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PORCINE PARVOVIRUS AND PORCINE CIRCOVIRUS 2 ASSOCIATED POST-WEANING MULTI SYSTEMIC WASTING SYNDROME AND REPRODUCTIVE FAILURES IN CROSSBRED INDIAN PIGS

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ABSTRACT

We conducted a study to find out the concurrent occurrence of porcine circovirus 2 (PCV2) and porcine parvovirus (PPV) in pigs affected with reproductive disorders and post weaning multisystemic wasting syndrome along with the genetic characterization of the identified agents. Out of 150 samples which were screened by PCR, 57 were stillbirths and mummified, 41 were pre-weaned piglets and 52 were post-weaned animals. The co-infection of viruses was detected in 9 out of 150 pigs (6%) by conventional PCR. The pathological lesions in the co infected cases were more severe, indicating a synergistic action between the PCV2 and PPV. BLAST analysis of VP2 gene sequence of two isolates of parvovirus named IVRI-312-2015 and IVRI-350-2015 showed maximum identity (98-99%) with different isolates of China, Hungary, Romania and Germany. In the present study, we report cases of both reproductive failures and post-weaning multi systemic wasting syndrome (PMWS), due to concurrent infection with PCV2 and PPV in Indian pigs.

Keywords: Co-infection, PCV2, Porcine parvovirus, Reproductive failure, Pigs

INTRODUCTION

Raising pigs is an important activity for the livelihood of poor and marginal society in India. A wide variety of pathogens which cause diseases in pigs pose a special challenge to the skills of the clinicians. In recent years, Porcine circovirus type 2 associated diseases (PCVAD) is emerging as a new threat to the pig population worldwide. Porcine circovirus 2 (PCV2), the essential causative agent of PCVAD is now considered as one of the most important viral pathogens of pigs. Porcine parvovirus (PPV), an ubiquitous infectious cause of reproductive failure in swine, is endemic in swine populations throughout

the world (Mengeling, 1992) and its main manifestation is reproductive failure (Kresse *et al*., 1985; Bolt *et al.,* 1997). The co-infection by PCV2 and PPV has been identified in naturally (Ellis *et al*., 2000) and experimentally infected pigs (Kennedy *et al.,* 2000). Although PCV2 is the necessary cause of post-weaning multi systemic wasting syndrome (PMWS) in swine, a variety of co-factors, including other infectious agents like PPV are thought to be necessary in the complete manifestation of disease (Rajkhowa and Saikumar, 2012). The present study was conducted to estimate the incidence of PPV and PCV 2 associated post-weaning multisystemic wasting syndrome and reproductive failures in crossbred Indian pigs.

MATERIAL AND METHODS

A total of 150 heart, lungs and lymph nodes samples of pigs were collected from postmortem facility, Department of Veterinary Pathology, ICAR-Indian Veterinary Research Institute and various slaughterhouses in different regions in Uttar Pradesh, India. The samples were collected on ice for molecular analysis and stored at - 80º C until further use. All organs were collected in 10% buffered neutral formalin for histopathological examination and processed according to standard protocol for Haematoxylin-Eosin (H&E) staining. In the present study, the samples were selected for screening by Polymerase Chain Reaction (PCR) based on its gross and histopathological lesions suggestive of PCV2/PPV infection. Tissue samples from post mortem cases which showed pale heart, hydropericardium, congested or/and haemorrhagic lymph nodes and all stillborn and mummified foetuses were selected for PCV2/PPV screening.

Total viral DNA was extracted from the pooled sample using QIAamp DNA Mini Kit (QIAGEN, Germany) according to manufacturer's protocol. The DNA extracted was tested by PCR using the primers PCVLF and PCVLR (Larochelle *et al.,* 1999). The positive samples were preceded further for sequencing. Porcine parvovirus was screened with PPVF and PPVR (Arnauld *et al*., 1998) and confirmed by sequencing analysis. The genetic characterization of the virus was done by phylogenetic analysis. Phylogenetic analysis based on VP2 gene of PPV and construction of phylogenetic tree was done by maximum likelihood method with 1000 bootstrap replicates. The characterisation of the PCV2 isolate was done with blast analysis in NCBI.

RESULTS AND DISCUSSION

Out of 150 samples which were screened by conventional PCR, 57 were

Table 1. Gross and histopathological lesions noticed in concurrent PPV and PCV2 infection

