Original Research

Seroprevalence of Porcine Circovirus-2 Associated Reproductive Failure in Pigs of Mizoram, India

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Abstract

Reproductive failure associated with PCV2 is caused by porcine circovirus (PCV) which is characterised by acute systemic illness of the dam, abortion, mummified foetus, stillborn piglets or weak born piglets. Porcine circovirus type 2 (PCV2) is the primary causative agent of porcine circovirus associated disease (PCVAD). The virus preferentially targets the lymphoid tissues, which leads to lymphoid depletion and immunosuppression in pigs. The present study was carried out to determine the pathology and diagnosis of porcine circovirus-2 associated reproductive failure in pigs of Mizoram. Based on PCR analysis, out of 268 serum samples collected across the state, 44 (16.4%) were positive for PCV2.

Key words: Mizoram, Porcine Circovirus 2, Porcine Circovirus Associated Disease, Reproductive Failure, Swine

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Introduction

The swine population plays a major role for the socio-economic status of the state of Mizoram. Swine population is affected by various health problems, of which reproductive problems is one of the major concern. PCV2 associated reproductive failure is caused by Porcine circovirus (PCV), a single stranded DNA viruses with diameter 17nm, belonging to the family *Circoviridae* (Tischer *et al.*, 1982) and may clinically result in acute systemic illness in the dam, abortion, increased nonviable foetuses at parturition (mummified and stillborn piglets), or weak born piglets (O'Connor *et al.*, 2001; Park *et al.*, 2005; West *et al.*, 1999). PCV2 can be transmitted both horizontally through faecal-oral (Sato *et al.*, 2000) or respiratory routes, (Magar *et al.*, 2000) and vertically (West *et al.*, 1999). Postweaning multisystemic wasting

syndrome (PMWS) is believed to be caused by PCV2, a pathogenic strain of PCV (Allan *et al*., 1999). PMWS is a disease that can affect nursery and fattening pigs, and is reported in pigs throughout the world (Segalés *et al*., 2002; Wen *et al*., 2005). PCV2 was also found to be associated with porcine dermatitis and nephropathy syndrome (PDNS) (Wellenberg *et al*., 2004), respiratory disease complex (Kim *et al*., 2003) and reproduction failure (Sanchez *et al*., 2001).

PCV2 consists of two major open reading frames (ORFs), ORF1 (Rep gene) and ORF2 (Cap gene). ORF1 is essential for virus DNA replication while ORF2 (Cap gene) encodes a major capsid protein (Liu *et al*., 2001; Nawagitgul *et al*., 2000). The ORF2 of PCV2 may have a potential causative role in the PCV2 related disease (Larochelle *et al*., 2002). The seroprevalence of PCV2 virus has been reported in different countries in Asia, Europe, North America, Oceania and South America (Patterson and Opriessnig, 2010). In India, PCV2 infection among Indian pigs and its association with reproductive problems and PMWS was established very recently. It has been reported from Northern India (Mukherjee *et al*., 2018). In North-eastern India, especially Mizoram, the majority of livestock farmers are dependent on a healthy pig population for their livelihood. It stands clear that the health of the pig population should be given high priority, and the diagnosis of such reproductive problems becomes very crucial for the sustenance of the pig production.

Thus, the present study was conducted in College of Veterinary Sciences and Animal Husbandry, CAU (I) Selesih to investigate and better understand the seroprevalence of PCV-2 in the pig population in Mizoram India.

Materials and Methods

Collection of Serum Sample

In the present study a total of 268 serum samples were collected randomly from pigs irrespective of age, sex, breed and health status from all the 8 districts in Mizoram during the study period from August 2012 to March 2013. Approximately 4-5 ml of blood was collected using 10 ml sterile syringe; the blood was emptied into 15 ml sterile centrifuge tube. The collected blood was allowed to stand for about 1 hr without any disturbance in slanting position for clot formation, with a sterile stick the blood clot was scrapped off from the wall and was centrifuged at 3500 rpm for 5 mins for separation of serum. The collected serum was properly numbered and stored at -20^{o} C till further use.

Polymerase Chain Reaction

Total DNA was extracted using conventional procedures as per with certain modifications (Laltlankimi, 2013). Extracted DNA was stored at -20^{0}C till further use. The oligonucleotide primers for ORF2 gene region of PCV2 used in the study polymerase chain reaction (PCR) analysis were as per Larochelle *et al*., 1999 with the primers PCVLF (5'-TAGGTTAGGGCTGTGGCCTT-3') and PCVLR (5'-

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CCGCACCTTCGGATATACTG-3') which amplified a DNA fragment of 263bp. A 25µl reaction using 10X PCR buffer, 10pmol of each primers, 10mM dNTPs, 5U/µl Taq DNA polymerase was amplified. Known positive control DNA (a gift from IVRI, India) was also amplified. Thermal cycling was performed in thermal cycler (Eppendorf, Germany) with the cycling parameters of initial denaturation of 95°C for 5min followed by 30 cycles of 95^{0}C for 45 sec and 60^{0}C for 45 sec and final extension for 5 min at 72°C. Confirmation of PCR products was carried out in agarose gel electrophoresis using 1.5% agarose gel containing ethidium bromide.

Result

A total of 268 serum samples were tested by PCR for detection of PCV specific gene. Out of 268 serum samples, 44 (16.4%, 95% CI = 11.97- 20.83) were found to be positive for PCV2 antigens (Fig. 1). The district-wise details of PCR screening result is shown in Table 1 and Fig. 2.

