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Molecular epidemiology of Porcine Reproductive and Respiratory Syndrome Virus causing outbreaks in Karnataka

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ABSTRACT

We describe the molecular epidemiology of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) involved in outbreaks and massive spread of Porcine Reproductive and Respiratory Syndrome (PRRS) in Karnataka state of India during the year 2017. The study involved investigation of outbreaks in three districts.viz, Udupi, Dakshina kannada and Bengaluru in Karnataka. The disease was characterised by large scale piglet mortality with severe respiratory distress and abortions in pregnant sows. The study recorded death of 394 piglets, 131 adults and abortions in 82 pregnant sows. The organ samples collected from dead pigs were found negative for Classical swine fever virus by 5'UTR gene based Reverse transcription polymerase chain reaction (RT-PCR). RT-PCR targeting full length *ORF5* gene of PRRSV on spleen and lung samples of dead pigs yielded specific amplicon of 803 bp indicating the presence of PRRSV. The phylogenetic analysis of the nucleotide sequences derived from *ORF5* gene of PRRSV involved in the current outbreaks revealed 99.99% sequence homology with the highly pathogenic PRRSV of genotype 2 (North American type) from China and India (Mizoram state). Since pig husbandry plays a significant role in socio-economic upliftment of the poor and marginalised farmers in the country, it's time to put in place effective prevention and control measures for PRRS, before it cripples pig industry in India and its surrounding world. Present study is the first epidemiological report of PRRS outbreaks in South India.

Key words: Molecular epidemiology, Porcine Reproductive and Respiratory Syndrome Virus (PRRSV)

Porcine reproductive and respiratory syndrome (PRRS) is caused by Porcine reproductive and respiratory syndrome virus (PRRSV), an enveloped, plus sense RNA virus belonging to the family Arteriviridae; order Nidovirales (Quinn *et al.* 2011). PRRS is a devastating and economically very important infectious disease of swine characterised by abortions, stillborn and/or birth of weak piglets, and severe respiratory distress with high mortality rates in newborn and weaned pigs (Zimmerman *et al*. 2012; Rajkhowa *et al.* 2015). First recognized in 1987 in the United States of America, PRRS was soon a pandemic disease in North America, Europe and Asia (Bilodeau *et al.* 1991; Wensvoort *et al.* 1991; Baron *et al.* 1992; Kuwahara *et al.* 1994). The virus has two distinct genotypes.viz, genotype 1 (European) and genotype 2 (North American) (Quinn *et al.* 2011). Though, both the genotypes of PRRSV cause the same disease symptoms, genotype 2 induces more severe respiratory disease than genotype 1 (Rajkhowa *et al.* 2015). Genotypes 1 and 2 share 60% nucleotide identity between them (Allende *et al.* 1999; Forsberg 2005). There are a very few reports of PRRS outbreaks in India and the disease is mostly confined to North-Eastern parts of the country (Rajkhowa *et al.* 2015, Rajkhowa *et al.* 2016). Present study describes the clinical and molecular investigations of massive outbreaks of PRRS that killed hundreds of pigs in Karnataka during July, 2017. This study is the first epidemiological report of PRRS outbreaks in south India.

MATERIALS AND METHODS

Sample collection: Samples were collected from PRRS outbreaks in three districts, viz. Udupi, Dakshina Kannada and Bengaluru of Karnataka state, India during July, 2017. The details of the location of the farm, total number of pigs, mortality, abortions and number of animals from which samples were collected for laboratory diagnosis are depicted in Table 1. At each farm, organ samples from lungs, spleen, tonsil and mesenteric lymph nodes were collected from dead animals during post-mortem examinations. All the collected organ samples were transported to laboratory under cold chain conditions and were stored at –80°C until further processing.

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Table.1. Details of the pig farms that witnessed PRRS outbreaks in Karnataka state during July 2017.

