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BRIEF REPORT

Genetic diversity of group A rotavirus in swine in Canada

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Abstract Group A rotaviruses (RVA) in pigs have been poorly investigated in Canada. In a continued effort to fill this gap, ten finisher swine farms in Quebec, Canada, were sampled over a nine-month period. The presence of RVA was detected in healthy pigs on all farms investigated during the entire sampling period. The genotypes detected included G2, G5, G9 and G11; P[6], P[7], P[13], P[27] and P[34]; and I5 and I14. The predominant types were G2, P[13] and I5, which is different from previous global reports. Various fomites were consistently contaminated by RVA, suggesting that a resident viral flora remains in the farm environment and may play a role in the infection of incoming pigs. The results also suggest temporal or geographical specificities regarding strain distribution on pig farms.

Keywords Rotavirus A · Swine · Phylogeny · Genetic diversity · Zoonoses · Canada

Rotaviruses (RVs) are important etiological agents of severe neonatal diarrhea in humans and animals including cattle, swine, horses, dogs, cats, chickens and turkeys. The World Health Organization estimates that 453,000 children aged less than 5 years, most from developing countries, died from RV infection in 2008, which accounts for 5 % of global child death [54]. In Canada, RVs cause 10-40 % of childhood gastroenteritis, resulting in considerable direct medical costs, mainly from hospitalization, and societal costs such as loss of work time from parents [22, 26]. Economic losses due to this diarrheal disease are also recognized in livestock, such as swine, due to medical treatment and decrease in performance [49]. RV infection is known to cause a diarrheal disease in suckling and weaned pigs that is usually resolved in 2-3 days if not complicated by secondary microbial infections [57]. In fact, group A rotaviruses are one of the most frequent viral agents detected in diarrheic piglets from 1-8 weeks of age [50]. Asymptomatic RV infections are also known to occur in pigs of all ages [5, 27, 52].

RVs are non-enveloped viruses belonging to the genus *Rotavirus* within the family *Reoviridae*. RVs have an 11-segmented double-stranded RNA genome, which encodes six structural proteins (VP1 to VP4, VP6 and VP7) and five or six non-structural proteins (NSP1-NSP6) [10]. RVs are genetically highly diverse, with eight known serogroups (A-H) based on antigenic properties and nucleotide sequence of the inner viral capsid protein 6 (VP6) [40]. Although rotaviruses of groups A, B and C infect humans and animals, group A rotavirus (RVA) is considered the most important RV group because of its high prevalence and pathogenicity [10, 34, 37]. RVAs have two outer capsid proteins, VP4 and VP7, both of which are important for inducing neutralizing antibodies and protective immunity. A binary classification system for RVAs was established based on the nucleotide sequences of VP4 and VP7 genes and is continuously updated by the

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Rotavirus Classification Working Group (RCWG). So far, a total of 35 P types (VP4) and 27 G types (VP7) have been described [39].

Although specific G and P genotypes are generally restricted to specific animal species, implying host preference, a number of genotypes have been detected in multiple species, including humans, which suggests interspecies transmission [36]. For example, a number of studies have suggested a porcine origin of G3, G4, G5, G9, P[6] and P[7] segments detected in human, equine or bovine clinical specimens [14, 20, 21, 34]. In addition, a number of RVA segments detected in swine display human-like or bovine-like genotypes, indicating that reassortment events can occur between RVAs originating from different animal species [13, 17, 31, 32, 47]. Complete genome sequencing of RVAs also revealed a close evolutionary relationship between human Wa-like and porcine strains [35]. Overall, these findings suggest that swine represent a significant gene pool for human RVAs and those of other animal species and emphasize the need for surveillance studies to better understand the ecology of these viruses and their zoonotic potential. Since there is limited information about RVA strains circulating in the human and animal population in Canada, this study was designed to provide additional data on swine RVA diversity by investigating VP4, VP6 and VP7 genetic heterogeneity on ten finisher swine farms.

