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Molecular Detection of Porcine Parvovirus in Swine with Reproductive Failure

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ABSTRACT

Background: Porcine parvovirus (PPV) is considered to be one of the most important causes of reproductive failure in swine. Fetal death, mummification, stillbirths and delayed return to estrus are predominant clinical signs commonly associated with PPV infection in a herd.

Methods: In this field laboratory investigation during 2020-2022, the aim was to study the prevalence of PPV in swine reproductive failure in an organized farm in Chennai, Tamil Nadu. A detailed necropsy examination was conducted on 3 mummified fetuses and 17 still born fetuses and salient gross finding were accumulation of serosanguinous fluid in the cavities. Representative tissues samples from brain, lungs, heart, spleen, intestine, kidneys, mesenteric, inguinal lymph nodes and liver were collected on ice and in 10% neutral buffered formalin for histopathological, immunohistochemical and molecular studies.

Result: Out of the 20 fetal tissues analyzed by PCR for PPV genome, 19 samples were found to be positive for the NS1 gene. The identification of the amplified products was confirmed by direct sequencing of purified PCR products. Histopathological examination of the liver showed intranuclear inclusion bodies and necrotic myocarditis. Immunohistochemical studies showed PPV antigen distributed in the striated muscle cells of the heart, alveolar epithelial cells of lungs, hepatocytes of the liver and tubular epithelial cells of kidneys. The present study confirms the prevalence of PPV in fetal tissues in an organized pig farm in Tamil Nadu which warrants further surveillance.

Key words: Fetus, Pathology, PCR, Porcine parvovirus.

INTRODUCTION

The porcine reproductive diseases cause huge economic losses to the piggery farmers in terms of stillbirth, mummified foetus, embryonic death, infertility and abortion. Among the most common viral infectious agents detected in stillborn, mummified and aborted foetuses is the porcine parvovirus (Kim and Chae, 2004). The disease is ubiquitous in swine and is enzootic in conventionally raised herds. The occurrence of PPV in pigs was first reported in 1967 (Cartwright and Huck, 1967) and the virus is now considered to be endemic in most areas of the world and can be found in all pig herd categories. The disease has two main epidemiological patterns-endemic and epidemic infection. Endemic PPV infection may go unnoticed, especially if a farm fails in record keeping, analysis and lack of veterinarian's supervision on herd health status. In contrast, epidemic PPV infection in a susceptible herd is readily apparent and the economic impact is serious. In India, the disease was reported for the first time by Sharma and Saikumar (2010) in association with reproductive failure and neonatal mortality in crossbred pig. In South India, Aishwarya *et al.* (2016) reported the occurrence of porcine parvovirus from Kerala in domestic and wild pigs. The virus is transmitted to a new herd either oronasally, transplacentally or through clothing of farmers and rodents (Truyen and Streck, 2012). Boars might play a significant role in the dissemination of PPV by semen. The disease is caused by a small non-enveloped, single stranded DNA virus which is classified under the genus *Protoparvovirus* of the family *Parvoviridae* (Xiao *et al.,* 2013).

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The present work aimed to study the prevalence of porcine parvoviral infection in pigs with reproductive failures in the piggery unit of livestock farm at Postgraduate Research Institute in Animal Sciences, Tamil Nadu Veterinary and Animal Sciences University, Kattupakkam, Tamil Nadu.

MATERIALS AND METHODS

The study was conducted at the piggery unit of an organized livestock farm at Postgraduate Research Institute in Animal

Sciences, Tamil Nadu Veterinary and Animal Sciences University, Kattupakkam (Tamil Nadu). The total stock in the pig breeding unit was 895 and housed Large White Yorkshire (LWY) and TANUVAS KPM Gold 75% LWY + 25% desi breeds of different ages and of both sexes. The pigs were reared under intensive system and fed with concentrate and swill feeding. The vaccination program in the farm included live attenuated lapinized strain of classical swine fever virus (CSFV), foot and mouth disease inactivated vaccine and porcine circo virus vaccination. The pigs in the farm had a history of abortions, still births, mummified fetuses and weak piglets since the year 2013. Earlier porcine circo virus 2 was isolated and reported (Karuppannan *et al.*, 2016). In the present study, a total of 20 fetuses, 17 still birth and 3 mummified fetuses from 4 sows during the period from November 2019 to January 2020 were investigated for porcine parvo viral infection. A differential diagnosis by PCR tests was performed for the elimination of co-infection or association of CSFV, PCV-2 and porcine respiratory and reproductive syndrome and rule out their presence (Chen *et al.,* 2019).

