Rev. Salud Anim. Vol. 31 No. 3 (2009): 176-179

Short communication

PORCINE PARVOVIRUS INFECTIONS IN CUBA

Heidy Díaz de Arce, L.J. Pérez , Sara Castell, María I. Percedo, Patricia Domínguez, María T. Frías

Centro Nacional de Sanidad Agropecuaria (CENSA), Apartado 10, San José de las Lajas, La Habana, Cuba. E-mail: heidy@censa.edu.cu

ABSTRACT: **To obtain information about the porcine parvovirus (PPV) infection status of pigs in Cuba, tissue samples collected from ill pigs were evaluated using polymerase chain reaction (PCR). The PCR analysis showed the presence of PPV in 12 of the 34 (35.3%) field clinical samples assessed. Pigs from three of the seven swine herds of six different geographic regions were detected to be positive for PPV. A field PPV strain was isolated for the first time in the country. A swine herd showed 57 of 60 sera sampled (95%) positive for PPV specific antibodies by a commercial ELISA. This study is the first report of PPV infecting pigs in Cuban swine herds.**

(Key words: porcine parvovirus; polymerase chain reaction)

INFECCIONES POR PARVOVIRUS PORCINO EN CUBA

RESUMEN: **Con el fin de conocer el estatus de infección por parvovirus porcino (PPV) de cerdos en Cuba, se colectaron muestras de órganos de cerdos para su evaluación por reacción en cadena de la polimerasa (PCR). El análisis de PCR mostró la presencia de infecciones por PPV en 12 de las 34 (35.3%) muestras clínicas de campo evaluadas. Se detectaron como positivos a PPV tres rebaños porcinos del total de siete evaluados pertenecientes a seis diferentes regiones geográficas del país. Se aisló PPV de una muestra clínica de campo por primera vez en el país. Un rebaño porcino mostró 57 de 60 muestras (95%) como positivas para anticuerpos a PPV por un ELISA comercial. Este estudio constituye el primer reporte de PPV infectando cerdos en rebaños porcinos en Cuba.**

(Palabras clave: parvovirus porcino; reacción en cadena de la polimerasa)

Porcine parvovirus (PPV) is an autonomous parvovirus belonging to the genus parvovirus, subfamily *Parvovirinae*, family *Parvoviridae*; it is the major causative virus in a reproductive failure syndrome in swine characterized by stillbirths, mummified fetuses, early embryonic death, and infertility (1). Although acute infection of postnatal, non-pregnant pigs is usually subclinical, PPV has also been linked to skin lesion occurrence in piglets (2), interstitial nephritis in slaughter-aged pigs (3), and non-suppurative myocarditis in lactating piglets (4). PPV has been reported to occur worldwide with variable prevalence rates (1).

Recently, PPV has gained importance as an agent able to enhance the effects of porcine circovirus type 2 (PCV2) infection in the clinical course of postweaning multisystemic wasting syndrome (5, 6), an economically significant disease worldwide (7) and as emergent pathogen because genetic variability have been reported (8, 9, 10) and the new variant generated may have important consequences in the epidemiology and the pathogenicity of PPV infection as well as the effectiveness of vaccination against PPV (11). Because PPV causes reproductive failure and these other clinical and pathological conditions, 53 vaccines to this virus are marketed worldwide (1). In Cuba, the PPV infection status of pigs has not been investigated and there is no field strain available. Therefore, the aim of the current study was to obtain information about the PPV infection status of pigs in swine herds in the country.

Samples of the spleen, tonsil, lymph nodes and kidneys were collected from 33 pigs in seven swine herds from six Cuban provinces (Herd 1-Ciego de Ávila/Herd 2-Ciego de Ávila/Herd 3-Villa Clara/Herd 4-Pinar del Río/Herd 5-La Habana/Herd 6 F-Sancti Spiritus/Herd 7-Cienfuegos) during 2007 and 2008 and pooled. The animals selected showed clinical signs that included respiratory and wasting disorders as well as presumptive CSFV diagnosis.

Total DNA was extracted from 100 µl of each sample (10% tissue homogenate), with Wizard® Genomic DNA Purification Kit, (*Promega, Madison, WI, USA*) following manufacturer instructions.