Stool samples and viral RNA extraction

A total of 10 finisher swine farms (farms A-J) located in the province of Quebec, Canada, were part of a project investigating transmission patterns and epidemiology of enteric pathogens (bacteria and viruses) between the farms and a slaughterhouse through various stakeholders, using molecular fingerprints. Farms were situated within a 60-km radius and housed an average of 1300 animals. From June 2011 to February 2012, 4-5 composite fecal samples (approximately 20 g each) were collected twice: once between the months of June and August and once between the months of September and February. Each composite sample originated from three different pens that were randomly chosen within each farm to increase the range of RVA strains, with the hope that some might be specific for certain farms and act as tracers in the epidemiological investigation. There were a total of 98 composite samples in this study. Pens housed an average of 20 asymptomatic finisher pigs (between 12 and 22 weeks of age). Environmental samples were collected by wiping four different surface types (landing stages, doors, fan blades and portable solid panels) ranging from 20 cm^2 to 40 cm^2 with a sterile cloth. These were collected twice on each farm for a total of 80 samples. All samples were transported to the laboratory chilled on ice and stored at −80 °C until analysis. Twenty percent faecal homogenates were prepared in Eagle's minimum essential medium (MEM) (Invitrogen, Mississauga, ON, Canada), filtered twice with 0.45-µm and 0.22-µm filters (Sarstedt, Nümbrecht, Germany), and frozen at −70 °C. Environmental samples were homogenized in 15 mL of phosphate-buffered saline medium (PBS) (Invitrogen, Mississauga, ON, Canada), mixed with 0.1 gram of polyvinylpyrrolidone (Sigma-Aldrich, St-Louis, MO, USA), and spiked with a 100-µL suspension of murine norovirus (10^4 viral particles/mL) as an internal control. Following double filtration with 0.45-µm and 0.22-µm filters, samples were subjected to ultrafiltration by centrifugation at 5,000×*g* on Amicon® columns (Fisher Scientific, Ottawa, ON, Canada) until a final volume of 250 µL was obtained, and they were frozen at −70 °C if not used immediately. RNA extraction was performed using a QIAamp Viral RNA Mini Kit (feces) or RNeasy Mini Kit (environmental samples) (QIAGEN, Mississauga, ON, Canada) according to the manufacturers' protocols.

RT-PCR amplification

Partial VP4 (VP8*) and full-length VP6 and VP7 segments were amplified by reverse transcription PCR (RT-PCR) using a QIAGEN One Step RT-PCR Kit (QIAGEN, Mississauga, ON, Canada) with primers Con3-Con2 [12], GEN_VP6F-GEN_VP6R [36] and Beg9-End9 [16], respectively (see Table 1 for primer sequences). Viral RNA was treated for 3 min at 97 °C in order to denature the double-stranded rotavirus RNA segments and quickly chilled on ice. RT-PCR was performed using 10 µM of each primer in a 25-µL final volume. Reverse transcription was carried out at 45 °C for 30 minutes, followed by denaturation for 15 minutes at 95 °C. DNA amplification was performed for 40 cycles consisting of 30 seconds at 94 °C, 45 seconds at 45 °C, and 120 seconds at 68 °C, followed by a final elongation of 30 min at 68 °C. Amplification products were analysed using a QIAxcel capillary electrophoresis device (QIAGEN, Mississauga, ON, Canada). Samples that contained DNA fragments of the expected molecular weight (Table 1) were separated by electrophoresis in a 1 % agarose gel containing SYBR Safe (Invitrogen, Mississauga, ON, Canada) and visualized under UV light.

Sequencing and phylogenetic analysis

Amplicons of the expected molecular weight were extracted from the gel and purified using a QIAquick PCR Purification Kit (QIAGEN, Mississauga, ON, Canada)

Table 1 List of primers used in this study

Primer	Gene	Sequence (5′-3′)	Reference	Amplicon size
con3	VP4	TGGCTTCGCTCATTATAGACA (F)	Gentsch et al. [12]	877 bp
con2	VP4	ATTTCGGACCATTTATAACC (R)	Gentsch et al. [12]	
GEN_VP6F	VP6	GGC TTT WAA ACG AAG TCT TC (F)	Matthijnssens et al. [35]	1356 bp
GEN_VP6R	VP6	GGT CAC ATC CTC TCA CT (R)	Matthijnssens et al. [35]	
Beg9	VP7	GGCTTTAAAAGAGAGAATTTCCGTCTGG (F)	Gouvea et al. [16]	1062 bp
End9	VP7	GGTCACATCATACAATTCTAATCTAAG (R)	Gouvea et al. [16]	
M13F-20	—	GTAAAACGACGGCCAGT-3′ (F)	—	—
M13R-17	—	CAGGAAACAGCTATGAC-3′ (R)	—	

