# Isolation of circovirus from lesions of pigs with postweaning multisystemic wasting syndrome

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**Abstract** — Postweaning multisystemic wasting syndrome (PMWS), an apparently new disease, has been recognized in swine herds in western Canada. Young pigs with this disease have progressive weight loss, tachypnea, dyspnea, and jaundice, accompanied by interstitial pneumonia, lymphadenopathy, hepatitis, and nephritis.

We examined more than 400 pigs from more than 70 herds in Alberta, Saskatchewan, and Manitoba with cases of PMWS. A small virus was isolated from a range of tissues from 8 of 8 affected pigs examined. The agent was identified as a circovirus-like virus using electron microscopy, immunohistochemical staining with porcine and rabbit immune serum, and in situ hybridization. Immunohistochemical examination of tissues from more than 100 affected pigs has revealed widespread viral antigen, often contained in circovirus-like inclusion bodies, in lesions from numerous organs. Although Koch's postulates remain to be fulfilled, these results demonstrate a high degree of association between the presence of the circovirus-like virus and PMWS in affected swine.

**Résumé — Isolation du circovirus à partir de lésions chez des porcs présentant le syndrome de dépérissement multisystémique en postsevrage.** Le syndrome de dépérissement multisystémique en postsevrage (SDMP), une maladie apparemment nouvelle, a été reconnu dans des troupeaux porcins de l'Ouest du Canada. De jeunes porcs atteints de cette maladie ont présenté une perte progressive de poids, de la tachypnée, de la dyspnée et de la jaunisse, accompagnées de pneumonie interstitielle, de lymphadénopathie, d'hépatite et de néphrite. Nous avons examiné au-delà de 400 porcs sur plus de 70 troupeaux présentant des cas de SDMP en Alberta, en Saskatchewan et au Manitoba. Un petit virus a été isolé à partir d'une série de tissus chez 8 des 8 porcs examinés, atteints du syndrome. À l'aide de microscopie électronique, de coloration immunohistochimique à partir de sérum immun de porc et de lapin et d'hybridation in situ, l'agent a été identifié comme étant un virus semblable au circovirus. L'examen immunohistochimique des tissus de plus de 100 porcs affectés a révélé que l'antigène viral était largement répandu, qu'il était contenu dans des corps d'inclusion de type circovirus et qu'il était présent dans des lésions affectant plusieurs organes. Même si les postulats de Koch demeurent encore à être démontrés, ces résultats témoignent d'un fort degré d'association entre la présence d'un virus semblable au circovirus et le SDMP chez les porcs affectés.

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## Introduction

An apparently new disease, postweaning multisystemic wasting syndrome (PMWS), is being diagnosed with increasing frequency in "high health" pig herds in western Canada (1,2). These herds, usually comprising Landrace — large white cross pigs, are raised in total confinement and are free of common swine diseases, such as *Mycoplasma hyopneumoniae*, swine dysentery, and atrophic rhinitis, but not necessarily porcine reproductive and respiratory virus syndrome virus (PRRSV).

First recognized in a single swine herd in Saskatchewan in 1991, this disease is characterized clinically by progressive weight loss, tachypnea, dyspnea, and jaundice (2). Consistent pathologic changes include lymphocytic to granulomatous interstitial pneumonia, lymphadenopathy, and, less frequently, lymphocytic to granulomatous hepatitis and nephritis (1).

Porcine circovirus (PCV) was originally detected as a noncytopathic contaminant of porcine kidney (PK/15) cell lines (3). Along with chicken anemia virus (CAV) and beak and feather disease virus (BFDV) of psittacine birds, PCV has been classified into a new virus family called the Circoviridae (4). Circoviruses are small (15–24 nm) nonenveloped agents containing a unique single strand circular DNA genome of 1.76 kb to 2.31 kb (5). There are no recognized DNA sequence homologies or common antigenic determinants among the 3 currently recognized circoviruses (5).

Several surveys have documented a high seroprevalence of antibodies to porcine circovirus in swine

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populations in several parts of the world (6–8). However, until recently, there has been only scant evidence linking PCV infection with any disease in pigs (9–11). Herein, we report the identification and isolation of a circovirus from lesions of large numbers of pigs affected with PMWS.

## Materials and methods

### Porcine tissues

Tissues were collected at necropsy from more than 400 debilitated pigs exhibiting clinical signs (2) and multisystemic lesions (1) characteristic of PMWS. Affected pigs were submitted from multiple "high health" herds in western Canada. The age of affected animals ranged from 5 to 12 wk of age; there was no apparent sex predilection. Control pigs of similar ages ($n = 23$) were clinically normal animals or pigs that had died of unrelated diseases, and were from the same herds as, or different herds than, pigs with PMWS.

### Antisera

Rabbit anti-PCV sera, "Belfast-1" (12) and "Berlin" (kindly provided by Dr. I.A. Tischer, Koch Institute, Berlin, Federal Republic of Germany), were raised against porcine circovirus isolated from PK15 cells (PCV-CCL33). Porcine immune serum "Blue-2" was from a healthy pig from a herd in which PMWS had been diagnosed. This serum was shown to be free of antibodies to both porcine parvovirus (PPV) and PRRSV, as well as bovine viral diarrhea virus (BVDV).

