



Occurrence of post weaning multisystemic wasting syndrome in crossbred pigs of Uttar Pradesh and Uttarakhand, India

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ABSTRACT

Post weaning multisystemic wasting syndrome (PMWS) was first recognized in Canada in 1996 as a new emerging disease which caused wasting in post weaned pigs. Since then, PMWS has been recognized globally during the last decade. The present study was carried out to study occurrence of PMWS in pigs of organized pig farms in northern states of India i.e. Uttar Pradesh and Uttarakhand. A total 234 pigs out of 798 pigs in the age group of 5–12 weeks from a swine population of 1,298 were clinically examined over a period of 1 year. Thorough postmortem examination was carried out in 18 dead piglets and in 16 terminally ill piglets exhibiting clinical signs of PMWS, which were euthanized. Histopathology followed by immunohistochemistry and *in situ* hybridization with PCR techniques were used for diagnosis of the disease. This study is reporting high prevalence of PMWS in crossbred pigs of organized pig farms in Uttar Pradesh and Uttarakhand of India.

Key words: PCV-2, PMWS, Immunohistochemistry, *In situ* hybridization, PCR, Post weaning multisystemic wasting syndrome

In 1996 a new emerging disease, post weaning multisystemic wasting syndrome (PMWS), was first recognized in Canada (Clark 1997, Harding and Clark 1997). The disease is characterized by progressive weight loss, respiratory signs and jaundice. Affected herds are otherwise in good health, has a low morbidity but relatively high mortality rate among pigs in the age group of 5–12 week of age (Harding and Clark 1997, Allan and Ellis 2000, Kim *et al.* 2002, Pallares *et al.* 2002). Porcine circo virus 2 (PCV2) of the family *Circoviridae* was identified as its causative agent (Tischer *et al.* 1982, Mankertz *et al.* 1997, Mc Nulty *et al.* 2000, Cheung 2006). It has ambisense that encodes proteins by the encapsidated viral DNA, and by the complementary DNA of the replicative intermediate synthesized in the host (Segales *et al.* 2002, Cheung 2003, Mankertz *et al.* 2004). Incidences of PMWS and other PCV-2 associated infections have been reported from Europe, America and Asia. PMWS is considered a very significant disease in swine industry (Allan *et al.* 1998, Allan *et al.* 1999, Onuki *et al.* 1999, Fenaux *et al.* 2000, Mankertz *et al.* 2000, Wang *et al.* 2004, Cheung *et al.* 2007). Studies reported

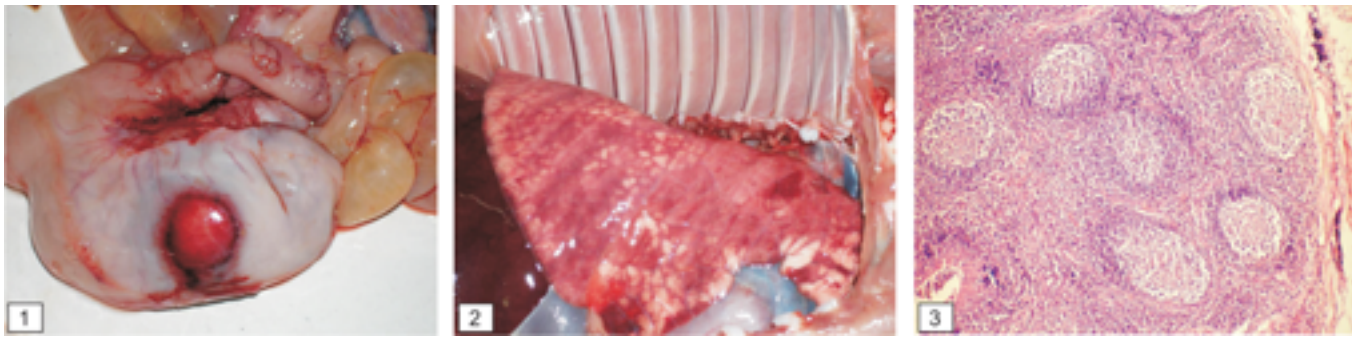
presence of PCV-2 associated reproductive failure (Sharma and Saikumar 2008, Sharma and Saikumar 2010) in swine population of India, but there is no report on occurrence of PMWS. During the period 2007–2008 several pig farms in northern Uttar Pradesh and Uttarakhand of north India experienced wasting syndrome with high mortality in post weaned piglets. This study was carried out to study occurrence of PMWS in pigs of organized farms located in Uttar Pradesh and Uttarakhand of Northern India.

