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Phylogenetic analysis of Indian isolate of porcine parvovirus and its associated pathology in neonatal crossbred Indian pigs

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Porcine parvovirus (PPV), the major cause of SMEDI (S, stillbirth; M, mummification, ED, embryonic death; I, infertility) syndrome (Thomson and Proszesky 1994), also leads to neonatal mortality and occasional abortions in sows. Porcine parvovirus is ubiquitous in swine and is enzootic in conventionally raised herds. It was first associated with reproductive losses in swine by Cartwright and Huck (1967). Subsequently, PPV infection was reported from most of the pig producing countries of the world. In India, it was reported for the first time by Sharma and Saikumar (2010) in association with reproductive failure and neonatal mortality in crossbred Indian swine.

Porcine parvovirus pathology: Litters (70) from a farm housing first-parity crossbred gilts (Landrace × local Indian breed) were closely monitored and observations on the number of piglets born alive, piglets born dead and piglets that died in the first week of life were recorded and analyzed. A detailed necropsy examination was conducted on the dead piglets and tissue samples (lungs, heart, liver, kidney, lymph nodes, spleen, tonsil, brain) were collected in 10% buffered neutral formalin fixative and on ice and stored at -70°C after proper labeling till further processing. Fixed tissues were subjected to histopathological processing and hematoxylin and eosin (HE) staining as per standard procedures. Individual sections were microscopically examined and the histopathological alterations were recorded and digitally micro photographed. The tissues from mummified fetuses were not processed for histopathology due to advanced autolysis.

Phylogenetic analysis: PPV nucleotide sequence of the VP 2 gene fragment of 226 bp was analyzed using MEGA4 software by Neighbor-Joining method (Tamura *et al*. 2007). The details of the PCR reaction and the tissues showing amplification of the fragment were described by Sharma and Saikumar (2010). The PCR reaction consisted of 50 µL mixture consisting of 10× PCR buffer (5.00 µL), 50 mM $MgCl_2$ (1.50 µL), 2.5 mM deoxyribonucleotide triphosphate (2.00 µL), 10 pmol/µL PPV1F primer (2.50 µL), 10 pmol/µL PPV1R primer (2.50 µL), Taq DNA polymerase (2.5 U), DNA (3.00 µL), and nuclease-free water to make up to 50 µL. The thermal cycling conditions consisted of initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 54°C for 30 sec, and extension at 72°C for 30 sec and final extension at 72°C for 10 min. The PCR amplicon was electrophoresed along with 100 bp molecular weight marker on a 1.5% agarose gel. The following 17 nucleotide sequences were used for the phylogenetic analysis: AY390557-VRI-I-South Korea, AY684864-225b-Germany, D00623-POVG-Spain, DQ675456-SR1-China, EF212027-N-China, EU790641-BQ-China, EU790642-ZJ-China, AY684871-27a-Germany, AY684868-21a-Germany, AY684865-15a-Germany, U44978-Kresse-USA, NC001718-NADL2, M38367-POVNADL2-USA, AY684867-143a-Germany, AY684872-IDT-Germany, AY742955-USA-CPV-436 and AY742935-CPVU6-Germany. The study also involved detection of PPV by other tests such as DNA probe based *in situ* hybridization. The presence of porcine circovirus 2 in the piglets was also investigated using pathological and molecular tools. The results of these tests were reported earlier by Sharma and Saikumar (2010).

In the present study, the overall incidence of reproductive failure in sows during 2 consecutive farrowings was low, but the pre-weaning mortality was high. Occasionally, some sows farrowed as few as 1 piglet. Out of the 371 piglets farrowed during the first farrowing, 3 were mummified foetuses, 11 were stillborn piglets and 138 piglets died within 7 days of birth. Out of the 223 piglets farrowed during the second farrowing, 6 were mummified foetuses, 2 were stillborn piglets and 56 piglets died within 7 days of birth. The clinical signs observed in weak undersized piglets included congenital tremors, splay legs, hind limb ataxia and diarrhoea.