### Virus isolation

Tissues for virus isolation were processed immediately after necropsy or stored at −70°C. Fresh or frozen lymph nodes (approximately 1 $cm^3$ pieces) were homogenized, using a laboratory blender (Seward Medical, London, England), for 2 min in 25 mL of Hank's balanced salt solution containing 2.5% HEPES buffer and penicillin (100 IU/mL)/streptomycin (100 µg/mL) (H-H-P-S). The resulting homogenate was sonicated for 4 s at 20 kHz and clarified at 2000 × *g*.

The supernatant was then extracted with an equal volume of freon (JB EM Services, Point Claire, Dorval, Quebec) to eliminate enveloped viruses by mixing in a vortex shaker for 1 min. After centrifugation at 2000 × *g*, the aqueous layer was recovered and further clarified at 14 926 × *g*. This supernatant was used as inoculum to infect PK15 cells that have been shown to be free of porcine circovirus (kindly provided by Dr. A. Afshar, Animal Diseases Research Institute, Nepean, Ontario). Nearly confluent PCV-free PK15 cells were suspended in H-H-P-S at a concentration of 9 mL diluent per 25 $cm^2$ flask. To inoculate, 1 mL of tissue extract was mixed with 9 mL of cell suspension, incubated in at 37°C for 5 h, and then centrifuged at 1000 × *g* for 15 min. The cell pellets were resuspended in 1 mL of 300 mM glucosamine (13) in H-H-P-S and incubated at 37°C for 30 min. After diluting with 10 mL of fresh, modified Eagle's minimum essential medium (E-MEM), supplemented with 1% nonessential amino acids, lactalysate (0.5%), 2 mM L-glutamine, sodium bicarbonate (2%) (Gibco, Canadian Life Technologies, Burlington, Ontario), and 7.5% fetal bovine serum (FBS), cells were again centrifuged at 1000 × *g*. The resulting pellet was resuspended in 7 mL of fresh MEM (as described above) and seeded into a 25 $cm^2$ flask or into wells of a 96-well tissue culture plate for staining.

### Microplate immunocytochemical staining

For immunoperoxidase staining of cells in microplates, the tissue culture medium was removed and the cells were washed 3 times by immersion in 0.01 M phosphate buffered saline (PBS) (pH 7.2) containing 0.05% Tween 20. Cell monolayers were fixed in 80% acetone/0.01 M PBS (pH 7.4) for 15 min and dried using an electric fan. Cell monolayers were then incubated in 100 µL of blocking buffer containing 50 mM TRIS, 10 mM EDTA (pH 8.5), 2% skim milk, 0.05% Tween 20, and 1% Triton X-100. Blocking buffer was shaken off and 50 mL of primary porcine (diluted 1/5000) or rabbit (diluted 1/1000) anti-PCV serum was added to the wells. The plates were then incubated for 1 h at 37°C and washed 5 times in PBS/0.05% Tween 20, followed by the addition of 50 µL of secondary antibody, biotinylated goat anti-rabbit (Vector Laboratories, Burlingame, California, USA) diluted 1:1000 in blocking buffer containing 4% normal goat serum. For porcine polyclonal serum, biotinylated staphylococcal protein A (Zymed Laboratories, South San Francisco, California, USA), diluted 1:10 000 in blocking buffer containing 4% normal chicken serum, was used. Following incubation for 1 h at 37°C, plates were washed 5 times in PBS/0.05% Tween and stained using an avidin-biotin immunoperoxidase technique, according to the manufacturer's instructions (Vectastain ABC Elite, Vector Laboratories).

### Electron microscopy

Untreated and freon extracted tissue homogenates were centrifuged for 10 min at 14 926 × *g*. One hundred microliters of supernatant were then centrifuged over a 50 µL cushion of 10% (w/v) sucrose for 11 min at 90 700 × *g* in an airfuge (Beckman, Fullerton, California, USA). The resulting pellet was resuspended in 1 drop (approximately 50 µL) of water.

For examination of material from inoculated PK15 cell cultures, cells were scraped off flasks and pelleted by centrifugation at 2000 × *g*. After resuspending to 1/5 the original volume in water, cells were sonicated for 4 s and extracted with freon. The sample was then clarified at 14 549 × *g* and the procedure continued as for tissue homogenates.

To stain tissue homogenates or material derived from cell cultures, carbon-coated formvar (0.3%) grids were wetted with a drop of water containing 2% fetal bovine serum and dried. One drop of specimen was then added to the grid and allowed to stand for 30 s. Following removal of excess liquid, the specimen was air dried and stained with 0.5% phosphotungstic acid (PTA) in PBS (pH 7.4). For immune aggregation, 5 µL of specimen was mixed directly on the grid with 5 µL of diluted (1/40) immune porcine serum and the mixture was incubated for 30 min prior to air drying and staining with PTA.

For examination of material that had been previously processed for routine histological examination, individual cells with inclusion bodies or positive

immunohistochemical staining were identified, removed from the slides, and processed according to previously described methods (14).

