The emergence of porcine circovirus 2b genotype (PCV-2b) in swine in Canada

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ABSTRACT

Since late 2004, the swine industry in the province of Québec has experienced a significant increase in death rate related to postweaning multisystemic wasting syndrome (PMWS). To explain this phenomenon, 2 hypotheses were formulated: 1) the presence of a second pathogen could be exacerbating the porcine circovirus 2 (PCV-2) infection, or 2) a new and more virulent PCV-2 strain could be infecting swine. In 2005, 13 PMWS cases were submitted to the Québec provincial diagnostic laboratory and PCV-2 was the only virus that could be found constantly by PCR in all 13 samples. The PCR detection results obtained for other viruses revealed the following: 61.5% were positive for porcine reproductive and respiratory syndrome virus (PRRSV), 30.8% for swine influenza virus (SIV), 15.4% for porcine parvovirus (PPV), 69.2% for swine torque teno virus (swTTV), 38.5% for swine hepatitis E virus (swHEV) and 84.6% for *Mycoplasma hyorhinis*; transmissible gastroenteritis virus and porcine respiratory coronavirus (TGEV/PRCV) was not detected. Sequences of the entire genome revealed that these PCV-2 strains belonged to a genotype (named PCV-2b) that has never been reported in Canada. Further sequence analyses on 83 other Canadian PCV-2 positive cases submitted to the provincial diagnostic laboratory during years 2005 and 2006 showed that 79.5% of the viral sequences obtained clustered in the PCV-2b genotype. The appearance of the PCV-2b genotype in Canada may explain the death rate increase related to PMWS, but this relationship has to be confirmed.

RÉSUMÉ

Depuis la fin de l'année 2004, une recrudescence marquée du syndrome de dépérissement en post-sevrage (SDPS) avec une augmentation du taux de mortalité a été observé dans les élevages porcins du Québec. Deux hypothèses furent émises pour expliquer ces observations: 1) présence d'un second pathogène qui exacerbe l'infection primaire au circovirus porcin de type 2 (PCV-2) et 2) présence d'une nouvelle souche de PCV-2 plus virulente. Des échantillons de 13 cas cliniques de SDPS furent soumis au laboratoire de diagnostic provincial du Québec et seulement le virus PCV-2 a pu être détecté dans tous les échantillons. Par contre, d'autres virus ont été détectés par PCR. Entre autres, 61.5%, 30.8%, 15.4%, 69.2%, 38.5% et 84.6% des 13 cas cliniques de SDPS étaient positifs pour le virus du syndrome reproducteur et respiratoire porcin (PRRSV), le virus influenza porcin (SIV), le parvovirus porcin (PPV), le torque teno virus porcin (swTTV), le virus de l'hépatite E porcin (swHEV) et *Mycoplasma hyorhinis*, respectivement, alors que tous les cas étaient négatifs pour la présence du virus de la gastroentérite transmissible et du coronavirus respiratoire porcin (TGEV/PRCV). Le séquençage complet du génome des 13 virus PCV-2 a révélé que ces virus appartenaient à un génotype (nommé: PCV-2b) qui, jusqu'à présent, n'avait jamais été rapporté au Canada. Le séquençage complet du génome de 83 souches canadiennes du virus PCV-2 soumis à notre laboratoire de diagnostic en 2005 et 2006 a démontré que 79.5% des séquences virales appartiennent au génotype PCV-2b. L'apparition du génotype PCV-2b au Canada pourrait expliquer l'augmentation du taux de mortalité associé au SDPS mais cette relation de cause à effet reste à être démontrée.