Pathological studies

A detailed necropsy examination was conducted on the still born and mummified fetuses and representative samples of tissues *viz*. lungs, liver, heart, intestine, spleen, kidney, brain were collected in 10 per cent neutral buffered formalin and in ice. The tissue samples were processed by routine method for histopathological examination and stained with Haematoxylin and eosin (Suvarna *et al*., 2018).

Immunohistochemical studies

Immunohistochemical detection of PPV antigens was done in the tissue sections. 4-5 µm thick paraffin embedded tissue sections were cut and mounted on Superfrost Plus, positively charged microscopic slides. Antigen retrieval was carried out in citrate buffer (pH 8.5-9.0) at 80°C in water bath for 30 min using Retriever system (BioGenex Laboratories Inc., San Ramon, California, USA). After non specific and endogenous peroxidase blocking, the sections were incubated with 1:100 dilution of porcine parvovirus positive serum (VMRD, PPV) for PPV at 4°C at room temperature for 2 h. Tissue sections were treated with super enhancer for 20 min. Secondary antibody conjugated with HRP (BioGenex Laboratories Inc., San Ramon, California, USA) was added and incubated for 30 min at room temperature. Antigen antibody complex was visualized using impact DAB Peroxidase Substrate Kit (BioGenex Laboratories Inc., San Ramon, California, USA) followed by counterstaining with hematoxylin. Immunopositivity was determined by the presence of golden-brown deposits in the infected cells.

DNA extraction and PPV detection

Total DNA was extracted from tissues using QIAmp DNA Mini and Blood Mini kit (Qiagen, USA) following manufacturer's instructions. Initial screening of the samples were done by PCR targeting 265 bp fragment corresponding to NS1 region as previously described by Aishwarya *et al.* (2016).

Amplification and sequencing

PCR reactions were performed in 10µl volume reaction and in each reaction mixture 1 µl of DNA as template was added to 5 µl of Red dye master mix, 0.5 µl of each forward and reverse primer and final volume was made to 3 µl using nuclease free water. The cycling conditions for amplification of PPV were 95°C for 2 min (initial denaturation), 35 cycles of 95°C for 15 s (denaturation), 53°C for 30 s (annealing) and 72°C for 30 s (extension) followed by single cycle at 72°C for 1 min (final extension). Amplification products were checked on 1.5% agarose gel in the presence of ethidium bromide, electrophorosed and photographed under UV illuminator.

PCR products of expected size were purified using a commercial DNA gel extraction kit. The identification of the amplified products was confirmed by direct sequencing of purified PCR products in both direction with respective forward and reverse primers used for PCR amplification at sequencing facility of Eurofins, India.

Phylogenetics

The NS1 gene sequences of the isolated PPV strains were analysed with available GenBank sequences using MEGA 10 software as previously reported (Kumar *et al.,* 2018). The sequence obtained in this study and those downloaded from GenBank were aligned using the ClustalW program of MEGA 10 software. The sequences were then trimmed to correspond to the region. The evolutionary history was found out by the neighbor joining (NJ) method. The bootstrap consensus tree inferred from 1000 replicates was taken to represent the evolutionary history of the sequences analyzed. The evolutionary distances used to infer the phylogenetic trees were computed using the Tamura 3-parameter method. The reconstructed phylogenics for each alignment are shown in Fig.