### Immunohistochemistry

For immunohistochemical examination, tissues were fixed in 10% buffered formalin, routinely processed into paraffin blocks, and stained using a previously described automated technique (15). Briefly, for each tissue block, 2 serial 5-μm sections were incubated with either rabbit anti-PCV antiserum (Belfast 1-diluted 1/1000 and 1/2000) or porcine immune serum (Blue 2-diluted 1/500 and 1/1000). In addition, a range of tissues from affected pigs were tested for the presence of PRRSV, human influenza virus, bovine respiratory syncytial virus, bovine parainfluenza-3 virus, transmissible gastroenteritis virus, pestiviruses, *Leptospira* spp., *Chlamydia* spp., and *Sarcocystis* spp., using reagents and techniques routinely performed in our laboratory for diagnostic purposes (15,16). Following reaction with the primary antibody, tissues were reacted with appropriate secondary antisera. In the case of PCV immunostaining, it was biotinylated goat-anti rabbit IgG serum or biotinylated protein A, followed by avidin-biotin complex peroxidase reagents, as previously described for microplate immunoperoxidase staining. Controls included serial sections of each block stained with the omission of primary antisera and with the substitution of primary antisera with species and isotype-matched irrelevant polyclonal or monoclonal antibodies.

### In situ hybridization

The bacterial plasmid pPCV 1 containing the replicative form (RF) of the PCV genome (17) was used as the source of PCV-specific DNA. An analogous bacterial plasmid, pCAA 1, containing 2/3 kbp RF (18) of the avian circovirus, chicken anemia agent virus (CAV), was used as a negative control. Stocks of both plasmids were stored in glycerol and used for the production of plasmid preparations by the alkaline lysis method (19) for subsequent use as the template DNA probe preparation.

Circovirus probes, representative of the complete genome of both PCV and CAV, were produced from target bacterial plasmid preparations (1 mg each) and random hexanucleotide primers, using a commercial kit (DIG DNA labeling kit, Boeringer Mannheim, Laval, Quebec) in accordance with the manufacturer's instructions. The randomly primed digoxigenin(DIG)-labeled probes were resuspended in a final volume of 50 μL to 100 μL of sterile water prior to subsequent use for in situ hybridization. Semiconfluent coverslip preparations of PCV-free PK15 cells were inoculated with tissue homogenate from affected pigs and glucosamine-treated, as described above. Following incubation for a further 48 h, cell monolayers were fixed in 10% neutral-buffered formalin (pH 7.0) for 6 h at room temperature, washed in PBS, and incubated with proteinase K (Sigma Chemical, St. Louis, Missouri, USA) at a concentration of 0.5 mg/mL in 0.05 M TRIS-HCl, 5 mM EDTA (pH 7.6) for 5 min at 37°C. The preparations were then placed in 1% glycine in autoclaved distilled water for 30 s, and then washed twice for 10 min in 0.01 M PBS (pH 7.2). The coverslip preparations were then air-dried, probes were applied, and clean coverslips were applied. The preparations were then placed in a hot air oven at 90°C for 10 min, removed and placed on an ice block for 1 min, incubated at 37°C for 18 h, washed briefly in 2× sodium chloride/sodium citrate buffer (SSC) to remove the coverslips, and washed twice for 5 min in 2× SCC. The preparations were then washed twice for 5 min in PBS and placed in 0.1 M maleic acid buffer (0.15 M NaCl, pH 7.5) for a further 10 min before incubation for 20 min in 1% blocking reagent (Boehringer Mannheim). The cultures were then incubated with a 1/250 dilution of monoclonal antibody to digoxigenin (Boehringer Mannheim) for 1 h at 37°C, washed in maleic acid buffer for 10 min, and washed in PBS for 10 min prior to the addition of biotinylated anti-mouse IgG (Zymed Laboratories) for 10 min at room temperature. The preparations were washed in 0.01 M PBS (pH 7.2) for 5 min before the application of a streptavidin peroxidase conjugate (Vector Laboratories) for 10 min at room temperature. Following a further wash, a fresh solution of amino-ethyl-carbanizole was applied for 3 min at room temperature, then the preparations were washed in PBS and mounted.

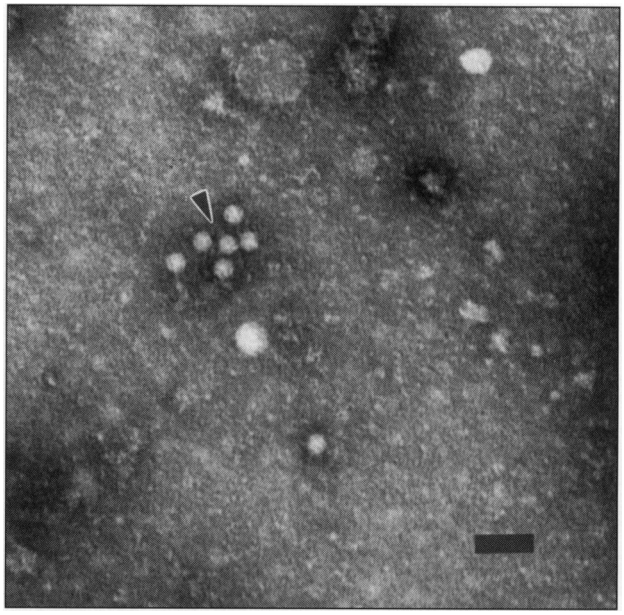

**Figure 1.** Electron micrograph of small circovirus-like particles (arrow) in homogenate of lymphoid tissue from a pig with postweaning multisystemic wasting syndrome. Negative staining. Bar = 50 nm

Controls included the testing of all samples with a negative probe (CAV) and the use of cell cultures known to contain the PK15 cell isolate of PCV (PCV-CCL33), as positive controls, and PCV negative cell cultures, as negative controls.

