Postweaning Multisystemic Wasting Syndrome (PMWS) in the Philippines: Porcine Circovirus Type 2 (PCV2) Detection and Characterization

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ABSTRACT. Four swine facilities located in Northern Luzon, in the Philippines, showed lesions and clinical signs similar to those described in post weaning multisystemic wasting syndrome (PMWS). Post-mortem and histopathological examination revealed the presence of typical lymphoid lesions caused by porcine circovirus type 2 (PCV2). An in situ hybridization (ISH) technique allowed the detection of PCV2 nucleic acid associated with the lesions in target tissues. PCV2 DNA from paraffin embedded tissues was extracted, purified and sequenced. The phylogenetic analysis of the sequences obtained confirmed their identity, and grouped them into a PCV2 subgroup, together with some Canadian, French and Dutch isolates. This report is the first description of the presence of PMWS in the Philippines.

KEY WORDS: PCV2, PMWS.

Porcine circovirus type 2 (PCV2) has been demonstrated to be the causal agent for post weaning multisystemic wasting syndrome (PMWS) [2, 3]. Since the first cases of the disease reported in Canada in 1991 [4], and its association with PCV2 in 1998 [5], PMWS has been reported in most swine producing countries, including East-Asian countries such as Japan, Korea and Taiwan [1].

The Philippines is estimated to have a total of 380,000 sows under intensive production. From an epidemiological point of view, the country is enzootic for pseudorabies, hog cholera and foot and mouth disease [12]. The presence of PMWS disease in the Philippines, although suspected, was previously unknown. The objective of this report is to describe the first cases of PMWS in the Philippines.

Four farrow-to-finish facilities housing 1500, 800, 550 and 1500 breeding sows were studied (named A, B, C and D, respectively). All four farms were located in Northern Luzon, which is one of the major pig-producing areas in the Philippines. Clinical parameters were recorded, and a group of affected animals (n=9), from 6 to 8 weeks old were necropsied (2 individuals per farm, except farm D from which there were 3). To perform the histopathological studies, samples of lung, lymph nodes, spleen, liver, intestines and/or kidney were fixed by immersion in 10% buffered formalin, embedded in paraffin, sectioned at 4 µm thick, and stained with hematoxylin and eosin. The same samples were used to perform an in situ hybridization (ISH) technique for detecting PCV2 according to Rosell et al. [10].

DNA from some of the available paraffin embedded tissues was extracted using the Qiagen mini kit (Qiagen Inc., Valencia, CA, U.S.A.) following the manufacturer’s instructions. Specific primers for porcine circovirus type 1 (PCV1) were used, and a fragment of the ORF1 gene was amplified [8, 9]. For PCV2, a fragment of the ORF2 gene was amplified as previously described [8, 9]. In short, the reaction mixtures contained 200 nM dNTPs, 1.5 mM MgCl2, 500 nM of each primer and 1.25 U of TaqPolymerase (Ecogen S.R.L., Barcelona, Spain). The reaction conditions were as follows: initial denaturation at 95°C for 3 min, followed by 35 cycles of 95°C during 30 sec, primer annealing (58°C for PCV1 and 64°C for PCV2) for 1 min and extension at 72°C for 7 min. A final 7 min extension step at 72°C was included. The amplified products were run in a 2% agarose gel, and visualized by staining with 0.5 mg/ml of ethidium bromide. PCR products were purified with a commercial kit (QIAquick®PCR Purification Kit, QIAGEN GmbH, Germany) and sequenced with both forward and reverse PCV2 primers by using the Dyenamic ET terminator cycle sequencing kit (Amersham Biosciences Europe GMBH, Freiburg, Germany). A BLAST analysis [2] was performed to establish the identity of the PCR sequences obtained, which were then aligned with the PCV2 sequences available in the GenBank database by using MegAlign software from DNASTAR Inc. (Madison, WI, U.S.A.).

An unrooted tree was derived from the sequence similarity comparison (MegAlign, DNASTAR Inc., Madison, WI, U.S.A.).

At the time of the clinical inspection, the swine herds studied showed a remarkable increase in mortality rates (11%, 9%, 8% and 13% for farms A, B, C and D, respectively) in the growing pig units. The clinical picture was initially detected in 6- to 7-week-old animals, and prolonged for at least 4 weeks. Clinical signs observed coincided with those described in the literature for PMWS: growth retardation, lethargy, dyspnea, anemia and diarrhea. Around 2 to 3% of such growing pigs also showed hemorrhagic skin lesions, compatible with porcine dermatitis nephropathy syndrome (PDNS) on farms A and D; most of these pigs died within the first week after the detection of skin lesions.

Post-mortem examination revealed enlargement of both inguinal and mesenteric lymph nodes. Fibrinous polyserosi-
Fig. 1. Mesenteric lymph node. Moderate to marked lymphocyte depletion together with histiocytic infiltration in the lymph node parenchyma. Concomitantly, a high numbers of intracytoplasmic inclusion bodies typical of PCV2 infection can be seen in the picture (arrows). Hematoxylin and eosin stain. Original magnification: × 20.