Introduction

Postweaning multisystemic wasting syndrome (PMWS) is a swine disease initially identified in Canada in 1991 (1). Now, it is known as a worldwide disease, with outbreaks being observed in swine herds of North and South America, Europe, and Asia (1). The disease affects 5- to 12-week-old piglets and is characterized in part by weight loss, dyspnea, jaundice, and enlarged lymph nodes, as well as by degeneration and necrosis of hepatocytes, multifocal lymphohistiocytic pneumonia, lymphocytic depletion, and multinucleated giant cell formation (2). The etiological agent responsible for PMWS has been identified as a circovirus particle and named porcine circovirus 2 (PCV-2) (3-5). The PCV-2 is a small nonenveloped virus that possesses a single-stranded ambisense circular DNA genome about 1.76 kb in length (6-9). Viral DNA possesses at least 3 functional open reading frames (ORF): ORF1 encodes the Rep proteins involved in virus replication (10-12), ORF2 encodes the nucleocapsid (NC) protein (13), and ORF3 encodes a protein that induces apoptosis and is also involved in viral pathogenesis *in vivo* (14, 15). Today, it is now recognized that the clinical expression of PCV-2 infection in swine is more complex than previously established, since it can play a pivotal role in several syndromes: porcine dermatitis and nephropathy syndrome (PDNS), porcine respiratory disease complex (PRDC), reproductive failure, granulomatous enteritis, necrotizing lymphadenitis, exudative epidermitis, and congenital tremor (16, 17). Consequently, to describe and name all those syndromes in a more convenient terminology, it is now accepted to refer to “porcine circovirus associated disease (PCVAD)”.

At the end of 2004, the swine industry in the province of Québec experienced a significant increase in death rate related to PCVAD. At that time, no statistical analysis supported this observation and furthermore no data indicating the extent of the increase in death rate was

available. Consequently, an epidemiological survey that included producers (for a total of 245 producers) that annually sold on the market 15% (1 000 000 pigs) of the entire Québec pig production was conducted by Dr Camille Moore, a private veterinary practitioner in Québec, to provide valuable information on the severity of the mortality increase (18). This study included all types of production and revealed an increase of 2.39% in the mortality rate in Québec pig farms in 2005 (7.57%) compared with 2004 (5.18%). More specifically, weaning-finishing production had a mortality rate average of 7.53% in 2005 compared with 5.31% in 2004. Similarly, finishing production had a mortality rate average of 7.66% in 2005 compared with 4.88% in 2004. Interestingly, 56% of the producers indicated that their production had a clinical, pathologic, or laboratory diagnosis of PCVAD at the time of the survey, which was held at the end of 2005. To explain this situation, 2 hypotheses were formulated based on the facts that coinfection with other pathogens is usually necessary to produce the clinical disease and gross lesions typical of PMWS (19-23) and that it is usual in virology to observe pathogenicity variation between different virus isolates (24-26): namely, 1) that the presence of another pathogen that could exacerbate the PCV-2 infection and 2) that a new and more pathogenic PCV-2 strain was present. Consequently, following the immediate urge to understand what was going on, a PCV-2 genotype, which has never been reported previously in Canada but which has already been identified in Asia and Europe, was identified.

Materials and methods

Clinical cases definition.

Thirteen PMWS cases that occurred in 2005 and originated from the province of Québec were selected because they presented clinical signs related to the PMWS definition and were from affected herds with an increased mortality rate. Those PMWS cases (named: FMV-05-6302, FMV-05-6317, FMV-05-6505, FMV-05-6507, FMV-05-7098, FMV-05-7386, FMV-05-7388, FMV-05-7389, FMV-05-7390, FMV-05-7537, FMV-05-7539, FMV-05-8037, and FMV-05-8574) had been submitted initially to the Québec provincial animal pathology laboratory (Institut national de santé animal – Ministère de l'agriculture, des pêcheries et de l'alimentation du Québec) for histopathologic evaluation to confirm the clinical diagnosis made by veterinarians. Samples (lung, lymph nodes, liver, spleen, kidneys) from 2 to 4 piglets were submitted for each PMWS affected herd.

Virus isolation.

Four cell lines (PK15A, ST, HRTG-G, and MDCK) were used for the isolation of different porcine viruses. The PK15A (porcine kidney) cells were used to isolate PCV-2. The PK15A cells, a subclone of PCV noninfected PK15 cells (27), were maintained in Earle's minimal essential medium (MEM; Invitrogen Corporation, GibcoBRL, Grand Island, NY, USA), supplemented with 10% fetal bovine serum (FBS), 300 U/mL of penicillin, 300 mg/mL of streptomycin, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 2.5 μg/mL of amphotericin B, and 10 mM HEPES buffer. The ST (swine testis) cells were maintained in Dulbecco's modified Eagle's medium (DMEM; Invitrogen Corporation, GibcoBRL) ,