Of the total 70 litters studied, PPV was detected in 5 (7.14 %) litters, in association with cases of reproductive failure and neonatal mortality. PPV is a cell-free circulating virus and after circulation in plasma, it crosses the placental barrier and replicates only in the foetal tissues. Lesions are observed only in the foetuses with or without foetal death. PPV induced foetal death followed by mummification occurs when

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infection preceeds development of foetal immunocompetence (at 70 days of gestation). PPV infection rarely results in abortion in sows (Harding 2004). Porcine parvovirus was detected in one mummified foetus in a litter where only 4 mummified foetuses were farrowed and 4 piglets from 4 different litters which died within a day of birth. All the 5 litters represented typical reproductive failure in sows, characterized by mummified foetuses, small litter size and weak nonviable neonatal piglets. Similar association of PPV with reproductive failure was reported by others (Johnson and Collinngs 1971, Donaldson-Wood *et al*. 1977, Sorensen and Askaa 1981, van Leengoed *et al*. 1983, Obaldia 1991, Huvsman *et al*. 1992, Hwang *et al*. 1998).

Porcine parvovirus pathology: All the 6 six piglets in the first PPV infected litter died within a day of birth. The second PPV infected litter comprised only 2 piglets. One of the piglets died within a day and the other after 3 days of birth. The gross findings in all the piglets were almost similar with minor differences. Small amount of straw coloured fluid was observed in the thoracic cavity. Lungs were non-collapsing, edematous, congested with focal haemorrhages on the apical and diaphragmatic lobes. Heart revealed thickened opaque pericardium, presence of increased pericardial fluid and pale myocardium (Fig. 1).

Liver lesions varied from intense congestion to pale/ yellowish discoloration (Fig.2). Kidney lesions were also variable and revealed congestion, petechiae and yellowish discoloration on the surface (Fig.3). Spleen showed patchy areas of congestion. The mucosa of stomach was highly edematous and contained traces of curdled milk. Congestion was observed in the intestinal mucosa. Tonsils revealed marked congestion. Brain revealed severely congested meningeal vasculature.

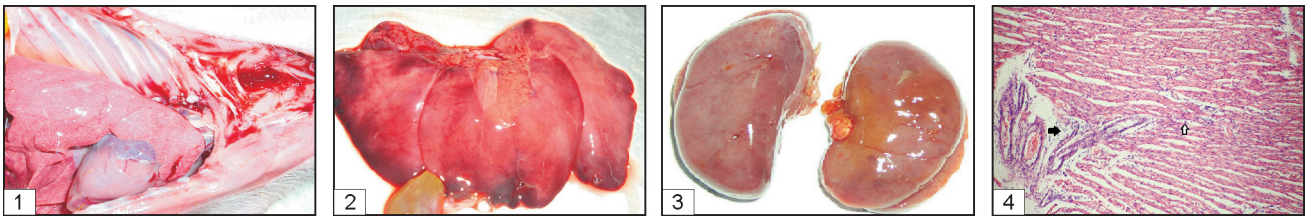
Histopathologically, lungs revealed mild interstitial pneumonia. Majority of the bronchioles contained inflammatory exudate in lumen comprising mainly of mononuclear cells, fibrin and desquamated epithelial lining cells. Other changes in lungs included degeneration of alveolar and bronchiolar wall at some places, proliferation of alveolar macrophages, mild thickening of interstitium due to proliferation of macrophages and mononuclear cells, marked congestion of blood vessels and moderate haemorrhages around bronchioles at places. Heart revealed edema and degeneration of myocardiocytes, diffuse areas of mild mononuclear cell infiltration and presence of leucocytes in areas adjacent to engorged blood vessels (Fig. 4).

Liver revealed severe degeneration of hepatocytes, congested sinusoids, moderate to severe haemorrhages in the parenchyma and presence of fat vacuoles of variable sizes in hepatocytes of the centrilobular areas. The renal cortex in the periphery revealed multiple areas of diffuse haemorrhages in the interstitium. Mild focal interstitial nephritis due to infiltration of mononuclear cells, degeneration of epithelial lining cells of proximal convoluted tubules and haemorrhages in the interstitium were observed. In some parts, the glomeruli were severely shrunken leading to relative increase in the periglomerular space and the tubules appeared collapsed. Spleen revealed multiple areas of small to medium diffuse haemorrhages and mild depletion of periarteriolar lymphoid sheaths. Brain showed focal gliosis in the subependymal region, focal accumulation of glial cells in the cerebral cortex and severe meningeal congestion.

The third PPV infected litter comprised 4 mummified foetuses. The foetuses were of different sizes, which might have died in utero at different stages of gestation as was evident by variation in organ sizes. The visceral organs were in advanced stage of autolysis in all the foetuses. The clinical, gross and histopathological findings in the present study were similar to those reported by earlier workers (van Leengoed *et al*. 1983, Narita *et al*. 1975). The additional two PPV infected litters showed co-infection with porcine circovirus 2 (Sharma and Saikumar 2010).