## Results

To date more than 400 young pigs with PMWS from more than 70, primarily large, high health, herds have been examined by gross and histopathological assessment. A range of tissues from 131 of these cases were examined using immunohistochemistry. Electron

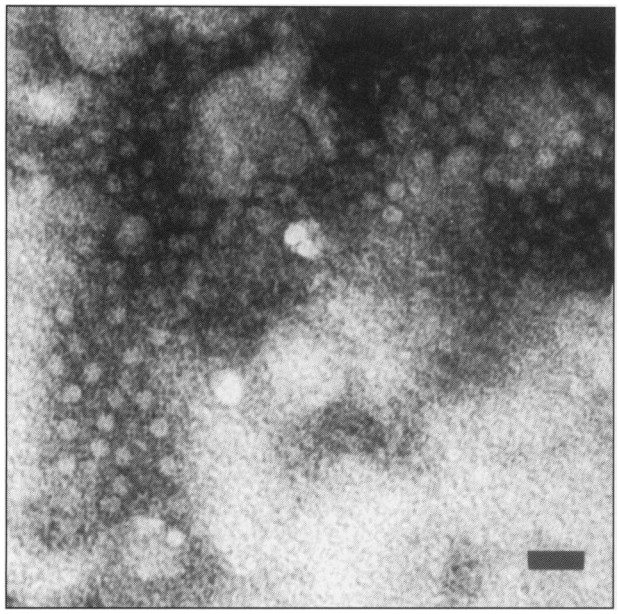

**Figure 2.** Electron micrograph of homogenate of lymphoid tissue from a pig with postweaning multisystemic wasting syndrome. Immunoagglutination of small circovirus-like particles using immune porcine serum. Negative staining. Bar = 50 nm

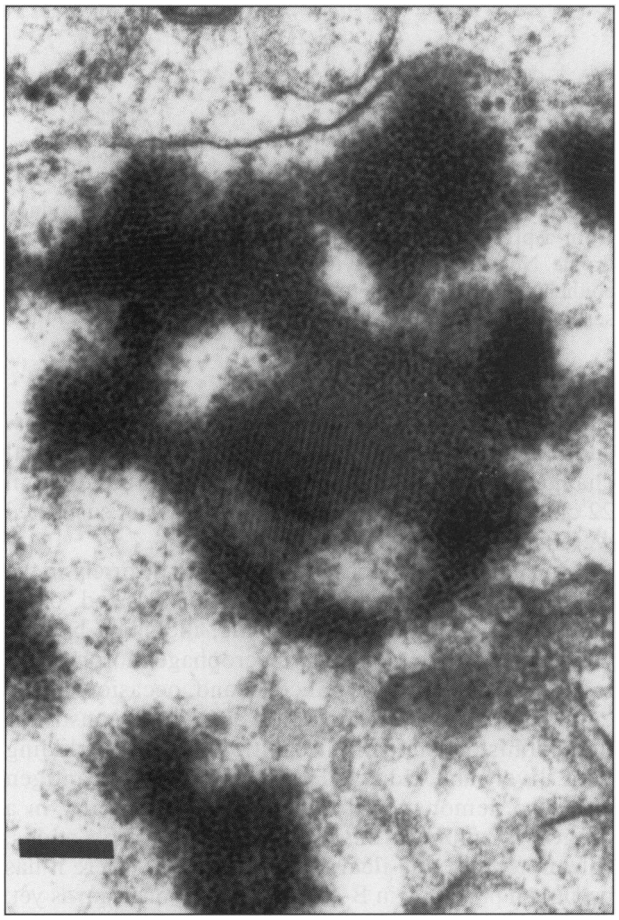

**Figure 3.** Transmission electron micrograph showing paracrystalline array of circovirus-like particles in cytoplasmic inclusions in lymphoid tissue from a pig with postweaning multisystemic wasting syndrome. An individual stained cell was removed from an immunohistochemically stained slide and processed for electron microscopy. Bar = 200 nm