MATERIALS AND METHODS

Sample collection: The present study covered 8 organized pig farms in northern Uttar Pradesh and 1 organized pig farm in Uttarakhand, maintaining 1 298 crossbred pigs. Out of 798 pigs in the age group of 5–12 weeks, 234 affected piglets were clinically examined for 1 year. Postmortem examination was carried out in 18 dead piglets and in 16 terminally ill piglets exhibiting clinical signs of PMWS, which were euthanized. Tissue samples comprising brain, tonsil, inguinal and other lymphnodes, heart, lung, liver, spleen, kidney, intestine were collected and preserved both in 10% buffered formalin for histopathological studies and at –80°C for molecular analysis.

Histopathological examination and indirect immunoperoxidase test: Fixed tissues were subjected to histopathological processing and staining as per Luna (1968).

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Figs 1–3. **1.** Haemorrhagic ulcer in the fundic wall of stomach (8-week-old piglet). **2.** Edematous, congested, mottled lung with rib imprints on surface (8-week-old piglet), **3.** Inguinal lymph node, Lymphoid depletion (8-week-old piglet) (H&E, 100 $\times$).

2 of the necropsied animals (Fig. 1). Adjoining to this 5–6 more developing ulcers with hemorrhagic borders were observed. Wall of small intestine appeared thickened whereas large intestine was thin walled and bloated. Liver was soft, enlarged, congested with yellowish discoloration and some of necrotic foci. Spleen was congested. Surface of the kidney revealed whitish foci with mild congestion. Cut surfaces revealed severe congestion of medulla. Tan, mottled,

congested, edematous and non-collapsing lung with rib imprints on it and patches of hemorrhages was a constant feature of the lung lesion (Fig. 2). These clinical and gross changes are in agreement with others (Harding and Clark 1997, Allan and Ellis 2000, Kim *et al.* 2002, Pallares *et al.* 2002, Opriessnig *et al.* 2007).

Microscopically examination showed severe lymphoid depletion in tonsil, inguinal lymph node and mesenteric lymph nodes (++ to +++). Depleted lymphoid follicles were infiltrated with histiocytes (Fig. 3). Intracytoplasmic inclusions, which were sharply demarcated, spherical, large, multiple, basophilic or amphophilic, grape like structures were seen in cytoplasm of histiocytic cells. Many eosinophilic like cells with eosinophilic staining of the cytoplasm were observed scattered in the parenchyma between the lymphoid follicles. Spleen showed mild lymphoid depletion (+ to ++) with congestion of the sinusoidal spaces.

Tissue sections from lung revealed severe interstitial pneumonia (+++). Inter alveolar wall was thickened due to severe mononuclear and histiocytic infiltration, congestion and edema. Formation of multinucleated giant cells (1–2 giant cells/ low power field) was observed in the thickened interstitium. Alveolar spaces were compressed and were filled with infiltrating mononuclear cells. Bronchiolar mucosal epithelial cells showed moderate to severe hyperplastic changes and lumen contained serous exudates with infiltrating cells. Severe mononuclear infiltration was observed in peri- bronchiolar region. Interlobular septae

