Histopathological and virological findings in emaciated pigs from Mexico: an exploratory study

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Abstract

The objective of this work was to detect the presence of three main pig respiratory viral agents (porcine rubulavirus [PorPV], porcine circovirus type 2 [PCV-2], and porcine reproductive and respiratory syndrome virus [PRRSV]) in tissues of emaciated piglets from the Bajío Region (Mexico). Necropsies and histopathological studies of 37 pigs with poor body condition were performed; viruses were detected by molecular biology methods and PCV-2 was further assessed by immunohistochemistry (IHC). Histopathologically, interstitial pneumonia was observed in 25/37 (68%) of the piglets. Also, a varying degree of lymphocyte depletion in lymphoid organs was found in 14/37 (38%) animals. Through polymerase chain reaction (PCR) and/or reverse transcription polymerase chain reaction (RT-PCR), from the 37 pigs, 16 were positive for PCV-2, 18 for PRRSV and 1 for PorPV. In accordance with these results, the infection and/or co-infection with PCV-2 and PRRSV were fairly frequent findings in piglets with poor body condition in Mexico, while the infection by PorPV was apparently negligible. Wasting of post-weaning piglets is a global pig farming problem that causes great economic losses and has been associated with diverse factors: microbial agents, environmental factors, nutritional factors, and management. When the Blue Eye Disease was first reported in Mexico, it was associated with severe wasting in post-weaning piglets. This study demonstrated that this disease does not seem to play such an important role in the wasting as was previously thought.

Wasting, histopathology, porcine rubulavirus, porcine circovirus type 2, porcine reproductive and respiratory syndrome virus, piglets

Wasting in nursery and fattening piglets is a significant problem that affects swine production worldwide. Specifically in Mexico, it is estimated that at least 10% of pigs reared under intensive conditions develop this clinical condition (García et al. 2008). The term wasting does not imply a diagnosis by itself but is a clinical term to describe a physical condition characterized by growth retardation and is usually of multifactorial origin. These factors include multiple microbial agents and environmental, nutritional and management effects. In many cases, wasting has been confused with the specific condition of porcine circovirus 2-systemic disease (PCV-2-SD, previously known as the post-weaning multisystemic wasting syndrome, PMWS). In addition to PCV-2-SD, there is a number of viral diseases that produce growth retardation in post-weaning pigs, porcine reproductive and respiratory syndrome (PRRS) being the most important one (Segalés et al. 2005). In certain geographic areas of Mexico, blue eye disease (BED) must be counted among those potential viral causes (Kirkland et al. 2012).

So far, wasting associated with porcine circovirus type 2 (PCV-2), porcine reproductive and respiratory syndrome virus (PRRSV) and porcine rubulavirus (PorPV) infections and

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co-infections has not been thoroughly described in Mexico. Therefore, the aim of this study was to detect PorPV in wasted piglets from farms of the Bajío Region of Mexico (BED endemic zone) and to differentiate the associated histopathological lesions as well as to discard the presence of PCV-2 and PRRSV as inducers of emaciation in pigs.

Materials and Methods

A convenience sampling (non-random) was performed in 22 pig farms from the Bajío Region of the states of Jalisco, Michoacan and Guanajuato in Mexico. A total of 37 necropsies of crossbred piglets (1–3 pigs/farm) were performed according to existing legislation (Norma Oficial Mexicana NOM-033-ZOO-1995, sacrificio humanitario de los animales domésticos y silvestres). Inclusion criteria were 6–16-week-old pigs displaying marked growth retardation (body condition scoring of 1 or 2).

Lung, mandibular and/or inguinal lymph nodes, and midbrain samples were fixed for 24 h in 10% buffered formalin, embedded in paraffin and cut to a thickness of 6 µm. Histological sections were stained with haematoxylin and eosin, and tissues were examined by light microscopy. Interstitial lung lesions were classified according to the criteria of Halbur et al. (1995) and lymphocyte depletion and granulomatous inflammation were scored according to the criteria of Grau-Roma et al. (2009).