In order to determine the extent of PPV infections in diseased pigs and to confirm the viral isolation in PK15A cell line, a seminested polymerase chain reaction (PCR) assay was carried out as described by (12). The primer pairs targeted an amplicon of 195 bp. Briefly, the amplification reaction was carried out in a volume of 50 µl containing 2 µl of DNA sample, 1x GoTaq® Flexi DNA Polymerase (Promega, Madison, WI, USA) [200uM of each dNTP, 1.5mM $MgCl_2$ (pH 8.5)] and 1µM of each primer. The PCR reaction was done under the conditions described by (12) in a thermal cycler (Eppendorf Mastercycler). The amplicons were visualized by electrophoresis on 2.0% agarose gel in TBE buffer (90mM Tris–borate, 2 mM EDTA) ethidium bromide stained. In addition, for detecting the possible presence of porcine circovirus type 2 (PCV2) and/or classical swine fever virus (CSFV) genome in the tissue samples, PCR assays were performed as described by (13 and 14), respectively.

The isolation of PPV from 10% (w/v) tissue homogenate suspension was performed in continuous pig kidney cells (PK15) which are known to support the replication of this virus. Tissue homogenate suspension from E6 sample selected to be PPV2 positive were inoculated onto cell cultures at 50–75% confluency using 200 µl/well in 2 mL growth medium (EMEM supplemented with 5% fetal calf serum) in 24-well multidish plates (Nunc, Denmark) and cultivated for two passages. After inoculation the plates were incubated at 37ºC in a 5% CO2–air atmosphere. The cells were observed for cytopathic effect (CPE) daily and one plate was after fixation stained with May-Grunwald Giemsa to observe viral nuclear inclusion bodies. Briefly, the medium was removed from the monolayer and washed two times with warm PBS, the monolayer was fixed with methanol for 5 minutes and stained with undiluted May-Grunwald solution for 5 minutes. Then the supernatant was discarded and Giemsa stock diluted 1:10 with tap water was applied for 15 minutes. Finally, the monolayer was washed with tap water and air dried.

A commercial ELISA (*Ceditest® PPV Strip Kit, Cedi-Diagnostics B.V., Lelystad, Netherlands*) for detection of antibodies against PPV was performed following the manufacturer recommendations on 60 sera from a swine herd located in *La Habana* province. The sample was representative of all age categories.

The PCR analysis showed the presence of PPV in 12 of the 34 (35.3%) field clinical samples assessed. In positive samples a PCR product of the expected size (195 pb) was obtained (Fig. 1).

Ten of the 12 PPV positive samples (83.3%) showed a concurrent infection with PCV2 and 8 of 12 PPV positive samples (66.7%) exhibited a concomitant infection with CSFV. Furthermore, seven tissue samples yielded PCV2, PPV and CSFV positive results showing that the three viruses were even found simultaneously infecting the same pig. Also, pigs infected with PPV were detected in three herds (Herd D/Pinar del Río; Herd E/La Habana and Herd G/ Cienfuegos) from the six regions examined.

In order to substantiate the PCR findings and with the purpose of possess our own PPV isolate for further studies, PPV from a sample selected to be PPV positive and PCV2/CSFV negative for PCR, was isolated in PK15A cell line.

During the first passage no CPE was observed. A second blind passage was performed and after 24 hours of infection the cells started to round up and subsequently become pyknotic and finally desintegrated. Once the CPE was observed the cells were subcultured and stained to demonstrate the PPV characteristic intranuclear inclusions (Fig. 1A-B). The field isolate was identified as PPV by the PPV specific PCR assay (Fig. 1C)

From the sera evaluated from the swine herd 5 located in the province La Habana in which PPV infections were detected as positive by PCR, 57 of 60 sera sampled (95%) yielded positive results. The three negative sera were obtained from a piglet, a breeding pig and a gilt, respectively.

In this study pigs with respiratory, wasting disorders as well as presumptive CSFV diagnosis were selected looking forward to detect not only potential PPV infections but also their possible association with PCV2 and CSFV because of PCV2/PPV co-infection has been reported as trigger factor of PMWS (5,15), and is a very likely circumstance in the field. Otherwise, classical swine fever is reported to be the most