supplemented with 2% FBS, 300 U/mL of penicillin, 300 mg/mL of streptomycin, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 2.5 μg/mL of amphotericin B, and 10 mM HEPES buffer. The HRT-G (human rectal tumor) cells were maintained in Roswell Park Memorial Institute (RPMI) 1640 medium (Invitrogen Corporation, GibcoBRL), supplemented with 300 U/mL of penicillin, 300 mg/mL of streptomycin, 10 U/mL of trypsin, 1 mM sodium pyruvate, 2.5 μg/mL of amphotericin B, and 10 mM HEPES buffer. The MDCK (Madin-Darby canine kidney) cells were maintained in 50:50 Hank's MEM: Earle's MEM (Invitrogen Corporation, GibcoBRL) supplemented with 300 U/mL of penicillin, 300 mg/mL of streptomycin, 10 mM HEPES buffer, 2.5 μg/mL of amphotericin B, and 10 U/mL of trypsin. All cells were maintained at 37^0C in 5% CO_2 atmosphere following their incubation with tissue homogenates. Virus isolation was attempted during 3 consecutive passages with pools of piglet samples (lung, lymph nodes, liver, spleen, kidneys) prepared from the 13 PMWS cases submitted to the Diagnostic Veterinary Virology Laboratory (DVVL) of the Faculté de médecine vétérinaire (FMV) of the Université de Montréal.

Polymerase chain reaction (PCR) diagnostic tests.

Viral RNA and DNA were isolated from 140 μL of homogenate suspensions prepared from piglet sample pools for each of the 13 PMWS cases by using commercial kits (QIAamp Viral RNA Mini Kit and QIAamp DNA Mini Kit; Qiagen, Mississauga, Ontario) according to the manufacturer's instructions. Subsequently, different PCR or RT-PCR diagnostic tests (in house or commercially available) were performed to identify porcine pathogens. A commercially available porcine reproductive and respiratory syndrome virus (PRRSV) real time PCR diagnostic test kit (Tetracore, Rockville, Maryland, USA) was used to identify the North

American PRRSV genotype according to the manufacturer's instruction. A PCV-2 nested PCR diagnostic test was performed to identify PCV-2 positive cases as previously described (28). Alternatively, a PCV-2 real-time PCR diagnostic test was developed by the molecular diagnostic service of the FMV to identify PCV-2 positive cases. Briefly, a set of primers (PCV-Foward: 5'-AGT GAG CGG GAA AAT GCA-3' and PCV1-AS6: 5'-CAC ACA GTC TCA GTA GAT CAT CC-3') was used to target the ORF1 gene of PCV-2 viral genome and gave an expected fragment of 226 bp in length. A specific fluorogenic PCV-2 DNA probe (5'-(FAM) TGC AGA CCC GGA AAC CAC (BHQ)-3') was then used to detect the newly synthesized PCR product. The transmissible gastroenteritis virus and porcine respiratory coronavirus (TGEV/PRCV) PCR diagnostic test was performed as previously described (29). It is well known that the conserved and variable regions of the 23S ribosomal RNA gene of *Mycoplasma* permit the identification of the cluster and subsequently the identification of the species (30). This strategy has been used, as previously described (31), to identify by PCR if *Mycoplasma* spp were present in the submitted samples and subsequently to identify which species (*hyorhinis* or *hyopneumoniae*) was present in positive cases. The presence of viruses that classified within the *Influenza A virus* genus, which includes the swine influenza virus (SIV), was determined with a RT-PCR assay targeting the M1 gene, using a specific primer set previously described by others (32). The swine torque teno virus (swTTV) was detected by using a nested PCR diagnostic test developed by McKeown et al. (33). The swine hepatitis E virus (swHEV) was detected according to a nested RT-PCR assay developed by Huang et al. (34), by using a commercial kit (QIAGEN OneStep RT-PCR kit; Qiagen) following the manufacturer's recommendations. The presence of the porcine parvovirus (PPV) genome was evaluated by a nested PCR, using 2 sets of primers (VPS1: 5'-TGG TGG ACC ATT TCT AAC TCC TAT AGT ACC-3' and VPAS1: 5'- GTT AAT AGT AAA CAC ATG AGA GCT TGT TTC-3'; VPS2: 5'-CAA TAC TGC ACC TGT ATT TCC AAA TGG-3'

and VPAS2: 5'-AAA ATT TTA TTG TTT TTT GGG GAT AAT TGG-3') that target the VP gene and gave expected fragments of 879 and 526 bp in length for wild type PPV and 1006 and 653 bp in length for the laboratory PPV strain (NADL-2).

