Retrospective serological survey of Porcine circovirus-2 infection in Mexico

Humberto Ramírez-Mendoza, Héctor Castillo-Juárez, Jesús Hernández, Pablo Correa, Joaquim Segalés

Abstract

Postweaning multisystemic wasting syndrome (PMWS) is considered a multifactorial emerging disease of which Porcine circovirus-2 (PCV-2) is the necessary infectious cause. However, retrospective studies have shown that PMWS is not a new disease and that PCV-2 has been circulating in pig farms for years. Most of these studies were performed in Europe and Asia; only a few were performed in North or South America.

A PCV-2 retrospective serological survey was carried out with 659 serum samples collected from pigs in Mexico between 1972 and 2000. Serological analyses were performed with an immunoperoxidase monolayer assay (IPMA). The overall prevalence of PCV-2 antibodies was 59% (387/659); the prevalence was 27% (24/90) for the period from 1972–1979; 44% (74/169) from 1980–1989, and 72% (289/400) from 1990–2000. Antibodies to PCV-2 were detected in at least 1 pig from all tested years since 1973. This study shows evidence of enzootic PCV-2 infection in Mexico for many years before the first description of PMWS in the country (in 2001), further supporting results obtained in other parts of the world. To date, this study provides the earliest evidence of PCV-2 infection in the North and South American continents.

Résumé

Le syndrome de dépérissement multi-systémique en post-sevrage (PMWS) est considéré comme étant une maladie multifactorielle en émergence pour lequel le circovirus porcin de type 2 (PCV-2) est la cause infectieuse essentielle. Toutefois, des études rétrospectives ont démontré que le PMWS n'est pas une maladie nouvelle et que le PCV-2 est en circulation sur les fermes porcines depuis plusieurs années. La plupart de ces études ont été effectuées en Europe et en Asie, et seulement quelques une en Amérique du Nord et en Amérique du Sud.

Une étude sérologique rétrospective pour PCV-2 a été effectuée sur 659 échantillons de sérum prélevés chez des porcs au Mexique entre 1972 et 2000. Les analyses sérologiques ont été effectuées par épreuve d'immunoperoxydase en monocouche (IPMA). La prévalence totale d'anticorps envers PCV-2 était de 59 % (387/659); la prévalence était de 27 % (24/90) pour la période de 1972–1979; 44 % (74/169) de 1980–1989, et 72 % (289/400) pour 1990–2000. Des anticorps envers PCV-2 ont été détectés chez au moins 1 porc pour toutes les années testées depuis 1973. Cette étude présente des évidences de la présence d'infection enzootique par PCV-2 au Mexique plusieurs années avant la première description de PMWS dans ce pays (en 2001), supportant ainsi des résultats obtenus dans d'autres parties du globe. La présente étude fournie, à ce jour, les premières évidences d'infection par PCV-2 en Amérique du Nord et en Amérique du Sud.

(Traduit par Docteur Serge Messier)

Porcine circovirus-2 (PCV-2), a single-stranded DNA virus that infects pigs, is classified in the family *Circoviridae* and genus *Circovirus*. This virus was initially linked to an emerging clinical and pathological condition of pigs from Canada (1,2) that was named postweaning multisystemic wasting syndrome (PMWS). Now it is known that PMWS is a multifactorial disease in which PCV-2 is the necessary infectious agent but is usually not sufficient to trigger the clinical condition (3). The virus also is known to be ubiquitous among domestic and wild swine (4–9). Moreover, evidence of PCV-2 infection was established as early as 1969 in Europe (10) and 1985 in North America (11). Retrospective studies have provided evidence of the existence of PMWS a decade before the disease was first described (10–15). Thus, it can be concluded that PMWS is not a new disease and that PCV-2 has been circulating in pig farms for years.

In Mexico, PMWS has been reported in domestic swine (16) and is considered to be of economic importance, yet no studies on its prevalence and associated production losses had been performed. Moreover, it is believed that PCV-2 is ubiquitous in pigs in Mexico, according to results for backyard pigs in Mexico City (4), as has been described in other countries (3). However, no retrospective studies on PCV-2 infection and PMWS had been performed in Mexico. The aim of the research described herein was to study PCV-2 infection by testing of serum samples collected from Mexican pigs between 1972 and 2000.