An immunohistochemical technique was performed using the avidin-biotin complex method in lymph nodes that showed mild to moderate lymphocyte depletion. The commercial monoclonal primary antibody 36A9 specific for the capsid protein of PCV-2 (INGENASA, Spain) was incubated overnight at 4 °C. ENVISION kit anti-mouse HRP (Dako, Glostrup, Denmark) was incubated for 60 min at room temperature. The development of labelling was performed with diaminobenzidine for 5 min. The slides were counterstained with Meyer's haematoxylin.

Frozen tissue homogenates (lymph nodes, spleen, tonsil, lung, and brain) were used to extract deoxyribonucleic acid (DNA) (QIAamp DNA Mini kit, QIAGEN, Germany) and ribonucleic acid (RNA) (RNeasy Mini Kit, QIAGEN, Germany) according to the manufacturer's instructions. Ribonucleic acid was used to detect PorPV (Wiman et al. 1998; resulting in an amplicon product of 374 bp) and PRRSV (Donadeu et al. 1999; with an amplicon product of 300 pb) genomes by reverse transcription polymerase chain reaction (RT-PCR) methods. Deoxyribonucleic acid was used to amplify the PCV-2 genome by polymerase chain reaction (PCR) (Ogawa et al. 2009; giving an amplicon product of 703 pb). Amplified products were analyzed by gel electrophoresis in 1.5% agarose Tris-acetic acid-ethylenediaminetetraacetic acid (EDTA, TAE), stained with GelRed™ (Biotium, CA, USA) as developing agent.

Results

Histopathological and viral detection findings are summarized in Table 1.

In total, 25/37 pigs had mild to moderate interstitial pneumonia with multifocal distribution; in some cases, foci of necrosis and/or groups of macrophages were observed. Suppurative bronchopneumonia was observed in 7/37 pigs. Four lungs displayed both interstitial and catarrhal-purulent bronchopneumonia. Broncho-interstitial pneumonia was found in 5/37 pigs. Finally, 2/37 animals had fibrino-haemorrhagic-necrotizing pleuropneumonia. A single pig displayed proliferative necrotizing pneumonia multifocally. Lymphocyte depletion was detected in 14/37 animals; 9 of them had slight lymphocyte depletion characterized by less than 25% of the lymphoid follicles loss and 5 showed moderate lesions with 25–75% loss of follicles. Mild to moderate macrophage infiltration located in intrafollicular areas was found in a multifocal distribution in lymph nodes of 4 pigs. One of these pigs showed the PCV-2 antigen in the lymph node. Multifocal necrosis was observed in 3 cases with moderate lymphocyte depletion, one of them being of generalized distribution together with sporadic mineralization and disseminated intravascular coagulation. Regarding the central nervous system, 2/37 pigs had non-suppurative meningoencephalitis. Finally, 8/37 pigs showed no apparent injury in any of the tissues observed.

Immunohistochemistry reaction showed one piglet positive to antigen PCV-2 among the 14 animals showing some degree of lymphocyte depletion. Labelling was abundant in the cytoplasm of numerous macrophages in lymph nodes.

A total of 16/37 (43%) pigs were PCR positive for PCV-2, 18/37 (49%) yielded a positive RT-PCR for PRRSV (North American strain) and 1/37 (3%) pigs for PorPV. PCV-2 and PRRSV co-infection was noticed in 7/37 (19%) piglets, while only 1/37 (3%) was positive both for PRRSV and PorPV. Finally, 10/37 (27%) animals were negative for all three viruses.

Table 1. Histopathological lesions and viral detection in emaciated pigs.

Infection status*	Number of pigs	Lung lesions	Lymphoid lesions	Brain lesions
PCV-2 only	9	CPBP (1) SIP (4) BIP (3) None (1)	MiLD (2) MiLD+GI (1) MoLD (2) MoLD+GI (0) None (4)	No
PRRSV only	10	CPBP (2) SIP (7) BIP (0) None (3)	MiLD (2) MiLD+GI (1) MoLD (0) MoLD+GI (1) None (6)	No
PCV-2 and PRRSV	7	CPBP (3) SIP (5) BIP (0) None (0)	MiLD (3) MiLD+GI (0) MoLD (0) MoLD+GI (0) None (4)	No
PRRSV and PorPV	1	CPBP (0) SIP (1) BIP (0) None (0)	MiLD (0) MiLD+GI (0) MoLD (0) MoLD+GI (0) None (0)	NSP (1)
None	10	CPBP (1) SIP (2) BIP (2) None (6)	MiLD (0) MiLD+GI (0) MoLD (0) MoLD+GI (1) None (9)	No

