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First report of Porcine Circovirus 2 (PCV2) circulation in Ecuador

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Abstract

This report describes the first detection of Porcine Circovirus Type 2 (PCV2) from pigs in Ecuador. Samples were collected from 162 animals from November 2010 to March 2011: 25 sick animals from commercial swine herds and 137 from slaughterhouses. To detect the virus we used polymerase chain reaction (PCR) and two primer set. Our procedure revealed PCV2 DNA in 24 of 25 clinically affected pigs from farms and 77 of 137 pigs from slaughterhouses. The nucleotide sequence analysis of nine amplicons confirmed the PCV2 detection with at least a 95% homology compared with other PCV2 found in the GenBank database. Additionally, microscopic lesions in tissues of clinically affected pigs suggested porcine circovirus infection.

Keywords. Porcine Circovirus Type 2, PCR, DNA, Ecuador

Resumen

Este reporte es la primera identificación de Circovirus porcino tipo 2 (PCV2) en cerdos del Ecuador. Desde noviembre 2010 a Marzo 2011 se colectaron muestras de 162 animales: 25 animales enfermos provenientes de granjas comerciales y 137 a partir de camales. Para detectar ADN de PCV2 utilizamos la reacción en cadena de la polimeraza (PCR) con dos pares de cebadores. Se detectó positividad en 24 de los 25 cerdos analizados a partir de granjas comerciales y en 77 de los 137 animales provenientes de camales. El análisis de secuencias de ADN obtenidas a partir de nueve amplicones confirmó la detección de PCV2 con al menos el 95% de homología en relación a otras secuencias de PCV2 encontradas en la base de datos del GenBank. Adcionalmente las lesiones microscópicas en los tejidos de cerdos clínicamente afectados, fueron compatibles con infección por circovirus porcino.

Palabras Clave. Circovirus porcino tipo 2, PCR, ADN, Ecuador

Porcine Circovirus 2 (PCV2) is the etiologic agent of porcine circovirus diseases (PCVD) (Allan et al. 2000) and has a worldwide distribution. PCV2 targets and replicates in lymphoid tissues causing lymphoid depletion, enlarged lymph nodes, and histiocytic replacement in lymphoid tissues. It affects young and mature pigs. In young pigs ages 5-12 weeks it causes progressive weight loss, respiratory signs, lymph node hypertrophy, diarrhea, and jaundice [1–3]. In mature animals PCV2 causes: dermatitis and nephropathy syndrome, enteritis, porcine respiratory disease complex, and reproductive disorders [4–6].

Virus transmission occurs both horizontally and vertically; PCV2 has been detected in nasal, rectal, urinary, salivary, semen, ocular and tonsillar secretions, and transplacental transmission may cause reproductive disorders [2, 6–9]. Usually, dissemination of PCV2 in a herd is hard to control due to absence of signs, the variety of transmission routes, and long periods of viral shedding.

This work was performed in Ecuador, a South American country in which circovirus vaccine is not available because the lack of disease reports. We used two PCR complementary protocols (2 primer sets followed by nucleotide sequencing of amplicons) to detect PCV2 in Ecuadorian swine herds. From November 2010 to March 2011, 137 swine animals were sampled from slaughter houses distributed along the Ecuadorian territory and 25 from industrial swine operations (5-26 week old with

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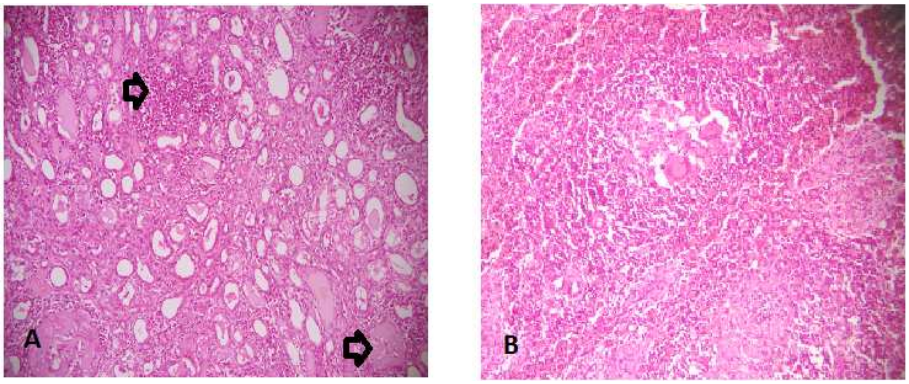