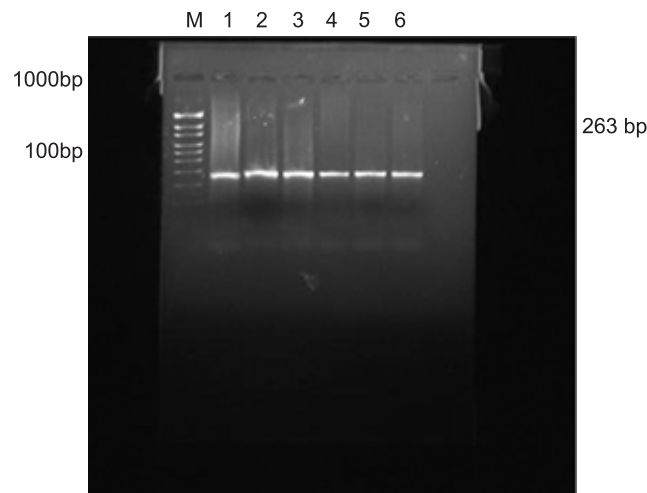
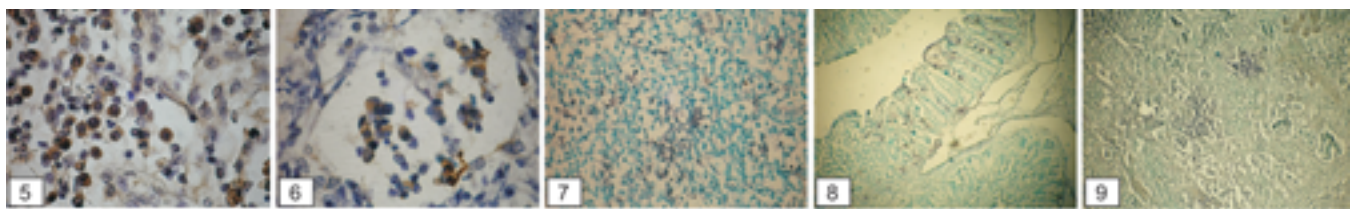


Fig. 4. Ethidium bromide stained 1.5% agarose gel showing amplification of 263 bp fragment of ORF2 gene of PCV-2. Lane: M, molecular marker; 1, tonsil; 2, inguinal lymphnode; 3, spleen; 4, intestine; 5, lungs; 6, liver.



Figs 5–9. **5.** Tonsil, PCV-2 antigen in cytoplasm of macrophages and lymphocytes in perifollicular region. DAB substrate, Mayers haematoxyline counter stain, Indirect IPT, 1000 $\times$; **6.** Lungs, PCV-2 antigen in cytoplasm of infiltrating macrophages in alveoli. DAB substrate, Mayers haematoxyline counter stain, Indirect IPT, 1000 $\times$; **7.** Tonsil, Hybridization signal in infiltrating mononuclear cells in perifollicular region. BCIP/NBT chromogens, Methyl green counter stain. PCV-2, DIG-ISH. 400 $\times$; **8.** Intestine, Hybridization signal in infiltrating mononuclear cells in lamina propria. BCIP/NBT chromogen. Methyls green counter stain. PCV-2, DIG-ISH. 100 $\times$; **9.** Lungs, Hybridization signal in infiltrating mononuclear cells. BCIP/NBT chromogen. Methyl green counter stain. PCV-2, DIG-ISH, 100 $\times$.

appeared thickened due to edema and mononuclear infiltration. Microscopic examination of liver revealed degeneration, vacuolation, karyomegaly of hepatocytes and disorganisation of liver plates (++ to +++). Lympho-histiocytic inflammatory infiltration and congestion was observed in the portal zone.

Moderate to severe lympho-histiocytic inflammatory infiltration was observed in the interstitium of kidney (++ to +++). Degeneration and desquamation of tubular epithelial cells were present with congestion and foci of hemorrhages. Tissue sections from the ulcerative lesions of stomach revealed complete necrosis of lamina propria. Heavy infiltration of mononuclear and neutrophils with oedema and congestion was observed in muscularis mucosa and sub mucosa. In small intestine the lamina propria was very much thickened due to mononuclear infiltration (++ to +++). Crypts of Lieberkuhns were compressed or occluded by infiltrating cells leading to cystic appearance of the glandular structures. In peyer's patches (GALT) severe lymphoid depletion was observed in the lymphoid follicles. The microscopic lesions of different organs described in our study were earlier reported as characteristic lesions of PMWS (Clark 1997, Harding *et al.* 1998, Cottrell *et al.* 1999, Harms *et al.* 1999, Rossel *et al.* 1999, Segales *et al.* 2000, Wellenberg *et al.* 2000 and Opriessnig *et al.* 2007).

