High-throughput sequencing revealed the presence of an unforeseen parvovirus species in Canadian swine: the porcine partetravirus

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CROSS-CANADA DISEASE REPORT /

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During the course of a research project on full viral genome sequencing of porcine reproductive and respiratory syndrome (PRRS) virus, 62 samples of swine sera were collected from piglets from various Canadian farms that were experiencing PRRS outbreaks. In order to lower the viral genome contamination of serum samples by the host genome (i.e. swine genome) for the high-throughput sequencing (HTS) procedure, viral particles were purified by ultracentrifugation (113 000 *g* on a 20% sucrose cushion) followed by a nuclease treatment (1 h at 37˚C with 500 µg/mL DNase and 250 µg/mL RNase A). The viral genome was then extracted with Trizol reagent (Invitrogen, Burlington, Ontario) according to the manufacturer's recommended procedure. Genomic material was subjected to random PCR amplification as previously described (1). Qualitative and quantitative evaluations of the random amplification products were respectively assessed by agarose gel electrophoresis and by real-time PCR (EZ-PRRSV™ MPX 4.0 Real Time RT-PCR Target-Specific Reagents for the Rapid Identification and Differentiation of North American and European PRRS Viral RNA, Tetracore, Rockville, Maryland, USA). Based on those results, a total of 11 samples, comprised of 6 samples from Québec, 4 from Ontario and 1 from Alberta, were selected and sent to the "Institut de biologie integrative et des systems" (IBIS) for HTS (Table 1). For each sample, a GS-FLX rapid library was produced from amplified genomic material using the manufacturer's instructions provided with the kit (Roche/454 Sequencing, Brandford, Connecticut, USA). Emulsion PCR and GS-FLX titanium sequencing was performed according to the manufacturer's instructions (Roche/454 Sequencing) at the Plateforme

d'Analyses Génomiques of the IBIS (Laval University, Québec City, Québec). Raw sequencing reads were assembled using the gsAssembler module of Newbler v.2.5.3.

In addition to PRRS virus genome sequences, HTS results revealed the presence of a swine parvovirus highly similar to the porcine partetravirus (a parvovirus also known as porcine hokovirus, porcine PARV4-like virus and porcine parvovirus 3) in one of the sera collected in Ontario (Table 1) (2, 3). Parvoviruses are small non-enveloped icosahedral viruses with a linear single-stranded DNA genome of about 4 to 6.3 kb. Based on this result, porcine partetravirus prevalence in Canadian swine herds was established to be 9.09% (Table 1). However, this estimate of the prevalence of porcine partetravirus in Canada may be biased because: 1) the sera were selected in regards to their PRRS virus positive status, 2) the amount of tested samples was low, and 3) the geographical origins of the tested sera did not accurately represent the overall Canadian swine industry. To our knowledge, the porcine partetravirus has never been isolated in cell culture, although virus isolation has been attempted by Lau et al (2008) and Streck et al (2013) (4, 5). Currently, 5 porcine parvoviruses (PPV) have been identified in swine: porcine parvovirus 1 (PPV1), PPV2, porcine partetravirus (PPV3), PPV4 and PPV5, which has been recently reported in the United States (2). PPV1 through PPV5 have substantial genetic divergence when they are compared to one another. The porcine partetravirus is genomically related to the human parvovirus 4 (60-65% nucleotide homology). The human parvovirus 4 was identified in 2005 from a plasma sample of a homeless drug user with an acute viral infection (6). The porcine partetravirus was reported for the first time in China in 2008 (5). Since then, the porcine partetravirus has been reported in other countries, including United States, Great-Britain, Romania, Hungary and

Germany (5,7-10). To our knowledge, the porcine partetravirus has not previously been reported in Canada.

The identified Canadian porcine partetravirus strain, named FMV10-1437266, possesses a genome length of 5 402 nucleotides (nt) (GenBank accession number KC992732) and has a GC content of 51.29%. After HTS procedure, the FMV10-1437266 genomic sequence was assembled from 938 reads, with a mean coverage of 48 reads per nt (minimum coverage of 8 reads and maximum coverage of 85 reads). The FMV10-1437266 partetravirus strain has a genome nearly 300 nt larger than that of the longest reported porcine partetravirus that was isolated in China in 2009 (GenBank accession number EU200677). This increased nt genomic length of the FMV10-1437266 gives a better insight into the inverted terminal repeats (ITR) found at both 5' and 3' ends of the porcine partetravirus. The ITR are involved in parvovirus genome replication (3). Genomic sequence comparison of the porcine partetravirus FMV10-1437266 with previously reported strain sequences gathered from the GenBank database revealed sequence identities varying between 95.4% and 99.3% (data not shown) which is in accordance with previous reports (4,8,10,11). A phylogenetic tree (Figure 1) was constructed by the neighbor-joining method with 1000 bootstrap replicates with the Geneious Pro (version 5.6.6) software. The genomic comparison was done using the longest common nt sequences between all compared partetravirus genomic sequences (which correspond to nt positions 292 to 5 074 of FMV10-1437266). Phylogenetic analysis revealed that the porcine partetravirus FMV10-1437266 is highly similar to a U.S.A. strain detected in Iowa in 2011 (GenBank accession number JQ425257). In U.S.A., porcine partetravirus has also been reported in North-Carolina (7).

The classical porcine parvovirus (PPV1) is an important pathogen associated with reproductive disorders of sows and is widely distributed around the world, including Canada (10). The identification of novel porcine parvoviruses (such as PPV2, porcine partetravirus, PPV4 and PPV5) raises concerns about their potential involvement in swine health and manifestations of clinical signs or disease. At present, the data are sparse and do not prove with certainty their involvement in swine diseases (12). Porcine partetravirus has not been found to be an etiological agent of swine disease and is currently considered to be non-pathogenic. Nonetheless, Dr Opriessnig's research team has revealed that the prevalence of porcine partetravirus in pigs with respiratory diseases, systemic/central nervous system diseases, and enteric diseases was 14.4%, 11.6% and 2.7%, respectively (7). These results suggest that the porcine partetravirus may be associated with diseases of these systems but this still needs to be proven. Porcine partetravirus has been detected in porcine blood pharmaceutical by-products such as plasma and Factor VIII (FVIII). Porcine derived FVIII is being used to treat a human autoimmune hemophilia in which patients possess antibodies against FVIII. Thus, the presence of porcine partetravirus in swine blood and subsequently in pharmaceutical by-products may represent a potential threat for human health (11). In swine, the porcine partetravirus was detected in adult pigs and nursery pigs, and was not detected in fetuses or suckling pigs (7). In the near future, experiments will be conducted to establish with precision the prevalence of porcine partetravirus in the Canadian swine population. Moreover, further experiments are needed to ascertain the involvement of porcine partetravirus in swine diseases.

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Figure 1

Phylogenetic tree of the genomic nucleotide sequences of porcine partetravirus strains. The phylogenetic tree was generated by the neighbor-joining method using the Geneious V5.6.6 software with a bootstrap resampling method (1 000 replications) using the longest common nucleotide sequences between all compared partetravirus genomic sequences. Bootstrap confidence levels are indicated at the nodes of the phylogenetic tree. Genbank accession numbers for the sequences of all viruses are provided as well as their countries of origins and their date of collection (year). The horizontal scale indicates the distances between strains; a distance of 0.0040 means that the strains possess 99.6% nucleotide identity. The arrow indicates the porcine partetravirus detected in the Ontario swine serum.