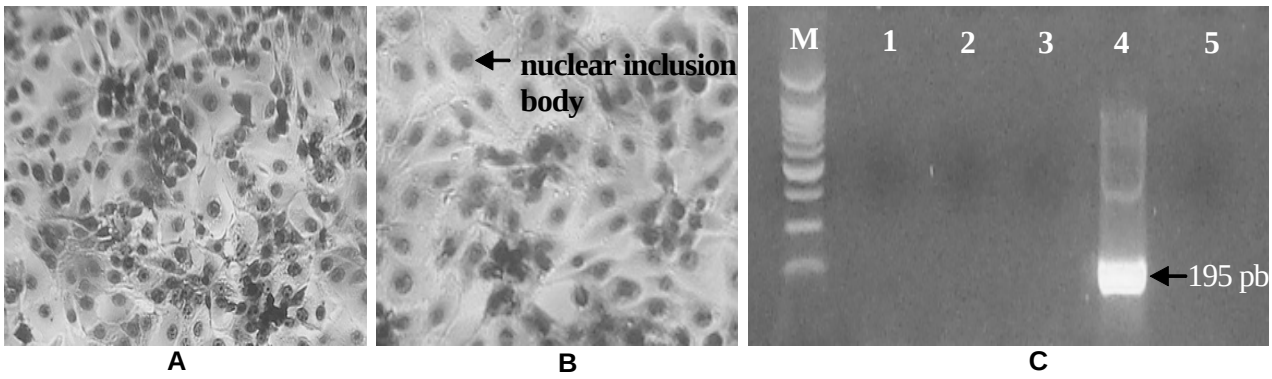

FIGURE 1. (A) Cell cultures non-infected (B) Cell cultures infected with PPV. Cytopathic effect, porcine kidney cell line (PK15), 24 hours after infection (May-Grunwald-Giemsa; x100). (C) PCR test for PPV detection M-molecular weight marker 100pb (*Promega*). Lane 1- nuclease free water (*Promega*); lane 2-negative control extraction (growth medium); lane 3- first passage, lane 4-second passage, lane 5-non-inoculated cells./ *(A) Cultivos celulares no infectados (B) Cultivos celulares infectados con parvovirus porcino. Efecto citopático, línea de células de riñón porcino (PK15), 24 horas después de la infección (May-Grunwald-Giemsa; x100). (C) Ensayo de PCR para la detección de parvovirus porcino M-marcador de peso molecular 100pb (**Promega**). carril 1-agua libre de nucleasas (**Promega**); carril 2-control negativo de extracción (medio de crecimiento); carril 3-primer pase, carril 4-segundo pase, carril 5-células no inoculadas.*

important disease in pigs in the country (16). This exploratory study reveals not only the presence of PPV but also PPV in concomitant infections with PCV2 which has been recently reported (17) and CSFV in pigs with respiratory, wasting disorders and presumptive CSFV diagnosis.

Our results suggest that PPV infections might be common in Cuban swine herds and could be a trigger factor in PMWS development. However, further studies are required to estimate the prevalence of PCV2 infections in the pig population and to determine the role of PPV infections in potential PMWS in pigs. These results could have been expected because PPV is nowadays highly prevalent in swine herds worldwide (1).

The isolation of PPV is reported to be somewhat difficult because several serial passages of the virus (18) may be necessary and isolation cannot be achieved for all PPV strains (19). The use of cell cultures at 50–75% confluency provided extensive opportunity for virus isolation due to the fact that PPV replication is enhanced by infection of mitotically active cultures (1). Many cells in such cultures are in S phase of their cell cycle, wherein the DNA polymerases of cell origin needed for viral replication are available (1). Besides, because replication of PPV is affected by mitotic activity, the effect of the serum contained in the growth medium on the cells is also especially important.

We observed the presence of the characteristic intranuclear inclusions caused by PPV replication after appropriate staining after the first 24 hours of cell infection even when cytopathic changes were still vague as reported by (18).

In this study, we tested the presence of specific antibodies against PPV in pigs from one herd in which we previously found PPV positive samples by PCR. The high percentage of positive samples (95%) suggests that PPV infections are widespread in this herd. Vaccines against PPV are not applied in the country. Therefore, the presence of antibodies in pigs reveals virus circulating in the field and this virus is expected to be mantained in the premises because the virion is extremely resistant to inactivation. It is stable between pH 3 and 9 and at 56ºC for 60 min. (20).

This preliminary study described the presence of PPV in swine herds in Cuba for the first time and must be followed for further studies about genetic diversity of PPV field strains, as well as the confirmation of the potential influence that PPV may have on reproductive parameters in pigs, its distribution and presentation in the field.

REFERENCES

1. Mengeling WL. Porcine parvovirus. In: Diseases of Swine, 9th Edition. Edited by B.E. Straw, J.J. Zimmerman, S. D'Allaire and D.J. Taylor. *Blackwell Publishing*, Ames, IA., 2006.

2. Lager KM, Mengeling WL. Porcine parvovirus associated with cutaneous lesions in piglets. *J Vet Diagn Invest.* 1994;6:357-359.

3. Drolet R, D'Allaire S, Larochelle R, Magar R, Ribotta M, Higgins R. Infectious agents identified in pigs with multifocal interstitial nephritis at slaughter. *Vet Rec.* 2002;150:139-143.