Sequencing and phylogenetic analyses.

The entire PCV-2 genome was amplified by PCR, using 2 sets of oligonucleotides (SEQ PCV-1NF: 5'-GGA CCC CAA CCC CAT AAA A-3'and SEQ PCV-1NR: 5'-CCC TCA CCT ATG ACC CCT ATG T-3'; SEQ PCV-2NF: 5'-TGT TTT CGA ACG CAG TGC C-3' and SEQ PCV-2NR: 5'-CCG TTG TCC CTG AGA TCT AGG A-3') that produced 2 overlapping PCR products at both ends of 1254 nucleotides (nt) and 1045 nt, respectively. The PCR products were purified by using a commercial kit (QIAquick PCR purification kit; Qiagen) according to the manufacturer's instruction. Both strands of the purified DNA PCR products were sequenced by using the same primer sets with standard automated sequencing methods (FMV Sequencing Laboratory, Bigdye terminator version 3.1, sequencer: ABI 310, Applied Biosystems; Foster City, California, USA). Resulting sequences were compared with other Canadian PCV-2 strains (8), as well as other PCV-2 sequences available in GenBank. Software (BioEdit Sequence Alignment Editor version 7.0.5.2, Ibis Therapeutics; Carlsbad, California, USA) using the CLUSTAL W alignment method was utilized and an unrooted phylogenic tree was constructed by using the distance-based neighbor-joining method. Bootstrap values were calculated on 1000 repeats of the alignment. The identification of the PCV-2 sequences used for the phylogenic tree and their respective GenBank accession number are indicated in Table 1.

Results

Identification of cofactors possibly involved in the appearance of PCVAD.

Interestingly, the 13 PMWS cases submitted to the DVVL presented characteristic microscopic lesions of PMWS at various degrees of intensity. The observed lesions were predominantly identified as marked lymphocytic depletion, multinucleated giant cell formation, appearance of inclusion bodies in histiocytes and multifocal lymphohistiocytic pneumonia. Detection results presented in Table 2 show that these 13 PMWS cases were positive, not only for PCV-2 but also for several other swine viral pathogens. In these PMWS cases, 8 out of 13 (61.5%) were positive for PRRSV, 4 out of 13 (30.8%) were positive for SIV, 2 out of 13 (15.4%) were positive for PPV, 5 out of 13 (38.5%) were positive for swHEV, and 9 out of 13 (69.2%) were positive for swTTV. All PCV-2 positive cases were PCR positive for at least 1 other viral pathogen. The 2 worst cases were simultaneously infected with PCV-2, PRRSV, SIV, swHEV, swTTV, and *Mycoplasma. hyorhinis* (Table 2). TGEV/PRCV was not detected by PCR in any of the 13 PMWS cases studied. No other virus, except for PCV-2, could be isolated from the HRT-G, MDCK, ST and PK15A cell lines (Table 2). At the 3rd passage, PCR positive results were obtained for *Mycoplasma* spp and subsequently for *M. hyorhinis* in the cell culture supernatants of 11 samples (Table 2). Cell culture had permitted the growth of *M. hyorhinis* to a level where it could be identified. To eliminate the possibility of a *M. hyorhinis* contamination, 8 μg/mL of tylosin was added in the cell culture medium and virus isolation was tried once again. Unfortunately, no beneficial effect on virus isolation was observed, except for a small improvement on PCV-2 isolation (data not shown).

Sequence analysis of recent PCV-2 Canadian strains.