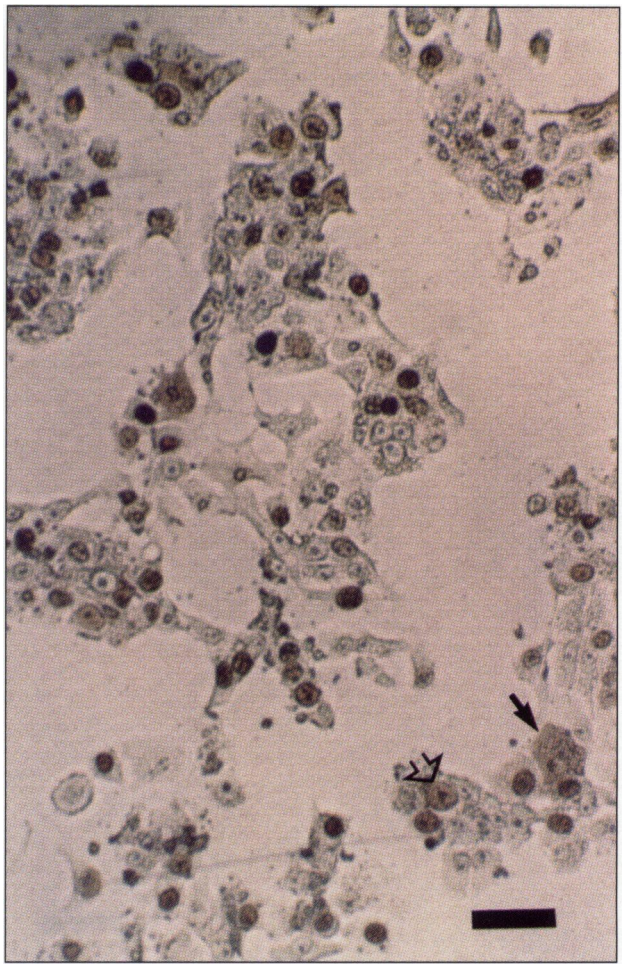

**Figure 4.** Immunohistochemical staining of porcine kidney cell culture 48 h after inoculation with tissue homogenate from a pig with postweaning multisystemic wasting syndrome. Note nuclear (open arrow) and cytoplasmic (solid arrow) staining. Immunoperoxidase stain using porcine immune serum. Bar = 20 μm

microscopic examination of tissue extracts from various organs of 5 affected pigs revealed small (15 to 17 nm size range) nonenveloped viral particles (Figure 1) that were agglutinated with immune porcine serum (Figure 2). To date, no other virus particles have been observed in untreated or freon-extracted tissues from affected pigs; however, extensive examinations have not been performed. Transmission electron microscopic examination of lymphoid tissue from 6 affected pigs revealed paracrystalline arrays of small viral particles, primarily in the cytoplasm of infected cells (Figure 3). Circovirus-like virus was isolated from one or more tissues, usually lung and lymph node, from 8 of 8 naturally affected pigs from which isolation was attempted.

Circovirus-like virus was demonstrated in PCV-free PK15 cells inoculated with homogenates of tissue from affected pigs by electron microscopy or microplate immunoperoxidase staining. At the dilutions of rabbit (1/1000) and porcine (1/5000) serum used, staining was predominately nuclear, although in heavily infected monolayers, or at lower serum dilutions, diffuse cytoplasmic staining was also observed (Figure 4). The staining of this circovirus-like virus with rabbit antisera,

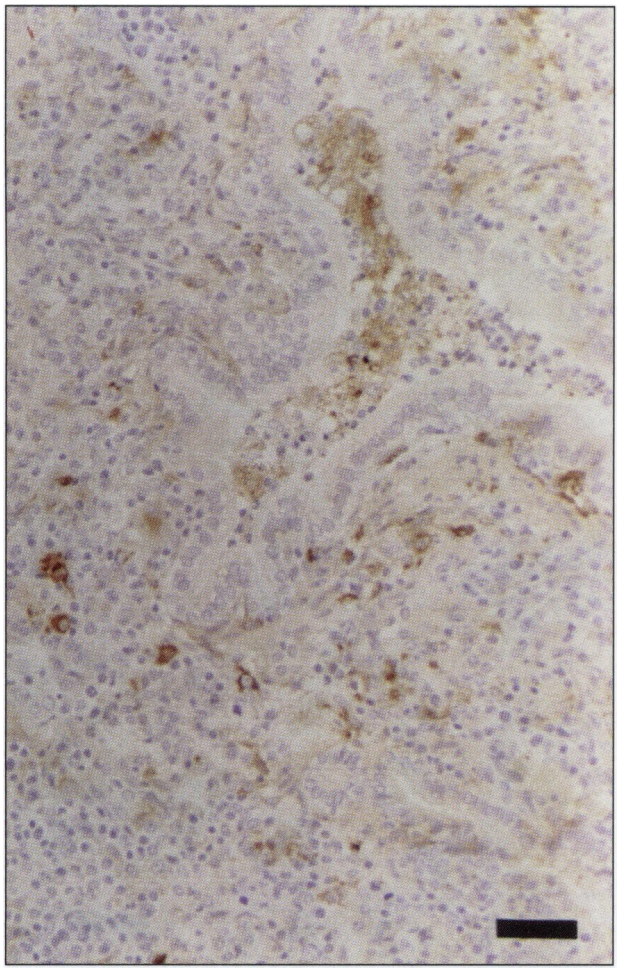

**Figure 5.** Immunohistochemical staining of section of lung with bronchointerstitial pneumonia from a pig with postweaning multisystemic wasting syndrome. Note positive staining in large cells (macrophages) in inflammed alveoli and parenchyma, and in cellular debris in bronchiole. Immunoperoxidase stain using rabbit serum. Bar = 40 μm

