Raichur		22	05	22.72	
	Total	270	77	28.5%	

state to south Karnataka is one of the inductive factors for high prevalence of CSF in these states, resulting in the observed high antigen and seroprevalence in comparison to north Karnataka. In north Karnataka most people do not prefer pork consumption for social and cultural reasons resulting less organized pig farming which might be one of the factors for the low prevalence of the disease in north Karnataka. Nandi *et al.* (2011) reported CSF seroprevalence of 63% from 12 different states of India during 2004–2010 and 53% prevalence in southern India alone. A compilation of data from OIE website indicates that there were 1576 outbreaks of CSF in India during 2005–2015 (OIE 2017b). The disease is also most frequently reported from Karnataka due to considerable density of pigs in the state. CSFV also shared antigenic similarity with other BVDV-1 and BVDV-2 of ruminants. Therefore, constant monitoring of swine population for CSFV is necessary (Podgorska *et al.* 2012).

Comparison of antigen ELISA and RT-PCR: Blood samples (151), which were collected from 14 outbreaks (based on clinical symptoms of high fever (106–108°F), huddling, incoordination, red patches on ear and inner side of abdomen, high mortality, and post mortem lesion on stomach, kidney and intestine), were used to compare the detection of virus by antigen (Ag) ELISA and RT-PCR. Eleven outbreaks were confirmed as CSF by Ag ELISA and 3 outbreaks were negative. Out of 151 blood samples, 39 samples were positive by Ag ELISA. However, 12 outbreaks were confirmed as CSF and 2 outbreaks were found negative for CSF by RT-PCR. Out of 151 blood samples, 61 samples were CSFV positive by RT-PCR (Table 2). The CSF outbreak at Khanahosalli, Bellary district was negative by Ag ELISA, but positive by RT-PCR even though pigs showed clinical signs of CSF. Outbreaks at Tumkuru and Raichur were negative for CSF by both Ag ELISA and RT-PCR. District wise detection of virus by ELISA and RT-PCR in blood samples from CSF confirmed outbreaks are depicted in Table 2.

Table 2. Details of blood samples found positive for CSF by RT-PCR and ELISA

District	No. of blood samples tested	No. of blood samples positive by	
		RT-PCR	ELISA
Kadagadal, Madikeri Taluq, Kodagu	13	08	07
Aagara, Kengeri Hobli, Bengaluru	08	08	05
Thathanoor, Hosakote Taluq, Bengaluru Rural	06	04	03
Hosallipalya, Hessaraghatta, Bengaluru Rural	11	07	05
Doddarasinakere, Maddur Taluq, Mandya	09	05	04
Kuduregere, Yelahanka, Bengaluru	14	07	04
Somashettyhalli, Yeshawantapur, Bengaluru	10	03	01
Navaratna Agrahara, Chikkajala, Bengaluru Rural	08	05	05
Hanamasagar, Kushtagi Taluq, Koppal	13	03	01
Bevoor, Bagalkot	16	04	02
Kodekal, Surapur Taluq, Yadgir	13	05	02
Khanahosalli, Kudligi Taluq, Bellary	10	02	00
Kyatasandra, Tumkuru	11	00	00
Lingasugur, Raichur	09	00	00
Total	151	61	39

The present study revealed that the blood represents the most appropriate sample for early detection of CSFV, since RT-PCR assay detected the presence of virus before the appearance of the disease symptoms. The virus has been shown to be predominantly associated with apoptosis of peripheral blood mononuclear cells (PBMC), including the white blood cells (Summerfield *et al*. 1998). Donahue *et al.* (2012) also reported that irrespective of virulence, whole blood and tonsil scrapings are the samples of choice for early detection of CSFV in live pigs by RT-PCR. In infected piglets, course of the disease was influenced by the virulence of the virus and symptoms may vary hence, the antigen ELISA, based on the detection of viral antigen (Depner *et al.* 1995) and molecular technique RT-PCR based become helpful for rapid detection of CSFV (Le Dimna *et al.* 2008). A challenge study revealed that virus in blood can be detected in blood collected on 6–7th day post infection (dpi) by antigen ELISA and RT PCR (Jasna *et al.* 2007, Raut *et al.* 2015), while, typical symptoms of CSF (anorexia, conjunctivitis, in-coordination, diarrhea, reddening of the base of ear etc) were observed 10–12 dpi onwards (Cariolet *et al.* 2008, Raut *et al.* 2015). So, it is very much necessary to create awareness among the pig farmers to submit blood samples along with tissue samples to the reference diagnostic laboratories for diagnosis of CSF. Virus isolation is standard procedure for CSFV detection (Terpstra 1996), however not suitable during outbreak conditions since, it is labour intensive and time consuming for results (Dewulf *et al.* 2004).

The results indicated that antigen ELISA is less reliable and considerably less sensitive than RT-PCR. Thus PCR methods offer a promising alternative in the diagnosis of CSF and differentiation of the virus from other pestiviruses (Podgorska *et al.* 2012). Barman *et al*. (2009) showed that nested RT-PCR could detect CSFV in highest number of samples (75.83%) when compared to ELISA (58.33%). These results further strengthen that RT-PCR is a far more sensitive test in detecting CSFV than ELISA. The ability of RT-PCR to detect CSFV in earliest stages of infection makes it a potential tool for surveillance in areas free of the disease and as the best diagnostic test during disease outbreaks (Dewulf *et al*. 2004).

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