The 659 samples corresponded to 22 unrelated farms, and the pigs were of various ages. The samples, stored at −20°C, at the Centro Nacional de Investigación Disciplinaria en Microbiología (CENID-Microbiología), in Mexico City, had originally been collected to

Departamento de Microbiología e Inmunología, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, México (Ramírez-Mendoza); Departamento de Producción Agrícola y Animal, Universidad Autónoma Metropolitana, Unidad Xochimilco, México (Castillo-Juárez); Laboratorio de Inmunología, Centro de Investigación en Alimentación y Desarrollo, Asociación Civil Hermosillo, Sonora, México (Hernández); Centro Nacional de Investigación Disciplinaria en Microbiología Animal; Secretaría de Agricultura, Ganadería Desarrollo Rural, Pesca y Alimentación, México (Correa); Centre de Recerca en Sanitat Animal, Departament de Sanitat i d'Anatomia Animals, Facultat de Veterinària, Universitat Autonoma de Barcelona, Bellaterra, Barcelona, Spain (Segalés).

Address all correspondence to Dr. Humberto Ramírez-Mendoza; telephone: +52-55-56225896; fax: +52-55-56225971; e-mail: betosram@yahoo.es

Received March 5, 2007. Accepted January 30, 2008.

Table I. Results of testing serum samples from Mexican pigs for antibodies to Porcine circovirus-2 (PCV-2)

Year	Total number of samples	Number of samples; PCV-2 antibody titer[a]				Seroprevalence (%)
		Negative	Low	Intermediate	High	
1972	15	15	0	0	0	0
1973	31	12	19	0	0	61
1977	24	20	3	1	0	17
1979	20	19	0	1	0	5
1980	19	18	1	0	0	5
1981	11	1	0	5	5	91
1983	10	1	8	1	0	90
1984	1	0	1	0	0	100
1985	6	1	4	0	1	83
1987	24	9	11	2	2	62
1988	51	39	7	3	2	24
1989	47	26	11	10	0	45
1990	3	3	0	0	0	0
1991	48	6	8	24	10	88
1992	32	21	5	4	2	34
1994	32	10	12	10	0	69
1995	32	6	5	17	4	81
1996	33	0	1	17	15	100
1997	32	1	13	14	4	97
1998	119	47	21	36	15	60
1999	37	16	6	11	4	57
2000	32	1	8	19	4	97
Total	659	272	144	175	68	59

[a] Low — titer of 1:20 to 1:80; intermediate — titer of 1:320 to 1:1280; high — titer of 1:5120 or greater.

monitor the results of vaccination and eradication programs against classical swine fever throughout the country. Samples were available for all years in the 1972–2000 period, except for 1974–1976, 1978, 1982, 1986, and 1993.

Antibodies to PCV-2 were detected by an immunoperoxidase monolayer assay (IPMA) (17). Briefly, trypsinized PK-15 cells free of PCV-2 and Porcine circovirus-1 (PCV-1) were seeded in 96-well plates and incubated for 5 d at 37°C in 5% CO_2. The cells were then fixed with methanol containing 1% hydrogen peroxide for 5 min at room temperature. After 3 washes with phosphate-buffered saline (PBS) containing 0.05% Tween 80 (PBS-T), the cells were incubated for 1 h with serum samples diluted 2-fold from 1:20 to 1:20 480 in PBS-T with 1% bovine serum albumin (BSA). Serum 211-P-PCRV (VMRD, Pullman, Washington, USA), a porcine hyperimmune serum against PCV-2 was used as a positive control with a known PCV-2 antibody titer of 1:1280. The plates or dishes were washed again with PBS-T and, after the addition of peroxidase-labelled protein A (0.6 µg/mL) in PBS-T-BSA, were incubated for 1 h at 37°C. Finally, the plates were washed with PBS-T, and aminoethylcarbazole was added to visualize the reaction. Porcine serum 211-N-PCRV (VMRD) was used as a negative control. With IPMA, which is widely cited in literature (3), there is apparently a cross-reaction between PCV-1 and PCV-2 antibodies (17).

The serological results were grouped as negative or as and positive with a low titer (1:20 to 1:80), intermediate titer (1:320 to 1:1280), or high titer (1:5120 or greater). The data were grouped, and the average prevalence in the 3 decades (1972–1979, 1980–1989, and 1990–2000) was compared with a chi-squared test under the hypothesis that the prevalence did not change over time, compared with the average for the complete data set. We used the arcsine transformation of the percentages in a linear regression with no intercept to test for changes in seroprevalence over time. Statistical analyses were performed with the use of SAS, version 9.1 (SAS Institute, Cary, North Carolina, USA).