Fig. 2. Mesenteric lymph node. High numbers of intracytoplasmic inclusion bodies in a severe case of PMWS. Note the grape-like appearance of the amphophilic inclusion bodies (of variable diameter) within the cytoplasm of macrophages (arrows). Hematoxylin and eosin stain. Original magnification: × 100.
tis, which is indicative of systemic bacterial co-infection, was also observed. Six of the sampled animals (66.7%) (at least one animal from each farm) had microscopic lesions on the lymph nodes, consisting of slight to severe lymphocyte depletion (Fig. 1), together with variable degrees of granulomatous (sporadically with multinucleate giant cells) inflammatory infiltration. Two of these animals also had a moderate to massive presence of spherical basophilic PCV2 inclusion bodies in the cytoplasm of macrophages (Fig. 2). The rest of the studied pigs did not have any lymph node lesions. One of the pigs had a marked multifocal to coalescent interstitial nephritis with sporadic presence of syncytial

Table 1. Summary of the tissues tested and results obtained by in situ hybridization and PCR

<table>
<thead>
<tr>
<th>ID Case</th>
<th>Farm</th>
<th>Examined Tissues</th>
<th>ISH results</th>
<th>PCR results</th>
</tr>
</thead>
<tbody>
<tr>
<td>3590</td>
<td>A</td>
<td>Lymph node</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Liver</td>
<td>–</td>
<td>NT&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spleen</td>
<td>–</td>
<td>NT</td>
</tr>
<tr>
<td>3591</td>
<td>A</td>
<td>Lymph nodes (n=2)</td>
<td>+++</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kidney</td>
<td>–</td>
<td>NT</td>
</tr>
<tr>
<td>3592</td>
<td>B</td>
<td>Lymph nodes (n=3)</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>3593</td>
<td>B</td>
<td>Small intestine</td>
<td>–</td>
<td>NT</td>
</tr>
<tr>
<td>3594</td>
<td>C</td>
<td>Kidneys (n=2)</td>
<td>–/+</td>
<td>–</td>
</tr>
<tr>
<td>3595</td>
<td>C</td>
<td>Lymph nodes (n=2)</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>3596</td>
<td>D</td>
<td>Lymph nodes (n=3)</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>3597</td>
<td>D</td>
<td>Lymph nodes (n=3)</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>3598</td>
<td>D</td>
<td>Lymph nodes (n=5)</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

<sup>a</sup> Amount of PCV2 nucleic acid: – (absence), + (low), ++ (moderate), +++ (high); – = absence of PCV2 nucleic acid.

<sup>b</sup> Not tested.
cells. All pigs with typical PMWS lesions in lymph nodes had a moderate to high amount of PCV2 nucleic acid detected by in situ hybridization (Fig. 3). Furthermore, a moderate amount of viral genome was detected in the kidney with marked interstitial lesions. A minimal amount of PCV2 nucleic acid was detected in the kidney of one pig from farm C. A summary of the tissues studied and their ISH result is included in Table 1.

PCV2 DNA was amplified from paraffin-embedded tissues from three animals. After sequencing the 657 bp amplicons obtained, a BLAST analysis confirmed the PCV2 identity of the amplified products. The alignment of the PCV2 amplicons with a PCV1 strain (GenBank accession number Y09921) showed a 29% divergence, whereas sequences from PCV2 strains available in GenBank shared a 90–99% homology with the Philippine strains. The phylogenetic tree resulting from the alignment of the sequences corresponding to the amplified regions of 35 PCV2 isolates (3 from this study and 32 from sequences available in the GenBank and one corresponding to PCV1) is shown in Fig 4.

This report summarizes the clinical, pathological and laboratorial findings obtained from cases of PMWS in 4 Philippine pig herds. This disease has not yet been reported in this country. The diagnostic approach described herein took into consideration the simultaneous presence of PMWS suggestive clinical manifestations, typical microscopic lesions in lymphoid tissues and the presence of PCV2 within the lesions. These three criteria are, nowadays, considered of paramount importance in diagnosing PMWS [11]. Two major clusters were found in the PCV2 DNA sequences examined: a large one containing European, Asian and some American isolates (usually grouped according to the different countries), and a second cluster containing some other American (Canada), European (France and the Netherlands) and the Philippine strains from this study.

Larochelle et al., have recently reported similar results from the alignment of 70 strains of PCV2 [7]. An interesting finding in our study is that the sequences from Asian countries (China and Taiwan) all were grouped in the opposite PCV2 major branch when compared with the Philippine isolates. These data indicate that the three Philippine isolates are closely related to each other, and to other known PCV2 isolates worldwide, but at the same time, they form a distinguishable minor branch. It has been suggested recently that although the genome of PCV2 is generally stable among different isolates, PCV2 isolates from different geographical regions vary in their genomic sequences [6]; whether these genetic variations have clinical relevance or not, remains to be investigated.
REFERENCES