

## POST WEANING MULTISYSTEMIC WASTING SYNDROME: AN EMERGING DISEASE OF SWINE- AN OVERVIEW

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**Abstract:** Post weaning multisystemic wasting syndrome is a worldwide disease of multifactorial origin and Porcine circovirus 2 (PCV2) has been identified as its essential infectious etiology. It mainly affects the nursery and growing piglets. Clinically, the symptoms include wasting, unthriftiness and paleness of the skin, respiratory distress, diarrhea and icterus. Microscopic lesions in lymphoid tissues mainly lymphocyte depletion with histiocytic infiltration are quite specific for PMWS. These clinical and laboratory findings mainly changes in the cytokine expression due to atrophy or depletion of organs along with lesions suggest that severely affected pigs become immunosuppressed. A vaccine against PCV2 is being used in some European countries but in India is not available.

**Keywords:** Post weaning multisystemic wasting syndrome, porcine circovirus 2, lymphoid depletion, immunosuppression, swine.

### Introduction

According to the 19<sup>th</sup> livestock census, the pig population of India is about 10.29 millions. Swine production is high in north eastern states of India where the pork is an important source of meat. The present production of meat in India is estimated at 6.27 million tons in 2010 (FAO, 2012), of which pork contributes 5.31% as a source of meat. PMWS infection causes wasting and increased condemnation of the carcasses leads to high economic losses. Post weaning multisystemic wasting syndrome (PMWS) was first discovered in Canada in 1995. It is an economically important disease because of the high mortality and production losses. PMWS is endemic in many swine producing countries and continues to be a major cause of wasting diseases of swine. Pigs affected with this syndrome show generalized lymphadenopathy, poor body weight, icterus, anaemia, diarrhea and skin lesions mainly in nursery and fattening pigs. Porcine circovirus is the causative agent of the PMWS and it is associated with various other disease syndromes referred to as porcine circovirus associated diseases (PCVAD). The term PCVAD refers to several disease entities which include PMWS, Reproductive disorder, Porcine Reproductive and Respiratory Disease complex, Enteritis,

porcine dermatitis and nephropathy syndrome. In which PMWS is the main syndrome causing leading economic losses (Lopes-Soria *et al.*, 2014).

### **Etiology**

Porcine circovirus (PCV), a member of the circoviridae family, was first detected as a contaminant of the pig kidney cell line PK-15 (Tischer *et al.*, 1982). Circoviruses are the smallest known DNA viruses with a diameter of 17 nm having a single-stranded, circular DNA genome of about 1.76 kb. The cell culture derived virus is Porcine circovirus 1 (PCV1) which is non pathogenic for swine. The virus associated with the various disease syndromes is referred to as PCV2. These two viruses share 76% nucleotide homology with each other. Both viruses are non-enveloped with icosahedral symmetry. Since, 1990's the world has seen a dramatic increase in PCV2 virus loads in pigs. The two other animal circoviruses included in this family are chicken anemia virus (CAV), psittacine beak and feather disease virus (PBFDV). These three circoviruses do not share nucleotide sequence homology or antigenic determinants with each other.

### **Occurrence**

Serological studies showed that the prevalence and incidence of PCV2 infection is more and affected up to 100% of pig herds in USA, Europe, North America, South-East Asia and Europe. Apparently, porcine circovirus 2 (PCV2) was widely distributed in swine population throughout the world. A retrospective serological analysis conducted in Canada, U.K, Spain, and Belgium to determine the presence of antibodies to PCV1 and PCV2 in serum reveals organisms circulating in the pig population at least 10 years before the PMWS reported. In India, PCV2 associated PMWS and reproductive problems was reported by Rajkhowa, (2008) in the farm having the history of increased mortality in piglets and decreased litter size at birth. Kumar *et al.*, (2014) reported that, PCV2 is an emerging viral pathogen in India.

### **Epidemiology**

PCV 2 is ubiquitous, both domestic and feral swine are natural hosts (Segales and Domingo, 2002). The nucleotide identity of PCV2 isolates from wild boars was identical to isolates from domestic swine and included both PCV2a and PCV2b genotypes. Non porcine species, including humans are not susceptible to PCV2 infection except in the mice. PCV2 was replicate within the mice and transmit between them to a limited degree. Oronasal exposure is primary route of transmission. PCV<sub>2</sub> was found in nasal, tonsillar, bronchial and ocular secretions, feces, saliva, urine, colostrums, milk and semen. Respiratory and oral secretions contain virus with higher loads in PCVAD affected pigs than healthy pigs. Transplacental

infection occurs in pregnant sows exposed to PCV2 three weeks prior to farrowing. Vertical transmission can result in birth of viraemic or persistently infected piglets. PCV2 can also transmit through semen.