RESULTS AND DISCUSSION

PPV is found in almost all pig-breeding countries and it is considered to be endemic in swine herds worldwide. The main clinical sign of PPV infection in non-immune sows is reproductive failure characterized by embryonic, fetal infection and death without any clinical signs in the dam (Mengeling *et al.,* 1979). The economic impact of the disease is due to the increase in repetition of heats, reduction of litter size and fertility (Collins *et al.,* 1992). Hence, profitable pig production depends partly on preventing the occurrence of such infectious diseases that affect swine reproductive performance. In the piggery unit under study, vaccination against PPV was not practiced as the disease was not reported earlier. However, Lalrinkima *et al*. (2021) in a seroprevalence study of PPV in pigs of the farm wherein high prevalence of PPV antibodies was recorded among adult males and females. Hence, further

identification and characterization studies on PPV were undertaken in the fetal tissues of still births and mummies. Seventeen still born and three mummified fetuses from 4 sows were studied. On necropsy examination, mummified fetuses were haematic type with brown black appearance, viscous membranous covering on the fetus. Mummified and still births had accumulation of abundant serosanguinous fluid in thoracic and abdominal cavities (Fig 1) with generalised congestion of viscera. Epicardial haemorrhage, distended dark red and wet lungs, pale soft liver and bile stained viscera were consistent findings. Microscopically, moderate lymphocytic broncho interstitial pneumonia with edema, diffuse vacuolar degenerative changes in hepatocytes with presence of intranuclear eosinophilic inclusions (Fig 2), focal lymphoplasmocytic interstitial nephritis and necrosis of myocardium were observed. Haemorrhage, neurophilic vacuolation and gliosis were observed in cerebral grey matter. Similar findings were reported by earlier workers (Narita *et al.,* 1975; van Leengoed *et al.,* 1983; Bolt *et al.,* 1997).

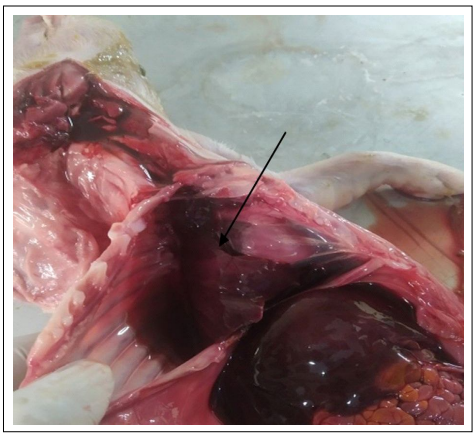


Fig 1: Still born fetus: Enlarged, friable and dark brown liver with accumulation of serosanguinous fluid in the thoracic cavity (arrow).

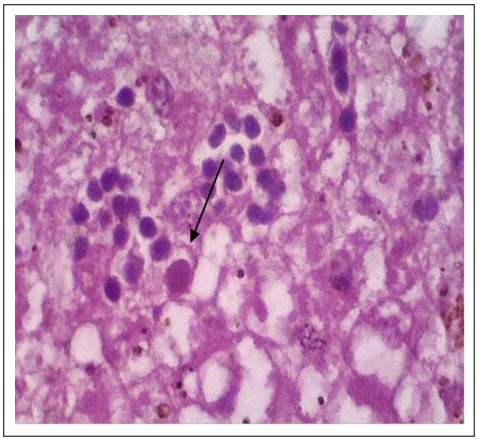


Fig 2: Still born fetus: Liver - Intranuclear eosinophilic inclusion (arrow) in the hepatocytes. HE 1000x.

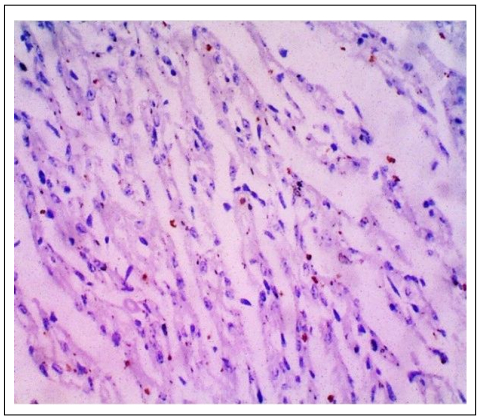


Fig 3: Still born fetus: Heart-intense intracytoplasmic immunopositivity for PPV antigen in striated muscle cells. IPT 400x.