District in Karnataka	Location and Type of farm Private/Govt.)	Total No. of pigs in farm	Mortality in the farm due to PRRS			No. of pregnant Sows aborted due to PRRS	No. of animals from which samples were collected for diagnosis
			Adults	Piglets	% Mortality		
Udupi	Kadekar (Private Farm)	210	09	48	27.14	13	07
Dakshina Kannada	Farm A Mangalore (Private Farm)	317	22	83	33.12	16	43
	Farm B Mangalore (Private farm)	87	52	35	100.00	06	30
	Farm C Mangalore (Govt. Farm)	231	17	109	54.55	23	65
Bengaluru (U)	Kodige Halli (Private Farm)	550	31	119	27.27	24	20
Total		1395	525		37.64	82	165

Reverse transcription polymerase chain reaction: Viral RNA was extracted from lungs and spleen samples collected at post-mortem using QIAamp viral RNA mini kit (Qiagen, Germany) as per the manufacturer's instructions.

For detection of the Classical swine fever virus (CSFV), the extracted RNA was subjected for reverse transcription polymerase chain reaction (RT-PCR) using the primers.viz, Forward 5'-CTAGCCATGCCCWYAGTAGG-3' and Reverse 5'-CAGCTTCARYGTTGATTGT 3' targeting amplification of 421 bp region on 5'UTR region of CSFV with cycle conditions of 50°C for 60 min for reverse transcription followed by 35 repeated cycles of denaturation at 94°C for 30 sec, 50°C for 30 sec and 72°C for 1 min with a final extension for 5 min at 72°C (Chandranaik *et al.* 2009). While for the detection of PRRSV, the extracted RNA was subjected for RT–PCR using set of primers.viz, Forward- 5'-TGACACCTGAGACCATGAGG-3' and Reverse-5'-GTGCAGAAGCCCTAGCAGTC-3' to amplify 803 bp region covering the full *ORF5* gene of PRRSV. RT-PCR was carried out in a reaction mixture containing 1 µl of each primer (20 pm/µl), 12.5 µl of RT master mix (procured from TaKaRa, Japan), 5 µl of extracted template RNA and 5.5 µl nuclease free water. The RT–PCR was carried out in a master cycler (Eppnedorf) with cycle conditions of 50°C for 60 min for reverse transcription followed by 35 repeated cycles of denaturation at 95°C for 1 min, 60°C for 1 min and 72°C for 1 min with a final extension for 5 min at 72°C (Rajkhowa *et al.* 2015). The PCR products were visualised by gel electrophoresis using 1.5% agarose.

Phylogenetic analysis: Gel purified amplicons from each place of outbreak (Udupi, Mangalore and Bengaluru) were subjected for nucleotide sequencing by Sangers method at M/s Bioserve Ltd, Hyderabad, India.

The deduced nucleotide sequences were BLAST analysed, aligned with the published sequences deposited in the GenBank database and the phylogenetic tree was constructed by maximum likelihood method using MEGA 6.2 software (Tamura *et al.* 2013; Chandranaik *et al.* 2014).

The processing of clinical samples and PCRs were carried out in BSL-3 laboratory of Diagnostic Virology Division of Institute of Animal Health and Veterinary Biologicals, Bengaluru, Karnataka, India.

RESULTS AND DISCUSSION

At all the pig farms that witnessed the outbreaks, the disease began with severe depression, ataxia and complete anorexia in pigs of all ages. Affected piglets showed huddling, high fever of 104 to 106°F, severe respiratory distress with coughing, reddening of the body with characteristic pinkish/bluish appearance of the ears. The pregnant sows had abortions, early farrowing, and delivery of stillborn or weak piglets or mummified foetus. The post-mortem showed pneumonic changes in lungs, severe haemorrhages and enlargement of liver, spleen and kidney. There were erosive lesions in stomach and intestines.

The organ samples collected from dead pigs were found negative for CSFV by RT-PCR. The RT-PCR targeting ORF5 region of PRRSV yielded specific amplicon of 803 bp indicating the presence of PRRSV in the tissues collected from dead pigs and thus confirming the pathogen involved in the outbreaks under this study.

The present investigation involving outbreaks in three districts in about 20 days recorded death of 394 piglets, 131 adults and abortions in 82 pregnant sows. In absence of any vaccination against PRRSV, the disease presented an epidemic form affecting all age groups, with death of 525 pigs. Further, the disease spread very rapidly between districts which were far apart. Similar PRRS disease patterns have been described in immunologically naive pig populations (Zimmerman *et al.* 2012). The symptoms of severe respiratory distress in piglets and abortions in pregnant sows and bluish discolouration of ears in most affected animals were characteristic features of PRRS described elsewhere in the world (Quinn *et al.* 2011).