### **Pathogenesis**

The pathogenesis of PCV2 infection and major cell types that support the viral replication is still not fully understood (Nauwynck *et al.*, 2012). However PCV2 antigen is detectable in the cytoplasm and nuclei of lymphoid and non-lymphoid tissues including smooth muscle cells and fibroblasts following infection. But it is still not clearly understood that lymphocyte depletion in affected piglets due to reduced production in the bone marrow or reduced proliferation in secondary lymphoid tissues or increased loss of lymphocytes in the bone marrow, peripheral blood or in lymphoid tissue via virus-induced necrosis or apoptosis. Yu *et al.*, 2007 analyzed the expression of Cap mRNA in tissues and leukocytes of PCV2 infected animals, the mRNA was detected in lymph nodes, tonsils and PBMC's. The presence of Cap mRNA can only reflect DNA transcription but provides no information on viral protein synthesis. While tested *in vitro* the macrophages and dendritic cells are not representing the primary site for viral replication. However in these cells it persists without loss of infectivity or the induction of cell death. It was suggested that migratory capacity of the dendritic cells will provide a vehicle for transport of the virus throughout the host. The primary etiological role of PCV2 was established under experimental infections in conventionally reared pigs, but it induces low grade lesions without manifestation of clinical signs of disease. Probably additional factors, mainly concurrent viral infections are necessary for manifestations of the disease (Balasch *et al.*, 1999). In young conventional piglets, the clinical signs and lesions of PMWS could be induced by co-infection with PCV2 and PPV, PRRSV or *Mycoplasma hyopneumoniae*

In the Flow cytometric analysis of the lymphocyte and monocyte, significant decrease in the number of CD3<sup>+</sup> and CD4<sup>+</sup> cells in pigs with PMWS compared to normal pigs, whereas number of CD8<sup>+</sup> cells was similar in both PMWS affected and healthy pigs. CD4<sup>+</sup> depletion, results in CD4<sup>+</sup>/CD8<sup>+</sup> ratio was reduced to 0.31 compared to 0.64 in healthy pigs. This selective loss of CD4<sup>+</sup> subset and inversion of CD4<sup>+</sup>/CD8<sup>+</sup> ratio, these are the hallmark of immunodeficiency in humans and cats infected by HIV (Cotran *et al.*, 1999) and FIV (English *et al.*, 1993) respectively. Changes in the cytokine expression includes an overexpression of IL-10 in thymus and general decrease of cytokines IL-2, IL-4, IL-10, IL-12 and IFN- gamma in other lymphoid organs. Thymic IL-10 mRNA over expression in PMWS

pigs is associated with depletion and atrophy of the organ. These changes lead to severe T-cell immunosuppression (Darwich *et al.*, 2002).

### **Clinical signs**

Clinical signs are non specific and variable. Affected pig shows, poor weight gain, wasting with or without respiratory signs, poor hairy coat, lethargy, icterus and emaciation. Some pigs show high fever (40-42<sup>0</sup> C), dyspnoea, anaemia, jaundice, mild diarrhea and poor response to antibiotic therapy. Superficial inguinal lymph nodes may become enlarged and palpable while some may become pale and yellow in color. A marked increase in mortality rate from single or multiple concurrent bacterial infections and viruses (Allan and Ellis, 2000) in post weaning and early finishing pigs. Pigs are viremic for 21 days or more and secondary infections may cause persistence of infection up to 42 days.

### **Macroscopic lesions**

Most striking lesions at necropsy are non-collapsed lungs and enlarged lymph nodes mainly superficial inguinal, submandibular, mesenteric and mediastinal lymphnodes. The livers are atrophic and discolored (icterus is an evident finding) while kidney cortices bear multifocal white foci (Non purulent interstitial nephritis). Serous atrophy of fat, soft rectal faeces (catarrhal colitis associated with diarrhoea), fibrino necrotizing colitis (Segales and Domingo, 2002) are the other features. Moderate to high numbers of PMWS affected pigs having bronchopneumonia and gastric ulceration of the pars oesophagea. It is not directly related to the effect of PCV2. Bronchopneumonia is associated with bacterial infections while gastric ulceration is multifactorial in origin. However the lesion in the stomach cause internal haemorrhage and it is the cause of death of a number of pigs with PMWS and it also responsible for the paleness of skin also frequently related to the disease. Brain lesions primarily of vasculitis predisposing to lymphohistiocytic meningitis have been described.