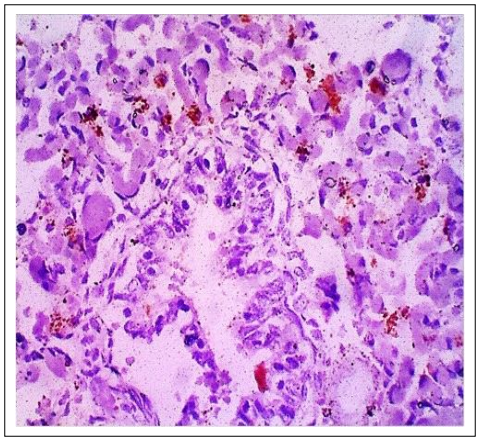


Fig 4: Still born fetus: Lung-intense intracytoplasmic immunopositivity for PPV antigen in alveolar epithelial cells. IPT 400x.

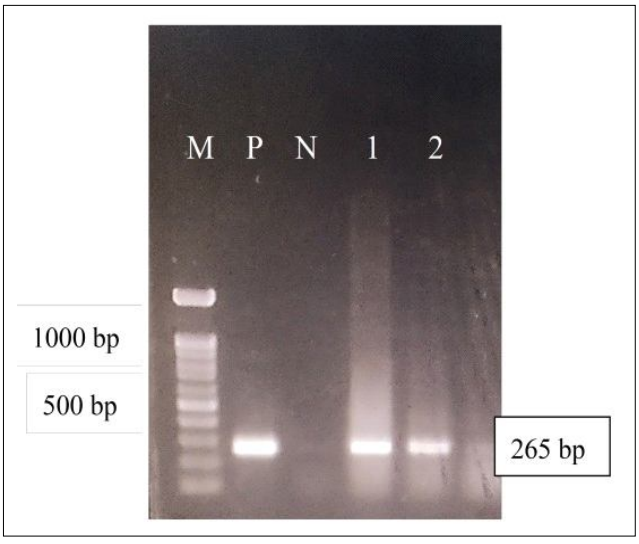


Fig 5: Electrophoresis of PCR products: Lane M 100 bp DNA ladder, Lane P (+C), Lane N (-C), Lane 1 & 2 (+PCV2) targeting 265 bp of NS1 gene.

Tissues samples were positive for the presence of PPV antigen by IHC. IHC has been used as a confirmatory diagnostic tool for PPV infection (Pescardor *et al.,* 2007; Sharma and Saikumar, 2010). Immunolocalization of PPV antigen was observed in the striated muscle cells of heart (Fig 3), alveolar epithelial cells of lungs (Fig 4), hepatocytes and renal tubular epithelial cells in fetal tissues. These findings were similar to that reported by other worker (Sharma and Saikumar, 2010).

Twenty fetal tissue samples were tested for PPV by PCR targeting NS1 gene of the virus (Fig 5). Of the samples tested, 19 were found positive by PCR with a percentage positivity of 95% unlike Aishwarya *et al.* (2016) who reported 5.26% (2/38) positivity for PPV in tissues samples. Serena *et al*. (2019) reported 7/131 samples from tissues of still birth and mummified fetuses were positive to PPV by PCR in Argentina. Jozwik *et al*. (2009) and Foerster *et al*. (2016) reported PCR positive for PPV in fetal lung and kidney