Gross lesions	Histopathological lesions
Stillborns: Oedematous and cyanotic (Fig.2). Mild thickening, oedema, and mild congestion of epicardium in a few cases. Myocardium showed mild to moderate degree of congestion. Myocarditis, pale myocardial areas, ventricular dilatation, hydrothorax, ascites and liver enlargement	**Stillborns:** Non-suppurative myocarditis and perivascular infiltration of mononuclear cells and macrophages. Intracytoplasmic inclusion bodies in infiltrating macrophages in the heart. Mild degeneration and oedema in all cases. Multiple areas of necrosis in some cases with severe infiltration of mononuclear cells.
Post weaned animal: Animal had stunted growth, rough hairs, arched posture and erythema and scabs of skin. Inguinal lymph nodes were mildly enlarged and pale. Hydro pericardium was noticed (Fig.3). Lung was pale and non-collapsible (Fig.4). Liver was mildly icteric. Small intestine was mildly thickened and corrugated.	**Post weaned animal:** Sections of heart revealed multifocal non-suppurative myocarditis, perivascular infiltration of mononuclear cells and mild endocardial thickening and infiltration of mononuclear cells. Lungs showed peribronchiolar lymphoid aggregation and infiltration of mononuclear cells and macrophages in inter alveolar septum. Liver showed mild vacuolar degeneration of hepatocytes with multifocal infiltration of mononuclear cells. In cerebrum, vaculitis, perivascular infiltration, multifocal gliosis were noticed.

stillbirths and mummified and 41 were pre-weaned piglets and 52 were post-weaned animals. The viruses (PCV2 and PPV) were detected in 9 out of 150 pigs (6%) by conventional PCR. Out of 9 positive, five were mummified foetus from same litter (Fig.1), other three were from stillbirth and one was 2.5 month age.

Lesions in stillborns and more number of mummified foetus gives an indication of the effect of viruses *in-utero* in sows and its involvement in causing reproductive problems. Co-infection of PPV and PCV2 results in more prominent cardiac lesions in piglets examined. The disease caused by the PCV and PPV co-infection may be more severe than that caused by PCV2 alone. In the litters that were co-infected by PCV2 and PPV, incidence of stillbirths and mummification were more, suggesting less survivability and more severity during co-infections. Out of the 19 post-weaned cases examined,

only one animal showed typical signs of PMWS. Detailed description of lesions was showed in Table.1.

Phylogenetic analysis of porcine parvoviruses based on nucleotide sequence of the VP2 and NS1 has been carried out by many researchers for molecular epidemiological studies (Shangjin *et al*., 2009). BLAST analysis of VP2 gene sequence of two isolates of present study named IVRI-312-2015 (Accession no: MK035431.1) and IVRI-350-2015 showed maximum identity (98-99%) with different isolates of China (Isolate GD2013 -KX242359), Hungary (NADL-2 M2- KF913346.1), Romania (WB-143-JQ249914.1) and Germany (693a-JN400519.1) (Fig.5). The VP2 region sequence of PPV obtained in this study was analyzed along with 18 sequences retrieved from NCBI database to construct a phylogenetic tree. The isolate IZN-312-2015 and IZN-350-2015 of the present study clustered with 2 other isolates of India (Accession no: DQ158864 isolate IZN-05 and JX495963 isolate PPV-07). Phylogenetic analysis of the sequence obtained during the study cluster with other published Indian sequences of VP2 of PPV indicated that they are closely related. BLAST analysis of full genome of PCV2 isolated revealed 99% homology with different isolates from China, Vietnam and recombinant strains (PCV2Izn-218-13 and PCV2Izn-89-13) from India.

Prevalence of concurrent affection of PPV and PCV2 and PPV alone in the current study was 6 %. Earlier studies on PPV from India showed a prevalence of 7.14 % in UP (Sharma and Saikumar, 2010), 41.1% in Punjab (Kaur *et al.*, 2016) and 5.26 % in Kerala (Aishwarya *et al*., 2016). The most commonly used method to detect PPV is PCR and sensitive detection

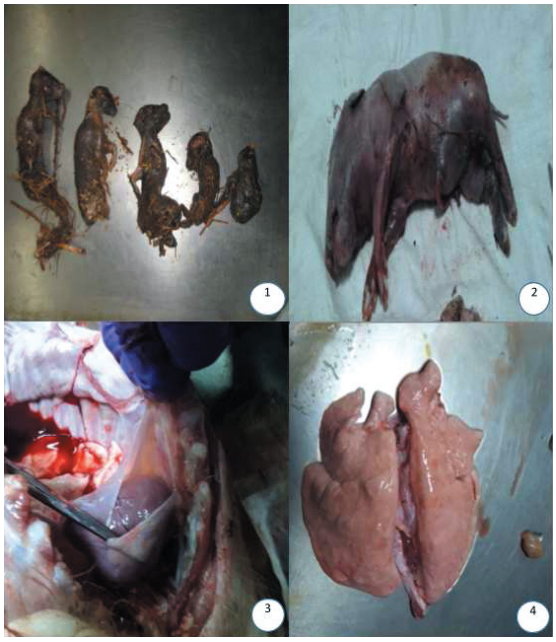

Fig. 1. Mummified fetuses which died at different stages in-utero.

Fig. 2. Stillborn: Oedematous and cyanotic piglet.