Porcine parvovirus phylogeny: The 226 bp VP2 gene fragment of PPV-Ind-Bly-05 is available on the web (accession no. DQ158864). The nucleotide sequence after BLAST analysis revealed 100 % homology with PPV strains, Tai'an (China), Embrapa (Brazil), N (China), 05-95 (Brazil), NADL-2 (reference strain), POVNADL-2 (USA) and POV G (Spain). The phylogenetic tree based on Neighbor-Joining (NJ) method with the percentages of confidence along the branches is shown in Fig. 5.

The tree was rooted to canine parvovirus strains, CPV-436 (AY742955) and CPVU6 (AY742935). The analysis of the phylogenetic tree showed that 2 strains from Germany, IDT and 143a, were very closely related and distinct from the others which formed a different group. Among the 14 viruses that formed a separate group, the Indian strain (DQ158864) was more closely related to



Figs 1–4. **1.** A day-old piglet showing straw coloured fluid in thoracic cavity, non-collapsible and edematous lungs, increased pericardial fluid, thick opaque pericardium and pale myocardium. **2.** Liver showing slight enlargement and diffuse, pale yellow discolouration. **3.** Kidneys showing pale discolouration and fine petechiae on the surface. **4.** Heart of a PPV infected piglet showing edema and degeneration of myocardiocytes, mild diffuse infiltration of mononuclear cells in the myocardium (white arrow) and infiltration of leucocytes in the vicinity of blood vessels (solid black arrow). H&E ×100.

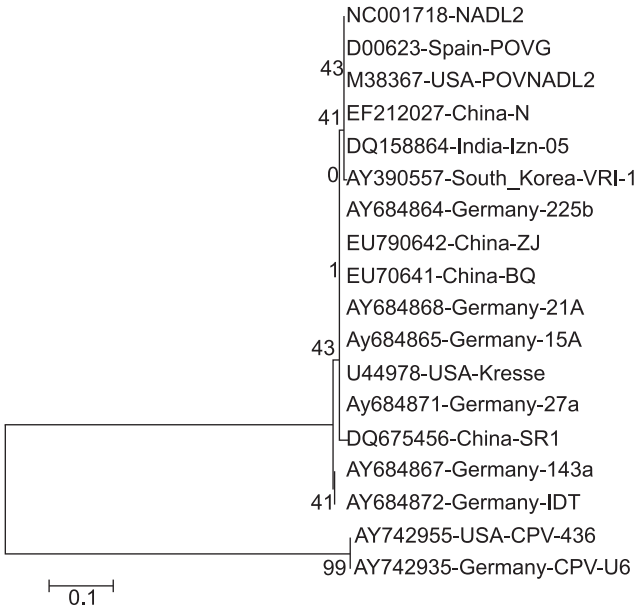


Fig. 5. Phylogenetic tree based on neighbor-joining (NJ) method for 226 bp VP2 sequences of PPV using MEGA 4 software.

viruses from USA (NADL2, POVNADL2), Spain (POVG), China (N) and South Korea (VRI-1). The other branch comprised of PPV strains from Germany (27a, 21A, 15A and 225b), China (SR1, BQ and ZJ) and USA (Kresse). Evidently in countries like USA, Germany and China more than one lineage of PPV are circulating. Phylogenetic analysis of PPV isolates from Brazil indicated circulation of 2 virus lineages and highlighted the need for close surveillance on PPV genetic drift, with an assessment of its potential ability to modify the antigenic make-up of the virus (Soares *et al*. 2003). Two different phylogenetic groups of PPV which could be further subdivided were reported by other workers as well (Shangjin *et al*. 2009). It is also apparent that a close relationship exists between PPV strains circulating in Asian, European and American continents. This observation corroborated earlier report of close association between the Asian and American strains (infectious NADL-2 and Kresse strain) of PPV (Zimmerman *et al*. 2006).

SUMMARY

Porcine parvovirus infection was diagnosed in 5 of the 70 litters in a farm housing first-parity crossbred gilts (Landrace × local Indian breed). The characteristic pathological lesions and high nucleotide homology with infectious strains of PPV helped to establish that porcine parvovirus was responsible for reproductive failure and neonatal morality in the population of cross-bred pigs studied. The Indian isolate of the PPV showed very close genetic relationship with PPV strains from USA, Spain, China and South Korea. Further studies are warranted at the national level to determine the prevalence of the virus in organized and unorganized pig herds and to develop an indigenous vaccine to minimize reproductive losses in pigs due to PPV infection.

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