Table I presents the grouped results and overall PCV-2 serological prevalence for each year and the entire study period. The number of PCV-2-positive samples was 24 out of 90 (27%) for the period from 1972–1979, 74 out of 169 (44%) for 1980–1989, 289 out of 400 (72%) for 1990–2000, and 387 out of 659 (59%) for the entire study period. Significant differences ($P < 0.0001$) in seroprevalence among the 3 decades were observed ($X^2 = 82.55$ with 2 degrees of freedom). The linear regression coefficient of the seroprevalence increase across the years was positive (adjusted $R^2 = 74.27\%$, $P < 0.0001$) and significant.

The data compiled in this retrospective study indicate that antibodies to PCV-2 have been present in the Mexican swine population

at least since 1973, 28 years before the first description of PMWS in the country (16). To date, this study provides the earliest evidence of PCV-2 infection in the American continents. The data agree with those from serological retrospective studies performed in other countries, in which PCV-2 antibodies were also detected before knowledge of the existence of PMWS — as early as 1969 in Belgium (10), 1973 in Northern Ireland (18), and 1985 in Canada (11) and Spain (13).

An interesting contribution of the present study is the time-period comparisons that showed the increase in PCV-2 seroprevalence over time. This situation might reflect a significant increase in virus circulation among Mexican farms during the studied period, but specific yearly results (and the fact that the exact ages of the studied pigs were unknown) prevent us from establishing this conclusion. In fact, a huge variation in PCV-2 seroprevalence was observed among each range of years; for instance, 1973 had a very high rate compared with the rest of the years within the 1972–1979 study period. This suggests a year effect or, more specifically, farm or age effects within a year. It is well known that PCV-2 seroprevalence may significantly vary among age groups within a farm and between farms (6,8). The overall increase in PCV-2 titer across the time periods is probably linked to an increase in virus dissemination or infectious pressure, or both, over time. The results allow one to speculate that an increased PCV-2 infectious pressure over time could have resulted in the emergence of PMWS by 2000–2001 or even before.

From the results of this study, it is possible to hypothesize that 1973 could be the year of PCV-2 introduction into the Mexican swine population, since no pigs had positive results in 1972. However, several facts prevent us from coming to this conclusion. First, a low number of animals were tested in 1972. Second, the original serum samples were selected for other study purposes (monitoring classical swine fever vaccination and eradication programs). And, third, most of the breeding pig population in Mexico during the early 1970s was imported from the United States, suggesting potential introduction of the virus with such genetic stock. If this last hypothesis is correct, it would not modify the hypothesis that our results represent the oldest evidence of PCV-2 infection in the American continents. Older samples would need to be tested to determine if 1973 was really the year of introduction of PCV-2 into Mexico.

It would be interesting to know if clinical signs compatible with PMWS were eventually observed in the studied PCV-2 positive pigs. However, given the main purpose of the sample collection and the lack of specificity of PMWS clinical signs (3), it is not possible to establish the existence of PMWS in Mexico during the period from 1973–2000. The proper way to assess the presence of the disease during said period would be by retrospective histopathological analyses of formalin-fixed, paraffin-embedded lymphoid tissues from pigs suspected of having the disease.

The serological technique used in this study may detect antibodies elicited by PCV-1 infection; titers of antibodies to PCV-2 and PCV-1 are usually strongly associated, the former being much higher than the latter (17). In fact, in another study using IPMA (19), only pigs with high titers of antibody to PCV-2 showed reactions indicating antibody against PCV-1. Moreover, the serological data are supported by virus detection in pigs: rates of infection with PCV-2 are high, reflecting this virus's ubiquity (3), whereas rates of infection with PCV-1 are very low (20). Therefore, although infection with PCV-1 might not be excluded as the potential cause of seropositivity in a low proportion of pigs, the available data indicate that positive IPMA results are really attributable to PCV-2 and hardly ever to PCV-1.

The results of the present study confirm that PCV-2 is a relatively old virus that was circulating among Mexican pigs for several decades before the first description of PMWS (16). The findings represent the earliest evidence of PCV-2 infection in pigs in the Americas. Although the results do not represent the seroprevalence of PCV-2 in all of Mexico (only 22 farms were tested), they suggest that PCV-2 infection is probably widely distributed among the commercial farms of this country, as has been assessed from backyard pigs in Mexico City (4).