Fig. 3. Heart (2.5 month): Hydroperi-cardium

Fig. 4. Lungs (1 month old): Pale, non-collapsible

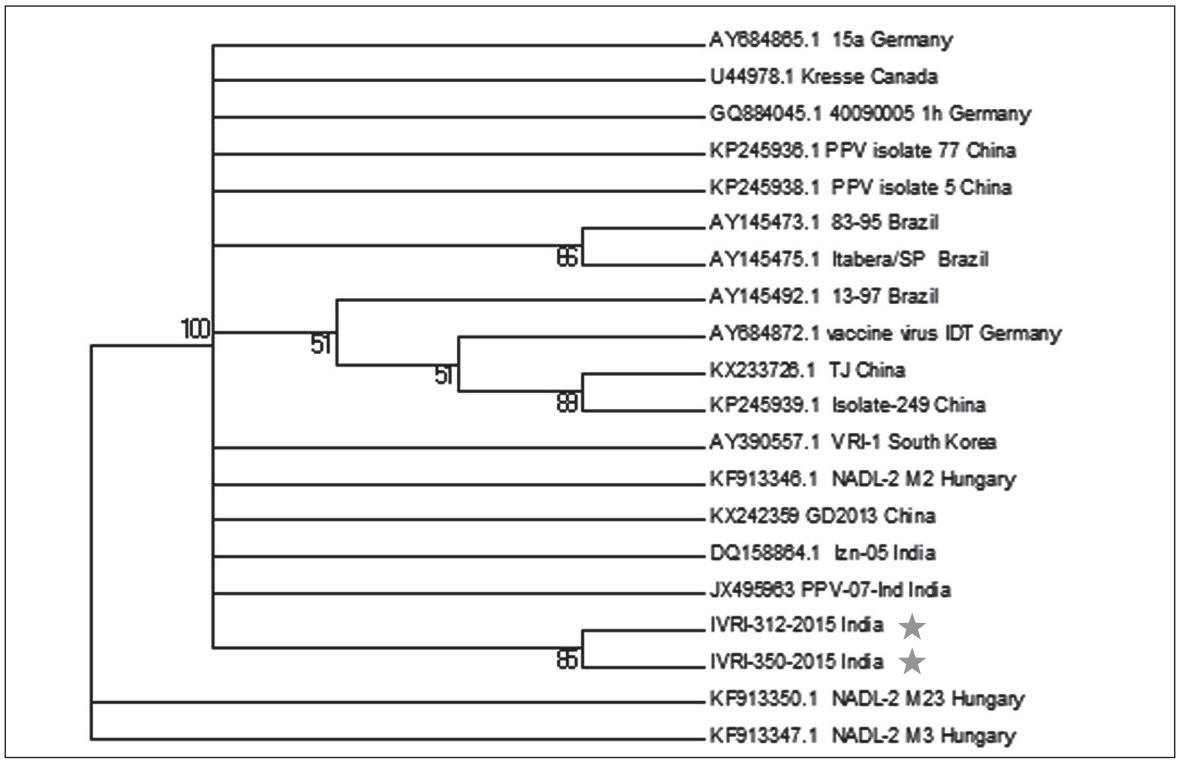

Fig. 5. Phylogenetic tree based on maximum likelihood method for 226 bp VP2 sequences of PPV using MEGA 6 software

of the virus by employing the test has been reported (Soares *et al*., 1997; Xu *et al*., 2012). Previous studies based on NS1 gene showing same results, showed more close relation with Chinese strains than Indian isolates (Aishwarya *et al*., 2016). In a study by Sharma and Saikumar (2015), the Indian isolate (Izn-05- DQ158864) showed clustering with viruses from USA (NADL2, POVNADL2), Spain (POVG), China (N) and South Korea (VRI-1).

This synergistic action of PCV2 and PPV in aborted swine foetuses had also been reported in natural cases in India (Sharma and Saikumar, 2010), Germany (Alterr *et al*., 2003) and in Brazil (Pescador *et al*., 2007). The hearts and lymph nodes were consistently positive in all stillborns and thus they may serve as target organs for diagnosing foetal infection (Kim *et al.*, 2004; Sanchez *et al*., 2004). Co-infection by PCV2 and PPV have been associated with natural (Ellis *et al.*, 2000) and experimental (Kennedy *et al*., 2000; Krakowka *et al.,* 2000) PMWS affected pigs and aborted swine fetuses (Altherr *et al.,* 2003). Porcine parvovirus replicates in cells of the monocyte-macrophage series and vascular endothelium and may produce

immune cell dysfunction, activation or immunosuppression causing an enhanced replication of PCV2 in affected pigs. Alternatively, PCV2 may initiate lymphoid depletion, resulting in an increased susceptibility to other viral or bacterial infections (Harding and Molitor, 1988; Kennedy *et al.,* 2000). This co-infection could be expected, since both viruses are worldwide distributed (Mengeling, 1992; Allan and Ellis, 2000).

SUMMARY

Prevalence of concurrent infection of PPV and PCV2 and PPV alone in the current study was 6%. In the present study, we report cases of both reproductive failures and PMWS, due to concurrent action of PCV2 and PPV in Indian pigs.

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