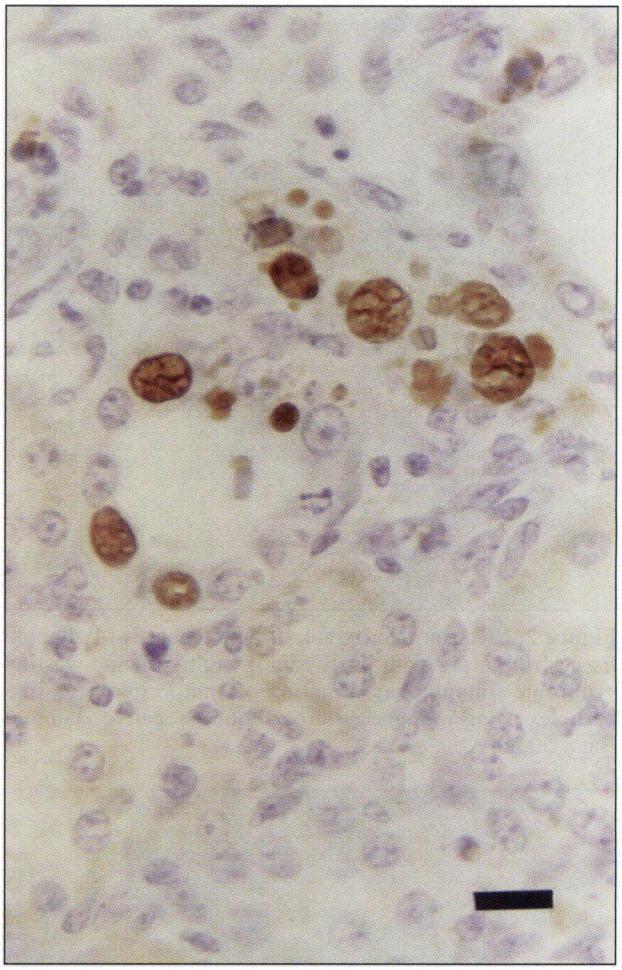

**Figure 6.** Immunohistochemical staining of section of kidney from a pig with postweaning multisystemic wasting syndrome. Note nuclear and cytoplasmic staining in renal tubular epithelial cells. Immunoperoxidase stain using porcine immune serum. Bar = 20 μm

Belfast-1 and Berlin, raised against PCV-CCL33 was less intense than the staining of PCV-CCL33 when equivalent dilutions of sera were used on cultures infected with the respective viruses. Although the porcine immune serum did not detect PCV-CCL33 *in vitro*, it reacted with PK15 cells inoculated with the tissues from pigs affected with PMWS to a dilution of >:1:36 450. Porcine kidney (PK15) cells inoculated with tissue from affected pigs did not react with antiserum or monoclonal antibodies specific for PPV or PRRSV, or pestiviruses.

Immunohistochemical detection of PCV in tissue has been attempted in tissues from 131 pigs; 77 using Belfast-1 rabbit antiserum, and 54 using Blue-2 porcine serum. In 20, clinically normal, control pigs from a high health herd with no reported clinical cases of PMWS, all tissues were negative using Belfast-1 as the primary antiserum. In 57 clinical cases stained with Belfast-1, tissues from 43 of 43 pigs with signs and lesions compatible with PMWS were positive for PCV antigen; tissues from 14 clinically ill pigs in which PMWS was not suspected were not stained with the antiserum. Similarly, of the 54 clinical cases stained with Blue-2, tissues from 27 of 27 pigs with signs and lesions compatible with PMWS were positive; while tissues from 5 pigs with gross and histopathological changes suggestive of early PMWS, and tissues from 22 clinically ill pigs in which PMWS was not suspected were negative.

Typically viral antigens were localized diffusely in the cytoplasm, or in polymorphic, variously sized, often multiple, usually intracytoplasmic, inclusion bodies, in large cells with monocyte/macrophage morphology, in multinucleated syncytial cells, and, occasionally, in epithelial cells. Viral antigen was also demonstrated immunohistochemically in multiple organs, including lung, liver, and kidney (Figures 5&6). Viral antigen was also demonstrated immunohistochemically in a range of lymphoid tissues, including lymph nodes, spleen, tonsils, and ileal Peyer's patches, where it has been seen primarily in B-cell dependent zones, in, as yet, unidentified cells (Figure 7). Immunohistochemical examination for PRRSV was attempted using lung and lymphoid tissue from more than 20 PCV-positive pigs with PMWS, and PRRSV antigen was demonstrated, along with PCV antigen, in the lungs of approximately half of these cases. However, in contrast to the

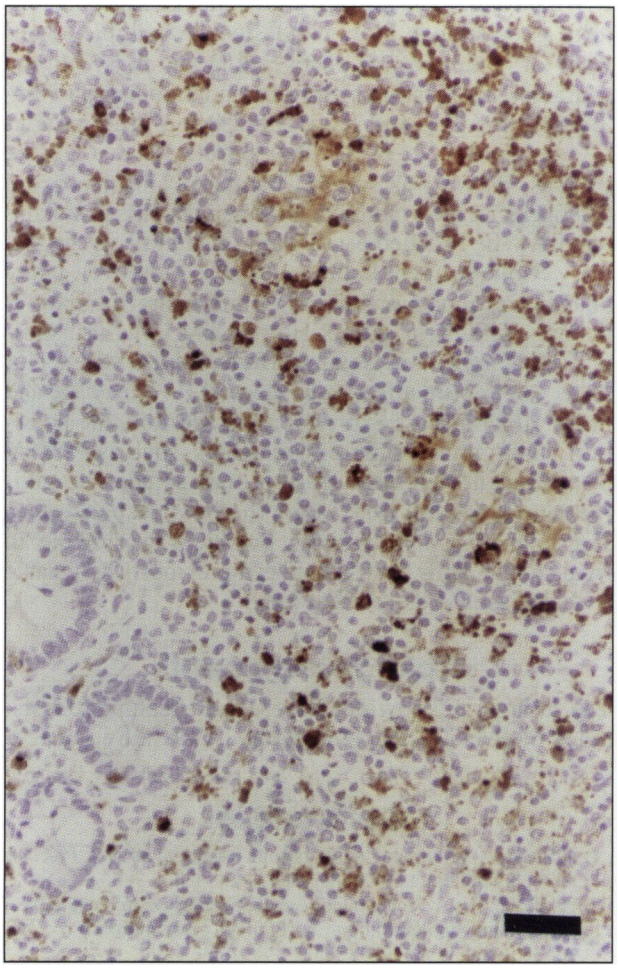

**Figure 7.** Immunohistochemical staining of section of ileum from a pig with postweaning multisystemic wasting syndrome. Note multifocal positive staining in lymphoid tissue. Immunoperoxidase stain using rabbit serum. Bar = 2.5 μm