*Determined by RT-PCR or PCR; PCV-2 - porcine circovirus type 2; PRRSV - porcine reproductive and respiratory syndrome virus; PorPV - porcine rubulavirus; CPBP - catarrhal-purulent bronchopneumonia; SIP - subacute interstitial pneumonia; BIP - Broncho-interstitial pneumonia; MiLD - mild lymphocyte depletion; MoLD - moderate lymphocyte depletion; GI - granulomatous inflammation; NSP - non-suppurative meningoencephalitis.

Discussion

In the present study, 73% of wasted pigs displayed infections or co-infections with PRRSV and PCV-2. It has been reported that both viral agents are two of the most important causes of growth retardation in postweaning pigs in Mexico (Reséndiz et al. 2012). In contrast, and despite the fact that the animals came from the PorPV endemic region of Mexico (Martinez et al. 2011; García et al. 2014), only 2 piglets had compatible brain lesions with PorPV and only one was RT-PCR positive for this virus. Therefore, the obtained results point out that PorPV played a relatively minimal role in the causation of wasting in the studied pigs. Although emaciation of piglets was reported in the first studies of BED (Stephano et al. 1988), it can be speculated that other agents may be involved in the wasting condition already at that time. As an example, a retrospective study in Mexico showed that antibodies against PCV-2 in pigs were present at least since 1972 (Ramírez-Mendoza et al. 2009).

The histopathological lesions and the presence of PRRSV found in the pigs sampled in this study further support serological studies in different regions of Mexico that demonstrate

a high seroprevalence of PPRSV (67%) in the country (García et al. 2014). Also, according to the obtained results, PCV-2 was detected in numerous piglets. In Mexico, as in many parts of the world, PCV-2 is a ubiquitous virus (Ramírez et al. 2007), but the prevalence of PCV-2-SD has never been determined. The present study cannot give insights on the prevalence of the disease associated with PCV-2; the chronic state of the selected animals prevented applying the recommended criteria to establish the diagnosis of PCV-2-SD, which includes an examination of animals in the acute-subacute phase of the disease (Segalés 2012). Based on the obtained results, only one pig fulfilled the diagnosis of PCV-2-SD by clinical signs, histopathological lesions and agent identification (PCV-2 antigen in these tissues and PCV-2 positive PCR). This particular pig was also positive by PRRSV RT-PCR and had lesions compatible with a bacterial pulmonary infection. In addition, pigs with moderate histopathological lesions compatible with PCV-2-SD, but negative by immunohistochemistry (and only one positive by PCR) suggests chronic ally affected animals. Again, the chronicity of the clinical condition may have prevented a potential diagnosis of PCV-2-SD and it is very likely that they represent animals in the convalescent phase of the disease.

It is important to highlight that many pigs had pulmonary lesions suggestive of bacterial infections. It is possible that potential immunosuppression or immunomodulation of viral origin (PCV-2 and/or PRRSV mainly) favoured the development of these infections, probably caused by *Pasteurella multocida*, *Mycoplasma hyopneumoniae,* and *Actinobacillus pleuropneumoniae*.

A significant number of analyzed pigs (22%) did not show histopathological lesions compatible with infections by any of the three viruses under study (PRRSV, PCV-2 and/or PorPV). Since wasting is by definition a chronic expression of disease, it cannot be ruled out that those infectious agents may have played a role at earlier stages and, at the moment of necropsy, some of the pigs did not show lesions already, or they might have cleared the virus. Moreover, growth retardation could be associated also with other aetiologies. In addition to these potential infectious agents causing wasting, other non-infectious causes may also be present, including the periweaning failure-to-thrive syndrome (Huang et al. 2012), digestive disorders of infectious or non-infectious origin, environment, nutrition, management, etc.

In summary, results obtained in this study suggest that PCV-2 and PRRSV are present very often (alone or in co-infection) in wasted pigs at Mexican farms. In contrast, the presence of PorPV and histopathological damage associated to this viral infection were very scarce in the studied animals.

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