Entire genome sequences obtained from the 13 PCV-2 cases were aligned and compared with PCV-2 reference strains (Table 1). As showed in Figure 1, the PCV-2 strains can be classified in 2 genotypes (PCV-2a and PCV-2b) and the nt sequence identity between both genotypes varies from 94% to 96%. Since the nt sequence identity between strains from PCV-2a genotype varied between 96% to 100% (Figure 1) (8), the overall PCV-2 nt sequences are quite conserved and their classification could be arbitrary. Consequently, it could be more appropriate to look at individual genes or encoded peptide sequences, or both than the entire nt sequences of the viral genome. The most variable protein between PCV-2a and PCV-2b genotypes was the NC protein, which is encoded by the ORF2 gene, with an amino acids (aa) sequence identity varying from 88% to 94% between both genotypes. The 2 other known proteins to be expressed by PCV-2 were less variable between both genotypes than the NC protein with an aa sequence identity between the 2 genotypes varying from 96% to 99% for Rep protein and from 92% to 98% for ORF3 protein. Since 2005, several PCV-2 positive cases submitted to the DVVL have been sequenced for different reasons. Most of those cases were provided because swine producers sustained important economical loss related to PCVAD in their herds, but some of them had no problem related to PCVAD at all. In fact, some producers just wanted to know the status of their herds and to know what type of PCV-2 was present. The number of cases that were sequenced, the province of origin and the strain genotypes are presented in Table 3. In summary, 83 PCV-2 positive cases originating from 4 provinces in Canada were submitted from early 2005 to June 2006 for sequencing and genotyping. The highest proportion of PCV-2 strains classified in PCV-2b genotype was found in the province of Québec with 50 out of 51 (98%). In Ontario, Manitoba, and Saskatchewan, 4 out of 7 (57.1%), 10 out of 23 (43.5%), and 2 out of 2 (100%)

263 were classified in PCV-2b genotype, respectively. Overall, 66 out of 83 (79.5%) PCV-2 entire
264 genome sequences in Canada clustered in the PCV-2b genotype.
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Discussion

The ST, HRT-G, MDCK, and PK15A cell lines were selected because they are known to be permissive to most of the porcine viruses As an example, the ST cells are known to permit the replication of porcine enteroviruses, PPV, TGEV/PRCV, and pseudorabies virus (PRV) (35); the HRT-G cells are known to permit the replication of coronavirus like the porcine hemagglutinating encephalomyelitis virus (36); the MDCK are known to permit the replication of SIV and several other viruses (35, 37); and the PK15A cells were used to isolate PCV-2, and they are known to permit the replication of other viruses like classical swine fever virus, african swine fever virus, vesicular exanthema virus, and vesicular stomatitis virus (35). As mentioned earlier, no virus could be isolated in those 4 cell lines, except PCV-2, from the 13 submitted PMWS cases. Also, no other common viral pathogen could be found by PCR in all 13 PCV-2 positive cases where PMWS disease was observed. Interestingly, even if PCR results from organ samples were negative for the presence of *Mycoplasma* spp, following virus isolation assays, most of cell cultures turned positive for *M. hyorhinis.* Following these results, we concluded that 84.6% of the PMWS cases were also positive for *M. hyorhinis* (Table 2) and that *M. hyorhinis* was the most prevalent pathogen found in the 13 PMWS cases but that the *Mycoplasma* spp PCR diagnostic test was not sensitive enough to detect the pathogen directly in the submitted samples. In fact, only 2 out of the 13 samples cell culture supernatants were negative for *M. hyorhinis* (Table 2). *Mycoplasma hyorhinis* is an extremely common contaminant in cell culture inoculated with swine tissues and *M. hyorhinis* has never been considered to be a major problem in the status of swine health. Nevertheless, it was recently implicated in pneumonia, causing lesions similar to those of *M. hyopneumoniae* (38). *Mycoplasma hyopneumoniae* has been known to be an important cofactor for the induction of PMWS in PCV-2 infected swine (22). Is it possible