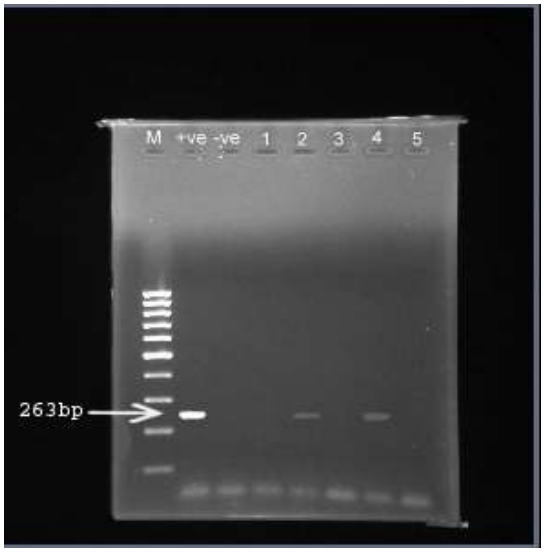

Fig. 1: 1.5% Agarose gel electrophoresis stained with ethidium bromide showing the 263 bp ORF2 gene fragment from serum sample.(M = marker, +ve = positive control, -ve = negative control,1-5= samples).

Table 1: District-wise percentage of PCR positive serum samples

S. No.	District of Collection	Total Samples	Total Number of Samples Positive for PCR
1	Aizawl	33	7 (21.2%)
2	Champhai	16	4 (25%)
3	Kolasib	33	4 (12.1%)
4	Lawngtlai	43	5 (11.6%)
5	Lunglei	54	10 (18.5%)
6	Mamit	47	9 (19.1%)
7	Saiha	32	4 (12.5%)
8	Serchhip	10	1 (10%)
Total		**268**	**44 (16.4%)**

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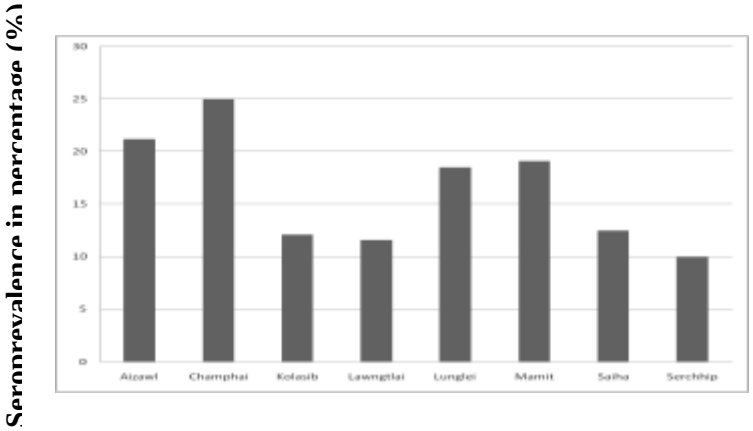

District wise distribution of PCV-2

Fig. 2: Graphical representation of district-wise distribution of PCV-2 in Mizoram

Discussion

Porcine Circovirus 2 (PCV2) has been associated with many diseases in the pig population all over the world. PCV2, the causative agent of PCVAD is now considered as one of the most important viral pathogen of pigs. Since PCV2 has been associated with reproductive loss- late term abortion, increase number of mummified foetus, stillborn piglets and weakborn piglets, it remains a serious economic problem for swine industry.

The present study was carried out to find the magnitude of the problem and occurrence of PCV2 associated reproductive problem in the organized and unorganized farm of the pig population in the state of Mizoram, India. Out of 268 samples, 44 (16.4%) tested positive for the PCV2 by PCR. In the present study, PCR technique was used for detection of the PCV2 infections in pigs. PCR is considered as a rapid tool in identifying PCV2 infections in pigs, though detection of PCV2 by PCR alone cannot diagnose PCVD in animals (Alarcon *et al*., 2011). Similar amplification of specific sequence of PCV2 by PCR using type specific primers has been reported previously (Bolin *et al.*, 2001; Rudan *et al.*, 2009). In India, studies on PCV2 was initiated by Kumar *et al*. (2006) where PCV2 was first detected from cases of postweaning multisystemic wasting syndrome (PMWS)-associated PCV2 systemic disease. Occurrence of PMWS in crossbred pigs of Uttar Pradesh and Uttarakhand, India has been reported (Rajkhowa, 2008; Rajkhowa and Saikumar, 2012). Genetic characterisation and phylogenetic analysis of PCV2 isolates from India was studied by Anoopraj *et al*. (2015). 20% of the 70 litters examined were found positive for PCV2 in an organized farm in Uttar Pradesh (Sharma, 2007).

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In the present study, Champhai district showed highest percent of positive result (25%), followed by Aizawl (21.2%), Mamit (19.1%), Lunglei (18.5%), Saiha (12.5%), Kolasib (12.1%), Lawngtlai (11.6%) and Serchhip (10%). Champhai district have the highest level of prevalence of the disease which may be due to its geographical location. Champhai is in connection to the border of Myanmar which could be assessing as the reason of high percentage of sero-pervalance of PCV2 associated reproductive failure in the district. There were implications that most of the local pigs were imported from Myanmar into Champhai district. Hence, it is strongly believed that introduction of the disease to Mizoram was from imported local pigs of Myanmar.

The studies on PCV2 associated reproductive failure in pigs of Mizoram may have incurred the inadequacy of sublime information or be it infrastructure, facilities and materials on exactly how to deal with the arising situation which is prevalent in Mizoram.

Conclusion

Despite the availability of effective vaccines, variant strains of PCV2 continue to emerge. Although tremendous progression has been made towards understanding PCV2 pathogenesis and immune interaction, many important questions remains unanswered and it seems a highly adequate and more substantial studies are still required.

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