Retrospective serological survey of antibodies to porcine circovirus type 1 and type 2

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Abstract

A retrospective serological survey was performed to determine the presence of antibodies to porcine circovirus type 1 (PCV1) and porcine circovirus type 2 (PCV2) in serum samples collected from sows at slaughterhouses in Canada in 1985, 1989, and 1997. Each serum sample was tested by indirect immunofluorescence on PCV-free PK15 cells, on PCV1-infected PK15 cells and on PCV2-infected PK15 cells. For the 3 years studied, sera positive to PCV1 and PCV2 were identified and the number of sera positive for PCV2 was greater than the number of sera positive for PCV1. The results indicated 1) that PCV2 appears to be the main PCV type circulating in the Canadian pig population, 2) that PCV2 had been circulating in the Canadian pig population at least 10 years before the postweaning multisystemic wasting syndrome (PMWS) was reported, and 3) that serological evaluation using PCV1 underestimates the seroprevalence of PCV2.

Résumé

Une étude sérologique rétrospective, visant à évaluer la présence d'anticorps envers le circovirus porcin type 1 (CVP1) et le circovirus porcin type 2 (CVP2) a été réalisée à partir de sérums de truies prélevés dans des abattoirs canadiens en 1985, 1989 et 1997. Chaque échantillon de sérum a été testé par immunfluorescence indirecte sur des cellules PK15 exemptes de CVP, sur des cellules PK15 infectées de CVP1 ainsi que sur des cellules PK15 infectées de CVP2. Pour les trois années considérées dans l'étude, des sérums positifs au CVP1 et au CVP2 ont été identifiés et le nombre de sérums positifs au CVP2 était supérieur à celui de sérums positifs au CVP1. Les résultats ont indiqué que 1) le CVP2 semble être le principal type de CVP circulant dans la population porcine canadienne, 2) le CVP2 circulait dans la population porcine canadienne au moins 10 ans avant le signalement du syndrome de dépérissement en post-sevrage et 3) l'évaluation sérologique utilisant le CVP1 comme antigène sous-estime la séroprévalence du CVP2.

(Traduit par les auteurs)

Porcine circovirus (PCV) is a small, non-enveloped, single-stranded DNA virus classified in the family Circoviridae. The virus was originally identified as a contaminant in the porcine kidney cell line, PK15 (1), and was found to be non-pathogenic when experimentally transmitted to pigs (2,3). Serological studies performed in different countries demonstrated that the virus is quite prevalent in the swine population (2,4–6). In 1997, a new pig syndrome, the postweaning multisystemic wasting syndrome (PMWS), was reported in western Canada and a PCV was found to be associated with this new condition (7–9). Similar syndromes have also been recently reported in France, the United States, Spain, Ireland, and Denmark (10–13). The PCV reported involved in cases of PMWS has been shown to be antigenically and genomically different from the PCV PK15 cell contaminant (9,12,14,15), and the term PCV type 2 (PCV2) was suggested to differentiate this putative pathogenic PCV from the non-pathogenic PK15 cell contaminant PCV, now referred to as PCV type 1 (PCV1) (15,16). Recently, field studies have demonstrated that PCV2, rather than PCV1, appears to be the main PCV circulating in pigs, and that PCV2 is not always associated with the clinical signs or the typical histological lesions reported for PMWS (17,18). Retrospective necropsy data has indicated that PMWS was present as early as 1991 in Western Canada (7). More recently, PCV2 was demonstrated by differential multiplex polymerase chain reaction from a field case in Québec dating back to 1994, several years before the identification of PMWS in this province (17). These latter findings, and the fact that past serological studies have essentially been performed using PCV from persistently infected PK15 cells (now referred to as PCV1), incited us to further investigate the presence of PCV2 in pigs in the years prior to the identification of PMWS. This was accomplished by performing a retrospective serological survey for the presence of antibodies to PCV1 and PCV2 in samples of swine serum banks of 1985, 1989, and 1997.

Serum samples were obtained from the Canadian national swine serum banks. These serum samples had been collected from sows slaughtered in abattoirs throughout Canada over the course of national swine herd surveys. The serum samples were conserved in a lyophilized state. Randomly selected subsets were tested:

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Table I. Identification of antibodies to PCV1 and PCV2 in porcine sera from 1985, 1989, and 1997 by indirect immunofluorescence

Year	n[a]	PCV1+/PCV2−[b]	PCV1+/PCV2+	PCV1−/PCV2+	Total PCV1+	Total PCV2+
1985	177	6 (3.4)	8 (4.6)	16 (9.0)	14 (8.0)	24 (13.6)
1989	145	2 (1.4)	58 (40.0)	47 (32.4)	60 (41.4)	105 (72.4)
1997	147	9 (6.1)	47 (32.0)	51 (34.7)	56 (38.1)	98 (66.7)

[a] Number of serum samples tested

[b] + or − at 1:20 dilution of serum

Data expressed as number (% of total)