Acknowledgments

This study was financed with funds from the National Autonomous University of Mexico (PAPIIT IN-226105-3) and from the Mexican government (SAGARPA-CONACYT 2004-C01-61). We are thankful to Marcia Castillo-Mendoza for her comments on the manuscript.

References

1. Clark EG. Post-weaning multisystemic wasting syndrome. Proc Am Assoc Swine Pract 1997;28:499–501.
2. Harding JCS, Clark EG. Recognizing and diagnosing postweaning multisystemic wasting syndrome (PMWS). J Swine Health Prod 1997;5:201–203.
3. Segalés J, Allan GM, Domingo M. Porcine circovirus diseases. Anim Health Res Rev 2005;6:119–142.
4. Ramirez-Mendoza H, Martinez C, Mercado C, Castillo-Juárez H, Hernández J, Segalés J. Porcine circovirus type 2 antibody detection in backyard pigs from Mexico City. Res Vet Sci 2007; 83:130–132. Epub 2006 Dec 8.
5. Vicente J, Segalés J, Höfle U, et al. Epidemiological study on porcine circovirus type 2 (PCV-2) infection in the European wild boar *(Sus scrofa)*. Vet Res 2004;35:243–253.
6. Larochelle R, Magar R, D'Allaire S. Comparative serologic and virologic study of commercial swine herds with and without postweaning multisystemic wasting syndrome. Can J Vet Res 2003;67:114–120.
7. Rose N, Larour G, Le Diguerher G, et al. Risk factors for porcine post-weaning multisystemic wasting syndrome (PMWS) in 149 French farrow-to-finish herds. Prev Vet Med 2003;61:209–225.
8. Sibila M, Calsamiglia M, Segalés J, et al. Use of a polymerase chain reaction assay and an ELISA to monitor porcine circovirus type 2 infection in pigs from farms with and without postweaning multisystemic wasting syndrome. Am J Vet Res 2004;65:88-92.
9. López-Soria S, Segalés J, Rose N, et al. An exploratory study on risk factors for postweaning multisystemic wasting syndrome (PMWS) in Spain. Prev Vet Med 2005;69:97–107.
10. Sánchez R, Nauwynck H, Pensaert M. Serological survey of porcine circovirus 2 antibodies in domestic and feral pig populations in Belgium. In: Proceedings of the ssDNA Viruses of Plants,

Birds, Pigs and Primates (ESVV) Meeting, 2001 Sept. 24–27; Saint-Malo, France. Ploufragan, France: AFSSA, 200l.
11. Magar R, Müller P, Larochelle R. Retrospective serological survey of antibodies to porcine circovirus type 1 and type 2. Can J Vet Res 2000;64:184–186.
12. Mori M, Sato K, Akachi S, Asahi S, Taniguchi S, Narita M. Retrospective study of porcine circovirus 2 infection in Japan: seven cases in 1989. Vet Pathol 2000;37:667–669.
13. Rodríguez-Arrioja GM, Segalés J, Rosell C, et al. Retrospective study on porcine circovirus type 2 infection in pigs from 1985 to 1997 in Spain. J Vet Med B Infect Dis Vet Public Health 2003;50:99–101.
14. Grierson SS, King DP, Sandvik T, et al. Detection and genetic typing of type 2 porcine circoviruses in archived pig tissues from the UK. Arch Virol 2004;149:1171–1183.
15. Staebler S, Sydler T, Buergi E, et al. PMWS: An emerging disease identified in archived porcine tissues. Vet J 2005;170:132–134.
16. Trujano M, Iglesias G, Segalés J, Palacios JM. PCV-2 from emaciated pigs in Mexico. Vet Rec 2001;148:792.
17. Rodríguez-Arrioja GM, Segalés J, Balasch M, et al. Serum antibodies to porcine circovirus type 1 and type 2 in pigs with and without PMWS. Vet Rec 2000;146:762–764.
18. Walker IW, Konoby CA, Jewhurst VA, et al. Development and application of a competitive enzyme-linked immunosorbent assay for the detection of serum antibodies to porcine circovirus type 2. J Vet Diagn Invest 2000;12:400–405.
19. Ellis J, Hassard L, Clark E, et al. Isolation of circovirus from lesions of pigs with postweaning multisystemic wasting syndrome. Can Vet J 1998;39:44–51.
20. Calsamiglia M, Segalés J, Quintana J, Rosell C, Domingo M. Detection of porcine circovirus types 1 and 2 in serum and tissue samples of pigs with and without postweaning multisystemic wasting syndrome. J Clin Microbiol 2002;40:1848–1850.