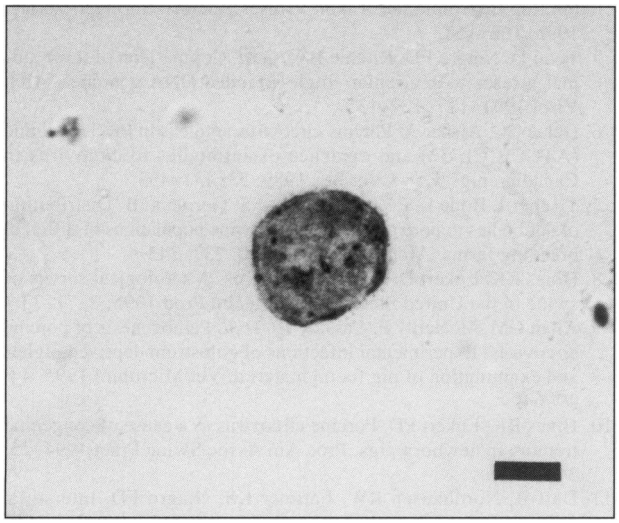

**Figure 8.** In situ hybridization on porcine kidney cell culture inoculated with tissue homogenate from a pig with postweaning multisystemic wasting syndrome. Note intense nuclear reactivity. Antisense whole porcine circovirus genomic probe. Bar = 2.5 μm

prominence of PCV antigen, PRRSV antigen was generally apparent only in scattered cells in affected tissues with dual viral infection (data not shown). Tissues from

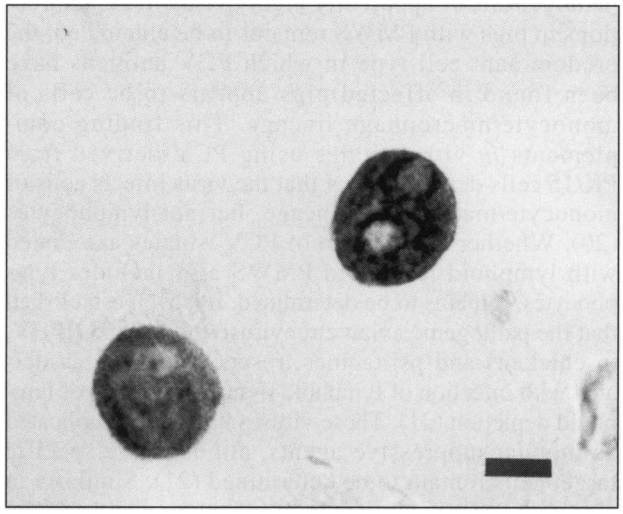

**Figure 9.** In situ hybridization on porcine kidney cell culture persistently infected with porcine circovirus. Note intense nuclear reactivity. Antisense whole PCV genomic probe. Bar = 2.5 μm

affected pigs were not stained by any other antibodies tested.

In addition, several bacterial species, including, *Escherichia coli* and *Streptococcus suis* have been isolated from the lungs of approximately 10% of pigs with pulmonary lesions typical of PMWS. As well, *Pneumocystis carini* has been observed in approximately 2% of lungs of affected pigs.

Following in situ hybridization, a signal was consistently seen in the nucleus of cells in cultures inoculated with tissue homogenates from affected pigs (Figure 8). No signal was observed when similar cultures were hybridized with the CAV probe or when the PCV probe was applied to known PCV-negative cell cultures (data not shown). The intensity and location of the signal generated in cell cultures derived from inoculation of material from diseased pigs was similar to that obtained following application of the PCV probe to cell cultures infected with the PK 15 PCV isolate (Figure 9)

## Discussion

Subsequent to the original isolation and characterization of PCV (3), sero-epidemiological studies have demonstrated that PCV infection is highly prevalent among domestic and wild swine populations throughout the world, including Canada (6–9). However, in contrast to the avian circoviruses, CAV and BFDV, until recently (1,2), there has only been scant evidence (9–11) that the circovirus of swine is associated with any naturally acquired disease. Our findings indicate a consistent association of PCV with lesions in multiple tissues from pigs with typical clinical signs and pathological changes of the newly described PMWS.