4. Bolt DM, Hani H, Muller E, Waldvogel AS. Non-suppurative myocarditis in piglets associated with porcine parvovirus infection. *J Comp Pathol* 1997;1172:107-118.

5. Allan GM, Kennedy S, McNeilly F, Foster JC, Ellis JA, Krakowka S, *et al*. Experimental reproduction of severe wasting disease by co-infection of pigs with porcine circovirus and porcine parvovirus. *J Comp Pathol.* 1999;121:1-11.

6. Krakowka S, Ellis JA, Meehan B, Kennedy S, McNeilly F, Allan G. Viral wasting syndrome of swine: experimental reproduction of postweaning multisystemic wasting syndrome in gnotobiotic swine by coinfection with porcine circovirus 2 and porcine parvovirus. *Vet Pathol.* 2000;37:54-263.

7. Segalés J, Allan GM, Domingo M. Porcine circovirus diseases. *Anim Health Res Rev*. 2005;6:119-142.

8. Shangjin C, Cortey M, Segalés J. Phylogeny and evolution of the NS1 and VP1/VP2 gene sequences from porcine parvovirus. *Virus Research*. 2009;140:209-215.

9. Soares M, Cortez A, Heinemann MB, Sakamoto SM, Martins VG, Bacci Jr M, *et al*. Genetic variability of porcine parvovirus isolates revealed by analysis of partial sequences of the structural coding gene VP2. *J Gen Virol.* 2003;84:1505-1515.

10. Zimmermann P, Ritzmann M, Selbitz HJ, Heinritzi K, Truyen U. VP1 sequences of German porcine parvovirus isolates define two genetic lineages. *J Gen Virol.* 2006;87:495-501.

11. Zeeuw EJ, Leinecker N, Herwig V, Selbitz HJ, Truyen, U. Study of the virulence and cross-neutralization capability of recent porcine parvovirus field isolates and vaccine viruses in experimentally infected pregnant gilts. *J Gen Virol.* 2007;88:420-427.

12. Kim J, Han DU, Choi C, Chae C. Simultaneous Detection and Differentiation between Porcine Circovirus and Porcine Parvovirus in Boar Semen by Multiplex Seminested Polymerase Chain Reaction. *J Vet Med Sci.* 2003;65(6):741-744.

13. Sandvik T, Grierson S, King DP, Spencer Y, Banks M, Drew T. Detection and genetic typing of porcine circovirus DNA isolated from archived paraffin embedded pig tissues. *Comp. Virol. Proceedings - ss DNA Viruses of Plants, Birds, Pigs and Primates, Saint-Malo,* 24-27 September, 2001.

14. Díaz de Arce H, Ganges L, Barrera M, Sobrino F, Frías MT, Núñez JI. An RT-PCR assay for the specific detection of classical swine fever virus in clinical samples. *Virus Research.* 2005;112:123-131.

15. Ellis J, Krakowka S, Lairmore M, Haines D, Bratanich A, Clark E, *et al.* Reproduction of lesions of postweaning multisystemic wasting syndrome in gnotobiotic piglets. *J Vet Diagn Invest*. 1999;11(1):3-14.

16. Díaz de Arce H, Ganges L, Barrera M, Naranjo D, Sobrino F, Frías MT, et al. Origin and evolution of viruses causing classical swine fever in Cuba. *Virus Research* 2005;112:123-131.

17. Pérez LJ, Díaz de Arce H, Domínguez P, Percedo MI, Frías MT. First report of porcine circovirus type 2 infections in Cuba. Res Vet Sci. 2009 in press.

18. Cartwright SF, Lucas M, Huck HA. A small haemagglutinanting porcine DNA virus. I. Isolation and properties. *J Comp Pathol.* 1969;79:371-377.

19. Kim J, Chae C. A comparison of virus isolation, polymerase chain reaction, immunohistochemistry, and in situ hybridization for the detection of porcine circovirus 2 and porcine parvovirus in experimentally and naturally coinfected pigs. *J Vet Diagn Invest.* 2004;16(1):45-50.

20. Berns KI, Bergoin M, Bloom M, Lederman M, Muzyczka N, Siegl G, et al. Fifth Report of International Committee on Taxonomy of Viruses. In: Murphy FA, Fauquet CM, Bishop DHL, Ghabrial SA, Jarvis AW, Martelli G.P, Mayo MA, Summers MD. (Eds.), Virus Taxonomy. *Arch. Virol.* Suppl. 10, pp. 166-178, 1994.

(Recibido 21-9-2009; Aceptado 22-11-2009)