### **Microscopic lesions**

Characteristic lesions in affected pigs include lymphoid depletion with infiltration by large histiocytic cells and multinucleate cells in lymphatic tissues. Another key finding is presence of sharply demarcated, spherical basophilic cytoplasmic inclusions and granulomatous inflammation of PCV2 affected lymphoid tissues. In thymus, cortical atrophy was a prominent finding (Darwich *et al.*, 2003). In lungs, interstitial pneumonia, peribronchial fibrosis and fibrinous bronchiolitis occur in advanced cases. Hepatic lesions have been described from the form of mild lymphohistiocytic infiltration in portal zones, single cell necrosis of hepatocytes, swelling and vacuolation of hepatocytes cytoplasm and karyomegaly

and massive inflammation with apoptotic bodies, disorganization of hepatic plates and perilobular fibrosis. Foci of lymphohistiocytic inflammatory infiltrates may be seen in many tissues mainly in kidney, pancreas, intestines and sporadically myocardium of pigs affected by PMWS. Sporadically moderate to severe granulomatous enteritis with blunting of villi was noticed.

### **Diagnosis**

Generally accepted PMWS diagnosis at herd level should fulfill the following criteria, Growth retardation, wasting and lymph node enlargement along with significant increase in the mortality in the farm, moderate to severe characteristic histopathological lesions in lymphoid tissues, moderate to high amounts of PCV2 within the lymphoid lesions (Grau-Roma *et al.*, 2011). Virus isolation was done by inoculation of PCV1 free PK-15 cell lines with clarified organ suspension or plasma or serum. Since no cytopathic effects are induced in infected cell lines by the PCV2, it needs to be confirmed by PCR or immunohistochemical methods using antibodies. PCR is used to amplify the specific sequence of PCV2. Multiplex PCR used to detect and differentiate PCV1 from PCV2 in infected cells. Nucleotide sequencing and phylogenetic analysis will of help in determining genetic variation among PCV2 isolates from different geographic regions. *In-situ* hybridization and immunohistochemistry used to detect the antigen. Digoxigenin (DIG) labeled DNA probes in ISH used to differentiate PCV1 from PCV2. There are many varieties of serological tests that detect antibodies against PCV. Since subclinical infections with PCV2 are known to be widespread in pigs, the use of serology in the diagnosis of PCV2 related disease is limited. So, serology can be useful as a management tool in breeding herds. Antibody dynamics of PCV2 is of interest because of their potential role in monitoring PCV2 vaccination Serology is also used to assess the maternal immunity that interfere with vaccination (Fort *et al.*, 2009)

### **Prevention and control**

PCV's are highly resistant to inactivation by common disinfectants and detergents but the use of sodium hydroxide is effective in reducing the virus titers. Appropriate antimicrobial drugs and bacterins used against specific bacterial infections are necessary to reduce the co infecting bacterial agents. It is a multifactorial disease that can be controlled by use of PCV2 vaccines. All current vaccines are based on PCV2a strains (Kekarainen *et al.* 2010). For the reduction of PMWS mortality in herds, measures were taken to increase maternal immunity and decrease sow viraemia at farrowing. The oil-adjuvant, inactivated PCV2 vaccine used to be efficacious in breeding age animals with two dose and single dose vaccine 3 week old pigs

in Europe, Mexico. Autogenous vaccines from lymphoid tissue or lung homogenates of PMWS pigs and inactivated with 2% formaldehyde was found to be reduce mortality from 20% to 3%. Currently, vaccines are not available in India.

### **Conclusion**

PCV2 is an economically devastating emerging pathogen of porcine. As this virus causing immunosuppression, leaves the pigs more susceptible to other swine pathogens. Thymic IL-10 mRNA over expression in PMWS pigs associated with depletion and atrophy of the organ is a feature. It leads to severe T-cell immunosuppression. Characteristic histopathological changes and detection of PCV2 virus within the lesions and existence of compatible clinical signs are major criteria used for the diagnosis. Better management practices are needed to reduce concurrent infections and potential factors that induce immune stimulation. Use of several inactivated, oil-adjuvant, killed vaccines and autogenous vaccines are reducing the mortality.

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