These findings are in marked contrast to previous studies with PCV in which PCV and viral antigen were detected using virus isolation and fluorescent antibody techniques, respectively, in multiple tissues following experimental infection of sows with PCV of PK15 origin (PCV-CCL33); however, there were no apparent pathologic consequences of infection (9). Although the

pathogenesis of apparently highly virulent PCV infections in pigs with PMWS remains to be elucidated, the predominant cell type in which PCV antigens have been found in affected pigs appears to be cells of monocyte/macrophage lineage. This finding complements *in vitro* studies using PCV derived from PK/15 cells demonstrating that the virus infects cells of monocyte/macrophage lineage, but not lymphocytes (20). Whether the tropism of PCV isolates associated with lymphoid lesions in PMWS also includes lymphocytes, remains to be determined. It is well established that the pathogenic avian circoviruses, CAV and BFDV, in chickens and psittacines, respectively, are associated with infection of lymphoid tissue and lesions of lymphoid depletion (21). These viruses have been implicated as immunosuppressive agents, although the specific target cells remain to be determined (21). Similarly in pigs with PMWS, virulent PCV infections could account for the observed lesions in multiple lymphoid tissues, with the resultant immunosuppression predisposing to infection with secondary pathogens.

Our results of microplate immunoperoxidase staining of PCV and PCV-like virus isolates with polyclonal rabbit antisera and porcine immune serum are suggestive of there being significant antigenic differences between the PCV from PK/15 cells (PCV-CCL33) and our field isolates of PCV-like viruses from pigs with PMWS. Differences in the intracellular location of viral antigen, both intranuclear and intracytoplasmic *in vitro*, and predominately, or most often exclusively, intracytoplasmic *in vivo*, may be reflective of differences in virus morphogenesis in the natural target cell *in vivo* versus in cell lines *in vitro*. As well, cell cycle (affected by glucosamine treatment) and passage level of target cells and virus *in vitro* may also affect virus morphogenesis (13). Significant changes in the reactivity of the PCV-CCL33-infected cells with a panel of monoclonal antibodies following adaptation of the virus to growth and passage in Vero cells has been reported (23). These changes in reactivity became more apparent with passage, eventually resulting in total lack of reactivity of the virus with a number of monoclonal antibodies. Clearly, passage through xenogeneic cells resulted in changes in antigenic composition. Whether or not similar alterations occur *in vivo*, or as a result of serial transmission of the virus in susceptible pigs, remains to be determined.

The degree of mutability of PCV, *in vitro* and *in vivo*, and the implications that mutations in the PCV genome have for virulence also remain to be defined. The available evidence indicates that a small number of point mutations and resultant substitutions of 1 or 2 amino acids in another small DNA virus, feline parvovirus, allowed a shift in host range from cats to dogs (24). These mutations resulted in a virus that was highly pathogenic to dogs. Similarly, although circoviruses are structurally different from parvoviruses, one or more point mutations may have occurred in PCV, allowing for the generation of pathogenic variants from an apparently nonpathogenic, ubiquitous virus. Alternatively, there may have been significant genetic changes in PCV circulating in pig populations, resulting in the derivation of a "new," virulent, circovirus-like virus.

We have demonstrated by several techniques, a uniform association of PCV with lesions in multiple organs from large numbers of postweaning age pigs with the characteristic wasting syndrome and associated pathological changes, and have ruled out a predominant involvement of several other porcine and ungulate viruses, as well as several bacterial and protozoal pathogens, in the syndrome. However, Koch's postulates remain to be fulfilled establishing this virus as the primary etiologic agent in the syndrome. Moreover, many unanswered questions remain concerning the natural history of PCV in swine. How is the virus transmitted? How may inter-isolate differences in virulence or target cell tropism affect pathogenesis? How may age and breed affect disease expression? In addition, the possibility that immunosuppressive agents, such as PRRSV or an unidentified retrovirus, or management, or environmental factors, may alter the host response to a virus that is apparently highly prevalent, worldwide, in swine populations but has not been previously associated with disease needs further study. CVJ

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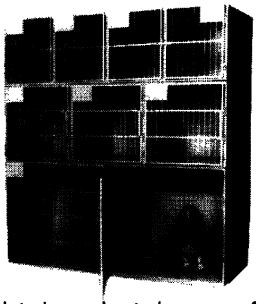

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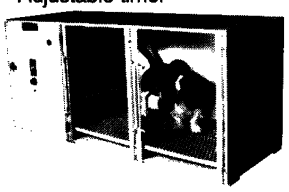

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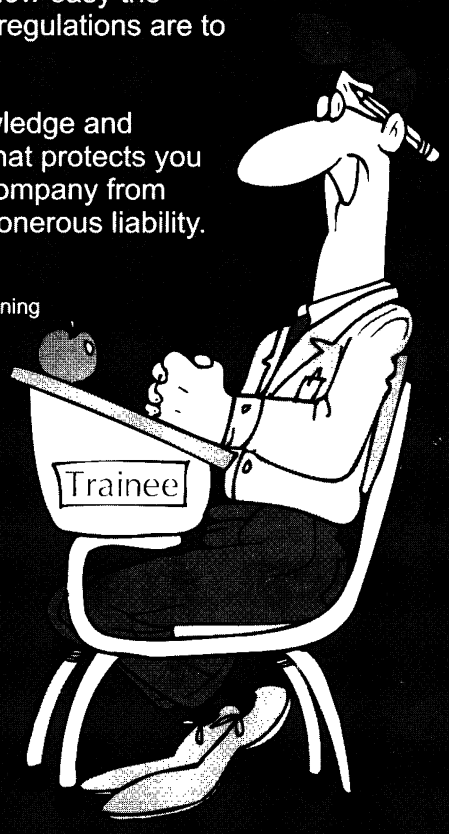


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