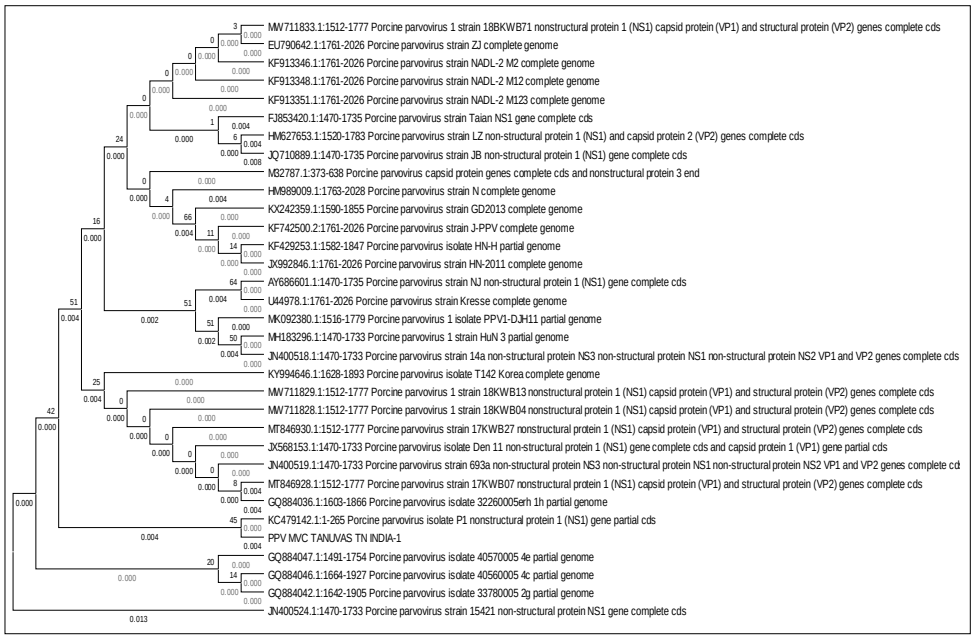


Fig 6: Phylogenetic analyses based on the amino acid sequence of viral nonstructural protein (NS1) for porcine parvoviruses by MEGA 10 software.

Table 1: Organ wise prevalence of PPV in still birth and mummified fetuses.

Sample no.	Types of samples	Heart	Lungs	Liver	Kidney	Spleen
KP - 6	Mummified fetus	+	-	+	+	+
KP - 7	Mummified fetus	+	-	-	+	-
KP - 8	Mummified fetus	+	-	-	+	-
KP - 9	Still birth	+	-	+	-	-
KP - 16	Still birth	+	-	+	-	+
KP - 17	Still birth	+	-	+	-	-
KP - 18	Still birth	+	-	+	+	-
KP - 19	Still birth	+	-	+	-	-
KP - 20	Still birth	-	+	+	-	-
KP - 22	Still birth	-	-	+	-	-
KP - 23	Still birth	+	-	-	-	-
KP - 24	Still birth	+	+	-	-	-
KP - 25	Still birth	+	-	-	-	-
KP - 26	Still birth	+	+	-	-	+
KP - 27	Still birth	+	-	-	-	-
KP - 28	Still birth	+	-	-	-	-
KP - 29	Still birth	+	-	-	-	-
KP - 30	Still birth	+	-	-	-	-
KP - 31	Still birth	+	-	-	-	-

tissues while high prevalence of PPV genome was detected in fetal heart tissue samples in our study (Table 1), which was corroborative with the findings of Streck *et al*. (2013). After preliminary identification of PPV by PCR, amplified PCR product was purified and sequenced. On BLAST and phylogenetic analysis, the sequence obtained in the study showed that they are closely related with other published Indian isolates P 1 (KC479142) of PPV (Fig 6). The positive isolates were related to the PPV 1 sequence (NADL-2), maintaining the amino acid differences in positions 436 (S-P) and 565 (R-K) (Serena *et al*., 2019). The prevalence of PPV genotypes 1-7 have been reported worldwide (Streck *et al.*, 2013; Xioa *et al.*, 2013; Schirtzinger *et al.,* 2015 and Xing *et al.*, 2018) in India (Sharma and Saikumar, 2015; Aishwarya *et al.,* 2016). PPV 1 is known as one of the most important causes of reproductive failure in swine (Streck *et al.,* 2013).

Transplacental infection of embryos and fetuses was the result of dams failing to develop an active immunity prior to pregnancy. Exposure of susceptible gilts to PPV near the time of conception can result in transplacental infection, death and resorption of their embryos (Mengeling *et al.,* 1980). However, Lalrinkima *et al*. (2021) in a seroprevalence study of PPV in pigs of the farm wherein high prevalence of PPV antibodies was recorded among adult males and females. Infected boars also can transmit the virus via semen and have been reported to constitute a potential source of transmission (Guérin and Pozzi, 2005).

CONCLUSION

The study reports the high prevalence of porcine parvovirus in fetal tissues from sows with reproductive failure and warrants a detailed surveillance of the virus in pig herds. The presence of PPV was confirmed by immunohistochemical and molecular studies and prevalence of PPV 1 is recorded.

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Conflict of interest

All authors declare that they have no conflict of interest.

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