177 serum samples from 1985, 145 from 1989, and 147 from 1997. Each serum sample was tested at a single 1:20 dilution in phosphate-buffered saline (PBS, pH 7.2) by indirect immunofluorescence (4) in 96-well microtiter plates containing PCV-free PK15 cells, PCV1-infected PK15 cells, or PCV2-infected PK15 cells. The PCV1 isolate originated from the PK15 CCL 33 cell line and the PCV2 isolate (LHVA-V53) was originally recovered from a pig demonstrating PMWS (18). The PK15 cells of all 3 preparations were found to be negative for porcine reproductive and respiratory syndrome virus (PRRSV), transmissible gastroenteritis virus/porcine respiratory coronavirus (TGEV/PRCV), swine influenza virus (SIV), and porcine parvovirus (PPV) by using specific monoclonal antibodies. For the indirect immunofluorescence assay, a positive and a negative control sera were added to each plate. The negative serum was collected from a PCV1 and PCV2 antibody-negative experimental control specific-pathogen-free (SPF) pig. The positive serum was from an SPF pig experimentally inoculated with PCV2 isolate LHVA-V53 (19). The PCV2-positive serum was collected at 34 d post-infection and titers to PCV2 and PCV1 were ≥ 1:1280 and 1:160, respectively, as determined by indirect immunofluorescence. Both sera were negative for antibodies to PRRSV, TGEV/PRCV, SIV (H1N1), and PPV. The conjugate used was goat anti-swine IgG-FITC (Jackson ImmunoResearch Laboratories, West Grove, Pennsylvania, USA) and was diluted 1:60 in PBS for the assay.

The results of the serological tests of 1985, 1989, and 1997 porcine sera are presented in Table I. Antibodies to PCV1 and PCV2 were detected in sera from all 3 years. The number of PCV1-negative/PCV2-positive (PCV1−/PCV2+) sera was greater than that of PCV1-positive/PCV2-negative (PCV1+/PCV2−) for the 3 years, but particularly for 1989 (32.4% PCV2+ vs 1.4% PCV1+) and 1997 (34.7% PCV2+ vs 6.1% PCV1+). A portion of sera from 1985 (4.6%), 1989 (40%), and 1997 (32%) were also antibody-positive for both PCV1 and PCV2. In this latter group of sera, it was observed that for a given serum, when the intensity of fluorescence was greater towards one PCV type, it was more often towards PCV2. This qualitative observation, however, needs to be confirmed by individual titration of these sera to both PCV1 and PCV2. The availability of immunoassays using PCV1 and PCV2 peptides or PCV1 and PCV2 monoclonal antibodies in a competition format should also serve in confirming the aforementioned observations. Experimentally, it has been shown that pigs inoculated with PCV2 can develop, in addition to high anti-PCV2 antibodies, lower titers to heterologous PCV1 (19,20). These experimental results and the present serological findings could imply that a significant portion of PCV1+/ PCV2+ sera were actually PCV2+ showing cross-reactivity to PCV1. The total number of sera antibody-positive for PCV2 (ie, PCV1−/PCV2+ plus PCV1+/PCV2+) was greater than that of sera antibody-positive for PCV1 (ie, PCV1+/PCV2− plus PCV1+/ PCV2+) for the 3 years studied. For 1989 and 1997, the total seropositivity to PCV1 was similar (41.4% and 38.1%, respectively), but markedly greater than that for 1985 (8.0%). Total seropositivity to PCV2 was also similar for 1989 and 1997 (72.4% and 66.7%, respectively), but markedly greater than that for 1985 (13.6%).

The results of the present retrospective serological study of Canadian porcine sera underline several interesting findings. Firstly, the present results indicate that PCV2 appears to be the main PCV type circulating in the Canadian swine population, rather than PCV1, which confirms our previous initial observations (17,18). Secondly, the results show that PCV2 had been circulating in the Canadian swine population at least 10 y before PMWS was reported as a new pathological entity. Thirdly, both PCV1+/PCV2− and PCV1−/PCV2+ serum samples were detected, underlining the fact that serological evaluation using PCV1 (persistently-infected PK15 cells) underestimates the seroprevalence of PCV2. A previous serological study in Canada (4) observed 26–55% of sera tested positive to PCV1 (CCL 33 PK15-persistently infected cells), which may be comparable to the figures of serum samples from 1989 (41.4% PCV1+) observed in the present study. However, when the indirect immunofluorescence assay in the present study was performed using PCV2 antigen, 72.4% of sera of 1989 were found to be positive. The inverse situation may also occur. Indeed, PCV1+/ PCV2− serum samples were also identified in the present study, though at a considerably lower frequency. It is thus suggested that both PCV1 and PCV2 serological assays, in a combined or separate fashion, should be performed to better assess the true seroprevalence of PCV and to better understand the respective potential pathogenic roles of both PCV1 and PCV2. The difference obtained between the number of PCV2 seropositive samples in 1985 compared to that observed in 1989 and 1997 was unexpected and should be further investigated. Testing of samples prior to 1985 would be necessary to ascertain this disparity.

It appears from the present serological results that PCV2, reported to be associated with PMWS, had been circulating in the swine population several years prior to the report of this new syndrome. Further research will be needed to appreciate and understand some of the reasons linked to the apparent emergence of PCV2 in the swine population and its association with PMWS. Among these, the potential appearance of virulent PCV2 strains, the current

management practices in the swine industry, as well as the contribution of individual, environmental, and microbial co-factors in PCV2-associated disease should be investigated more thoroughly.

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