that a dual infection with PCV-2 and *M. hyorhinis* led to the same outcome as an infection with PCV-2 and *M. hyopneumoniae*? At this time, no data are available to help us to answer this question. Nevertheless, it would be a fair assumption to believe that since some *M. hyorhinis* strains are able to induce pneumonia (38), they may influence the evolution of PCV-2 infection in swine. In the present situation, since the PCR diagnostic test was not able to detect *M. hyorhinis* in sample homogenates but only in cell culture supernatants, we can assume that the amount of *M. hyorhinis* was very low, suggesting that the degree of pathogenicity of the *M. hyorhinis* strains found in the PMWS cases was also very low. The 2 viruses that were found in higher proportion by PCR in PCV-2 positives cases were PRRSV and swTTV with 61.5% and 69.2% positive samples, respectively (Table 2). It is well known that PRRSV is a major pathogen that can lead to PMWS when present in PCV-2 infected swine (20, 23). The 61.5% PRRSV prevalence in PMWS cases is quite high but similar to what has already been reported by others (39, 40). Until now, swTTV has not been shown to be pathogenic in swine (33). Consequently, the potential role and effect of swTTV during coinfection with PCV-2 is even more obscure and unknown. Nevertheless, as previously reported by others (33), the overall 66.2% prevalence of swTTV infection in swine populations worldwide, is similar to our results in regards to the swTTV prevalence in Canada (Table 2). Interestingly, McKeown et al. (33) reported that in the province of Québec, all tested pig sera were positive for swTTV and that the overall swTTV prevalence in Canada was 79.1%. The swTTV prevalence value may vary a lot between countries (33%-100%), but despite this, it is still very high (33). The swHEV virus is more problematic, since it is known to be able to induce a subclinical infection in swine (41, 42) and mostly because it has to be considered as a zoonotic pathogen (43, 44). Similarly to swTTV, the prevalence of swHEV infection in the swine population is very high, as shown in Table 2, and it may vary a lot between countries (45, 46). Unfortunately, the potential role and effect of swHEV, as well as of swTTV,

during dual infections in swine with PCV-2 remains unknown. Since swTTV and swHEV are recently discovered viruses, many experiments still have to be accomplished to determine the effect of both viruses on animal health status and to determine their potential synergy during dual infections with PCV-2.

Sequence analysis of the entire genome of recent PCV-2 strains in Canada has helped to identify for the first time a new type of circulating PCV-2 strain in North America (Figure 1). In a previous study, Larochelle et al. (8) have shown that the PCV-2 strains circulating in Canada were all clustering in the PCV-2a genotype. The PCV-2 nt sequence identity between our 13 PMWS submitted cases was highly conserved, sharing similarities of 99% to 100%. Interestingly, all these 13 new PCV-2 sequences obtained in 2005 clustered in the PCV-2b genotype and, until now, no other older Canadian PCV-2 entire genome sequences have been reported and classified in the PCV-2b cluster, confirming the fact that a new type of strain has appeared in Canada (Figure 1 and Table 1). Although, even if these new Canadian PCV-2 strains clustered in PCV-2b genotype, other older and recent PCV-2b strains have already been reported in Asia and Europe (Figure 1 and Table 1) (47). Except in this report, no Canadian PCV-2 entire genome sequences have been reported between 2002 and 2005, so it is impossible to pinpoint exactly when the new PCV-2b genotype appeared in Canada. Nevertheless, preliminary results of restriction fragment length polymorphism (RFLP) and gene sequence comparisons reported by Carman et al. (48, 49) suggest that a new PCV-2 genotype, which seems to be related to PCV-2b genotype, appeared in Ontario in 2004 (48-50). France has experienced severe economical loss associated with PMWS in the past, and commercial exchange (swine importation) with Canada during those years may have favored the introduction of the PCV-2b genotype. Furthermore, the appearance of a new type of circulating PCV-2 strains (PCV-2b strains) seemed to coincide with an increased death rate and PMWS in swine herds across Canada and particularly in Québec (18,

50). It is now obvious that the PCV-2b genotype is more prevalent than the PCV-2a genotype in Québec and across Canada (Table 3). The same phenomenon, where the appearance of a new type of PCV-2 strain appeared and coincided with simultaneous increases in clinical PMWS cases, has also been reported in Hong Kong in 2005 (51). Unfortunately, the relationship between the presence of the PCV-2b genotype strains and the increase of clinical PMWS cases in Québec has still to be proven without any doubt. Some have argued that PCV-2b genotype strains are not more pathogenic than PCV-2a genotype strains, since they can be found in swine herds both with or without PMWS (47), an observation also made by our research team (data not shown). Nevertheless, the existence of variations in virulence could not be excluded, since others have reported the existence of PCV-2 pathogenicity variations between mutated viruses (15, 24). In conclusion, it is obvious that there is a new type of PCV-2 strain circulating in Canadian swine herds. However, experimental infections are needed to prove if this new type of PCV-2 strain is more virulent than previous PCV-2 strains found in Canada during the late 90's and early 2000's.