Detection of porcine circovirus-2 by PCR: Tissues bearing lesions from tonsil, inguinal lymph node, mesenteric lymph node, spleen, lung, liver, intestine and kidney were tested by PCR. PCV2 was detected in all the 16 euthanized piglets and 7 other dead piglets (67.65%), which showed an amplification of 263 bp (Fig. 4) fragment of ORF2 gene of PCV2. Similar amplification of specific sequence of PCV2 by PCR using type specific primers was reported earlier (Allan *et al.* 1999, Ellis *et al.* 1999, Larochelle *et al.* 1999, Kim and Chae 2003).

Indirect immunoperoxidase test for detection of PCV2 antigen in formalin-fixed, paraffin-embedded tissue sections: Labeling of PCV-2 antigen in large quantities were observed mainly in cytoplasm and occasionally in nuclei of a wide range of tissues including lymph nodes, spleen, tonsil, lungs, liver, kidneys and intestine. The positive cells showed a typical dark brown reaction product with diaminobenzidine (DAB). In lymph nodes, the antigen was demonstrated in the follicular regions of the cortex and paracortex and located in the macrophages/histiocytes and other reticuloendothelial cells. In tonsils strong positive IHC signals were observed in the cytoplasm of infiltrating histiocytes in depleted lymphoid follicles, mononuclear cells in peri follicular and inter follicular region and infiltrating mononuclear cells in lumen of crypts (+++) (Fig. 5). PCV2 antigen in spleen was observed in mononuclear cells of depleted PALS and inter follicular region (++).

In lungs the PCV2 antigen was detected mostly in the cytoplasm of infiltrating mononuclear cells in alveolar air

space, lumen of bronchi and bronchioles, alveolar septa and in the peri bronchiolar region (+++)(Fig. 6). Cytoplasm of multinucleated giant cells attached to the alveolar wall showed strong positive IHC signals for presence of PCV2 antigen. Abundant PCV2 antigen was observed in Kupfer cells and in mononuclear cells infiltrating interlobular septae and sinusoidal space of liver. Labeling of PCV2 antigen was also observed in the infiltrating mononuclear cells in the interstitium between the tubules (++) . In intestine strong positive signals for PCV2 antigen was observed in the mononuclear cells of GALT (in depleted follicles and in peri follicular area) and also in villous lining epithelial cells (+++).

In situ hybridization employing DIG labeled DNA probe for detection of Porcine Circo virus 2 in formalin-fixed, paraffin – embedded tissue sections: The PCV2 nucleic acid was detected in tonsil, inguinal lymph node, mesenteric lymph node, spleen, intestine and lungs. The positive cells exhibited deep purple or purple bluish color development with 4-nitroblue tetrazolium chloride/ 5-bromo-4-chloro-3-indolyl-phosphate (NBT/BCIP) substrate. Both intra nuclear and intra cytoplasmic labeling of PCV2 nucleic acid was observed in the infiltrating mononuclear cells mostly in inter follicular region and occasionally in depleted lymphoid follicles of lymph nodes (Fig. 7). In spleen, hybridization signal was observed in the cortical splenic parenchyma (red pulp). Intestine showed abundant hybridization signal in the infiltrating mononuclear cells presents in crypts of Lieberkuhn and in between the intestinal glands of thickened lamina propria (Fig. 8). In lungs PCV2 nucleic acid labeling was observed in the infiltrating mononuclear cells in alveolar air spaces and macrophages in the alveolar wall (+++) (Fig. 9).

Detection of both PCV2 antigen and nucleic acid in macrophages and mononuclear cells of target tissues by indirect immunohistochemistry and *in situ* hybridization employing DIG labeled DNA probe respectively and their distribution was consistent with those reported by Kim and Chae (2001).

The present study has revealed high prevalence of PMWS in the pig population of organized farms in Uttar Pradesh and Uttarakhand. Characteristic clinical signs, gross and microscopical lesions of PMWS were observed in piglets of 5–12 weeks old. Diagnosis of the disease in affected pigs was done by clinical examination, histopathology, PCR detection of PCV-2 nucleic acid in tissues and further confirmed by detection of both PCV2 antigen and nucleic acid by indirect immunohistochemistry and *in situ* hybridization employing DIG labeled DNA probe respectively in tissue lesions.

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