PRRS is an emerging transboundary animal disease of pigs in India. The literatures available on PRRS outbreaks in India are very scanty and as per the available reports, the

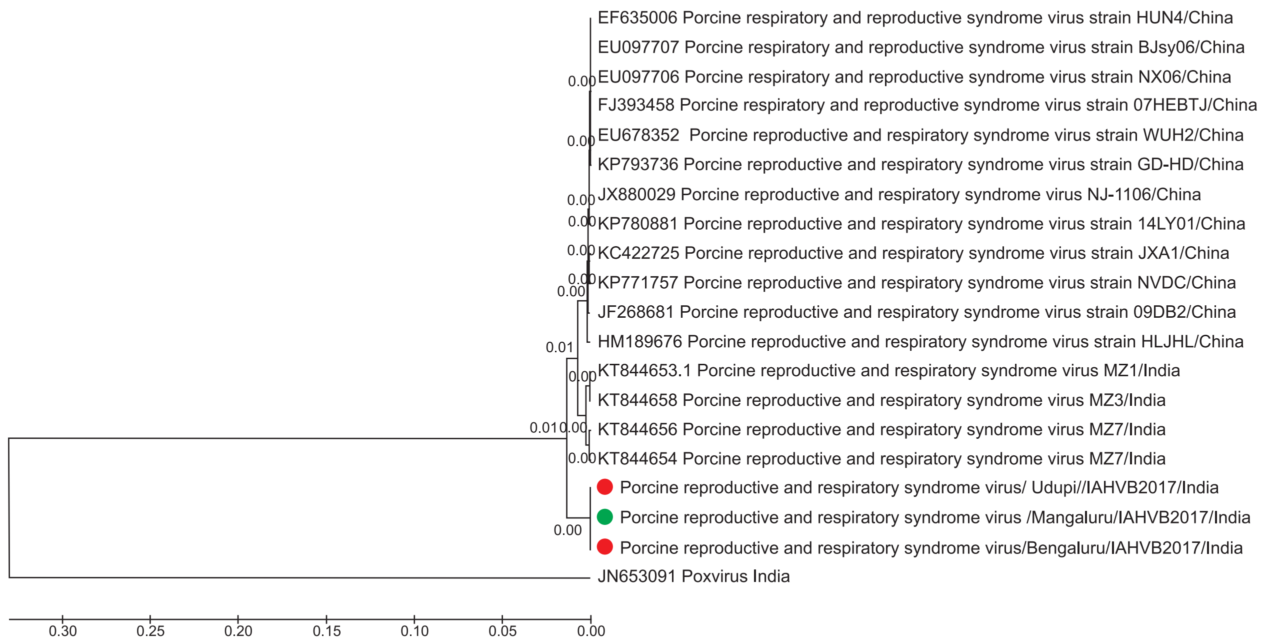

Fig. 1. Phylogenetic analysis of the deduced nucleotide sequences on the full-length *ORF5* gene.

outbreaks are mostly confined to north-eastern states of the country (Rajkhowa *et al.* 2015; Rajkhowa *et al.* 2016). Present study is the first epidemiological report of PRRS outbreaks in south India.

The phylogenetic analysis of the deduced nucleotide sequences on the full-length *ORF5* gene, revealed 100% sequence identity amongst PRRS viruses involved in outbreaks in three districts (Fig. 1), indicating common source of the virus in all the outbreaks under the study; possibly spread through movement of people and/or animal carrying vehicles and/or infected animals between these farms. Further, PRRSV involved in these outbreaks had 99.99% sequence homology with the highly pathogenic PRRS virus of genotype 2 (North American type) from China and virus isolates from Mizoram state of India; possibly indicating source and mode of transmission of PRRSV to Karnataka. It is important to note that highly pathogenic PRRS epidemics are being observed in China, causing devastating effect on Chinese pig industry, since 2009 (Yu *et al.* 2012) and North-Eastern states (which share international borders in eastern side of India) which constitutes for 28% of the piggery in the country (Rajkhowa *et al.* 2015). Rapid spread of infection in Karnataka is a matter of great concern for the Indian pig industry and with no trade barriers within the country; the virus may spread all across the country in a very short span of time. Since pig husbandry plays a very significant role in socio-economic upliftment of the poor and marginal farmers in the country, it is time to concretely put in place effective prevention and control measures for PRRS before it cripples piggery in India.

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