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Table 1. Identification of porcine circovirus 2 (PCV-2) strains with their geographic origin and GenBank accession number

Isolate ID	Geographic origin	GenBank accession number	Reference
FMV-05-6302	Canada/Québec	DQ220739	Gagnon et al., 2006
FMV-05-6317	Canada/Québec	DQ220728	Gagnon et al., 2006
FMV-05-6505	Canada/Québec	DQ220729	Gagnon et al., 2006
FMV-05-6507	Canada/Québec	DQ220730	Gagnon et al., 2006
FMV-05-7098	Canada/Québec	DQ220731	Gagnon et al., 2006
FMV-05-7386	Canada/Québec	DQ220732	Gagnon et al., 2006
FMV-05-7388	Canada/Québec	DQ220733	Gagnon et al., 2006
FMV-05-7389	Canada/Québec	DQ220734	Gagnon et al., 2006
FMV-05-7390	Canada/Québec	DQ220735	Gagnon et al., 2006
FMV-05-7537	Canada/Québec	DQ220736	Gagnon et al., 2006
FMV-05-7539	Canada/Québec	DQ220737	Gagnon et al., 2006
FMV-05-8037	Canada/Québec	DQ220738	Gagnon et al., 2006
FMV-05-8574	Canada/Québec	DQ220727	Gagnon et al., 2006
2A	Canada	AF027217	Hamel et al., 2000
2B	Canada	AF112862	Hamel et al., 2000
2C	Canada	AF109398	Hamel et al., 2000
2D	Canada	AF117753	Hamel et al., 2000
2E	Canada	AF109399	Hamel et al., 2000
Imp. 999	United States	AF055391	Meehan et al., 1998
Imp. 1010-Stoon	Canada	AF055392	Meehan et al., 1998
Imp. 1011-48121 (FRA1)	France	AF055393	Meehan et al., 1998
Imp. 1011-48285 (FRA2)	France	AF055394	Meehan et al., 1998
FRA3	France	AF201311	Mankertz et al., 2000
GER1	Germany	AF201305	Mankertz et al., 2000
GER2	Germany	AF201306	Mankertz et al., 2000
GER3	Germany	AF201307	Mankertz et al., 2000
SPA1	Spain	AF201308	Mankertz et al., 2000
SPA2	Spain	AF201309	Mankertz et al., 2000
SPA3	Spain	AF201310	Mankertz et al., 2000
412	Canada	AF085695	Wang et al., unpublished
M226	Canada	AF086836	Wang et al., unpublished
9741	Canada	AF086835	Wang et al., unpublished
B9	Canada	AF086834	Wang et al., unpublished
ISU-31	United States/Iowa	AJ223185	Morozov et al., 1998
MLTW98 (TA1)	Taiwan	AF154679	Kuo et al., unpublished
Tainan (TA2)	Taiwan	AF166528	Yang et al., unpublished
26606	United States/Utah	AF264038	Fenaux et al., 2000
26607	United States/Utah	AF264039	Fenaux et al., 2000
10489	United States/Illinois	AF264040	Fenaux et al., 2000
40856	United States/Missouri	AF264041	Fenaux et al., 2000
40895	United States/Iowa	AF264042	Fenaux et al., 2000
34464	Canada	AF264043	Fenaux et al., 2000
24657 NL	Netherlands	AF201897	Wellenberg et al., 2000
BF	China	AF381175	Lu et Yang, unpublished
HR	China	AF381176	Lu et Yang, unpublished
BX	China	AF381177	Lu et Yang, unpublished