Figure 1: Histopathoplogy of swine tissues infected with circovirus. (A) Kidney (100X) arrows show enlarged hyalinized glomeruli and interstitial lymphohistiocytic inflammatory infiltration with proteinaceous casts accumulation of inflammatory cells (B) Spleen (100X) showing lymphoid depletion with lymphohistiocytic and granulomatous inflamatory lesion.

apparently PCVD signs). Lung, lymph nodes, spleen, kidney and liver were collected from each animal.

Lung, lymph nodes, spleen, kidney and liver samples were pooled and homogenized and submitted to DNA extraction following a previously published protocol [10]. For PCR we used two different PCR protocols that amplify ORF2; which encodes for major structural capsid proteins. The first PCR protocol used primers 1443f-1150r and amplified a 481pb fragment of ORF2; the second PCR protocol used primers Fa2-Ra2 amplifying a 476pb fragment located at nucleotides 828 to 1584 and of a region of ORF1 and ORF2) [1, 11]. A sample was considered positive for PCV2 when an amplicon of the right size was observed when using one of the two primer sets. Nine amplicons were sent to Functional Biosciences Inc. (Madison, Wisconsin, USA) for nucleotide sequencing. Nuceotide sequenses were analyzed with MEGA 5.0 (www.megasoftware.net) and compared to homologous sequences and vaccine sequences available in GenBank using BLAST (http://www.ncbi.nlm.nih.gov).

The two PCR protocols used were complementary. From 101 positive samples 70 amplified with both sets of primers; 19 amplified only with primers Fa2-Ra2 and 12 samples amplified only with primers 1443-150. Our results showed positivity in 24 of 25 swines from comercial heards, and in 77 from 137 swine tissues collected at slaughter houses. Sequences analysis confirmed PCV2 detection with less than 95% homology compared with other GenBank PCV2 sequences.

Additionally tissues were fixed with 10% formaldehyde pH 7,2 and submerged in paraffin. Paraffin blocks were cut in sections of 2 μm, stained with hematoxylin and eosin, and examined by microscopy at 100x. Histopathology was performed in kidney, spleen and liver samples collected from 25 animals (industrial swine operations). All animals had lesions suspicious of PCVD infection: regional or generalized linfadenopathy, weight lost, dermatitis, and respiratory signs such as dyspnea.

This study reports for the first time the presence of Porcine PCV-2 in Ecuadorian swine herds. The results of the PCR test indicated high infection rates (62%) which is in agreement with reports in other Latin-American countries such as Brazil (70%) and Colombia, (63-100%) [12, 13]. Nucleotide sequences from amplicons confirmed the circoviral DNA in all the amplicons analized (data not shown). All tissues from symptomatic animals showed suggestive histopathology: microscopic examination evidenced enlarged and hyalinized glomeruli and lymphohistiocytic multifocal infiltration in the renal interstice (Fig1A), lymphoid depletion in the spleen (Fig1B) and cholangio hepatitis (data not shown).

The PCR protocols used in this research provided the evidence needed by sanitary authorities to initiate a vaccination program in Ecuador. Vaccination against PCV2 is essential to control this viral infection and to prevent economic negative impact due to the absence of an effective treatment [14]. The use of commercial vaccines has proven to give significant protection, increasing average daily weight and diminishing mortality rates [14, 15].

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