Table 1. continued

Isolate ID	Geographic origin	GenBank accession number	Reference
Imp. 1103	Canada/Alberta	AJ293867	Meehan et al., 2001
Imp. 1121	Canada/Saskatchewan	AJ293868	Meehan et al., 2001
Imp. 1147	UK	AJ293869	Meehan et al., 2001
IAF2897	Canada/Québec	AF408635	Racine et al., 2004
SH	China	AY291318	Feng et al., unpublished
ZJ	China	AY686764	Feng et al., unpublished
JXII	China	AY732494	Feng et al., unpublished
JS	China	AY691679	Feng et al., unpublished
SX04	China	AY604430	Li et al., unpublished
DG	China	AY682993	Wang et al., unpublished
ZC	China	AY682997	Wang et al., unpublished
ZS	China	AY596823	Da et al., unpublished
NL PMWS 4	UK	AY484416	Grierson et al., 2004
NL control 6	UK	AY484412	Grierson et al., 2004
AUT5	Austria	AY424405	Exel et al., unpublished
GD	China	AY613854	Song et al., unpublished
GD-ZJ	China	DQ017036	Song et al., unpublished
QD	China	AY291316	Xin et al., unpublished
Henan	China	AY969004	Liu et al., unpublished
375	Hungria	AY256460	Dan et al., 2003
HD	China	AY916791	Jiang et al., unpublished
Fd1	France	AY322000	de Boisseson et al., 2004
Fd2	France	AY321999	de Boisseson et al., 2004
Fd3	France	AY321984	de Boisseson et al., 2004

503

Table 2. Identification of viral swine pathogens in postweaning multisystemic wasting syndrome (PMWS) cases

	Polymerase chain reaction								Virus isolation	
PMWS cases	PCV-2	PRRSV	TGEV/ PRCV	SIV	PPV	swHEV	swTTV	*Mycoplasma hyorhinis*[b]	HRT-G/ MDCK/ST	PK15A[c]
FMV05-6302	+	-	-	-	-	-	+	-	-	+
FMV05-6317	+	-	-	-	-	-	+	+	-	+
FMV05-6505	+	+	-	-	-	-	+	+	-	-
FMV05-6507	+	+	-	-	-	+	+	+	-	+
FMV05-7098	+	+	-	-	-	-	+	-	-	-
FMV05-7386	+	+	-	+	+	-	-	+	-	+
FMV05-7388	+	+	-	-	-	+	-	+	-	+
FMV05-7389	+	+	-	-	-	-	-	+	-	-
FMV05-7390	+	+	-	+[a]	-	+	+	+	-	+
FMV05-7537	+	-	-	+[a]	-	+	-	+	-	-
FMV05-7539	+	-	-	-	-	-	+	+	-	-
FMV05-8037	+	-	-	-	+	-	+	+	-	-
FMV05-8574	+	+	-	+	-	+	+	+	-	-

[a] ***Swines influenza virus*** **have been confirmed by PCR to be H3N2 virus.**

[b] **Confirmed by PCR on the 3rd passage of cell culture supernatants.**

[c] **Only PCV-2 was isolated in PKA cells and it was confirmed by PCR at the 3rd passage.**

PCV-2 = porcine circovirus 2, TGEV/PRCV = transmissible gastroenteritis virus/porcine respiratory coronavirus, PRRSV = porcine reproductive and respiratory syndrome virus, SIV = swine influenza virus, PPV = porcine parvovirus, swHEV = swine hepatitis E virus, swTTV = swine torque teno virus, HRT-G = human rectal tumor cells, MDCK = Madin-Darby canine kidney cells, ST = swine testis cells, PK15A = porcine kidney cells

4

Table 3. Genotype classification of 2005-2006 porcine circovirus 2 (PCV-2) Canadian strains[a] following viral genome sequence analysis

Canadian Provinces	Number of PCV-2 sequenced	Genotype classification	
		PCV-2a	PCV-2b
Québec	51	1	50
Ontario	7	3	4
Manitoba	23	13	10
Saskatchewan	2	0	2
Total:	83	17	66

[a]Most of those viruses were obtained from PCVAD affected herds but some of them were obtained from herds that shown no clinical sign related to this disease.

505

506

FIGURE LEGENDS

Figure 1. Phylogenetic analysis of the complete genome of porcine circovirus 2 (PCV-2) strains. An unrooted neighbor-joining tree was constructed from aligned nucleic acid sequences of 27 Canadian reference strains (including the newly described 13 sequences identified with an arrow and the older sequences identified with an asterix) and 43 sequences found in GenBank. Original names, country of origin, and GenBank accession number are given in Table 1.