according to the manufacturer's instructions. Amplicons were cloned using a pGEM-T vector system kit (Promega, Madison, WI, USA). Between one and six clones for each sample were sequenced using the Big Dye v3.1 chemistry on a 3730xl instrument from Applied Biosystems (Foster City, CA). VP4 and VP7 clones were sequenced in one direction, while those of VP6 were sequenced in both directions due to the sequence length, using the plasmid M13F-20/M13R-17 primers (Table 1). Sequence alignments and editing were performed using ClustalW and Geneious Pro version 5.5.6 created by Biomatters (http://www.geneious.com/). Sequences were analyzed using the Basic Local Alignment of Sequences Tool (BLAST; http://www.ncbi.nlm.nih.gov/) with default values and the RotaC$^{2.0}$ automated genotyping tool for Group A rotaviruses to confirm RVA identity of all sequences [29]. Phylogenetic trees and molecular analysis were conducted using the software MEGA® version 5.0 using the neighbor-joining algorithm and the maximum composite likelihood model with all gaps ignored. Statistical support was obtained by 1000 bootstrap replications [53]. The MEGA® 5.0 software was used to calculate genetic distances between sequences using the p-distance algorithm.

Sequences from this study were deposited in the GenBank database under the following accession numbers: B48-B (VP7: KF501099); E83-A (VP7: KF501100); C57 (VP7: KF501101); D68-A (VP7: KF501102); B48-A (VP7: KF501103); D69 (VP7: KF501104); A11 (VP7: KF501105); G98 (VP7: KF501106); E85-036 (VP7: KF501107); C180-1 (VP7: KF501108); OB472 (VP7: KF534786); F432-3 (VP6: KF501109); F431-1 (VP6: KF501110); A260-4 (VP6: KF501111); D330-1 (VP6: KF501112); B42-B (VP4: KF501113); OE658 (VP4: KF501114); A4 (VP4: KF501115); E81-A (VP4: KF501116); H117-C (VP4: KF501117); D65 (VP4: KF501118); I157-A (VP4: KF501119); F93 (VP4: KF501120); A11 (VP4: KF501121); H119-B (VP4: KF501122); C182-A (VP4: KF501123); H117-A (VP4: KF501124); G98-A (VP4: KF501125); B42 (VP4: KF501126); H117 (VP4: KF501127).

A total of 48 out of 98 composite fecal samples (49 %) were confirmed positive for RVA by sequencing at least one of three investigated segments (VP4, VP6 or VP7). Positive samples were found on all farms (10/10). On eight farms, multiple G types and/or P types were identified (between 2 and 4 different G or P types). Five farms had two different I types (I5 and I14). RVAs were consistently found throughout the three sampling seasons on all farms (summer, fall and winter).

Sequence analysis of the VP7 segment

Based on a nucleotide cutoff value of 80 %, a total of 27 VP7 G genotypes have been established by the Rotavirus Classification Working Group (RCWG) [39]. Using the RotaC$^{2.0}$ automated genotyping tool for Group A rotaviruses [29] and Blastn, a total of 41 RVA strains from this study were successfully G-typed as G2, G5, G9 and G11 (Fig. 1a). For each genotype, a representative strain chosen among clusters sharing $\geq$ 95 % nucleotide (nt) identity was used in the phylogenetic tree reconstruction for more clarity.

A total of 24 out of 41 strains (59 %) were determined to be G2-type. Five are shown in Figure 1a, and these shared a mean nucleotide sequence identity of 92 %. Strains D69, E85-036 and A11 showed high nucleotide sequence identity (mean, 92 %) to porcine strain RVA/Pig-wt/CAN/CE-M-06-0003/2005/G2P[27] detected in Canada [25], whereas strains C180-1 and G98 clustered closely with Canadian porcine strain RVA/Pig-wt/CAN/F8-4/2006/G2P6[7] [30] (92 % mean nt identity). These five strains had a mean nucleotide sequence identity of 82 % to reference strain RVA/Pig-wt/THA/CMP034/2000/G2P[27] isolated from a piglet in Thailand [23].

A total of 8 out of 41 strains (20 %) belonging to genotype G5 were detected in this study, and four were used as representative strains in the alignments (Fig. 1a). The four G5 representative strains were genetically heterogeneous, sharing a mean nucleotide sequence identity of 88 %. Strain B48-B shared 94 % nucleotide sequence

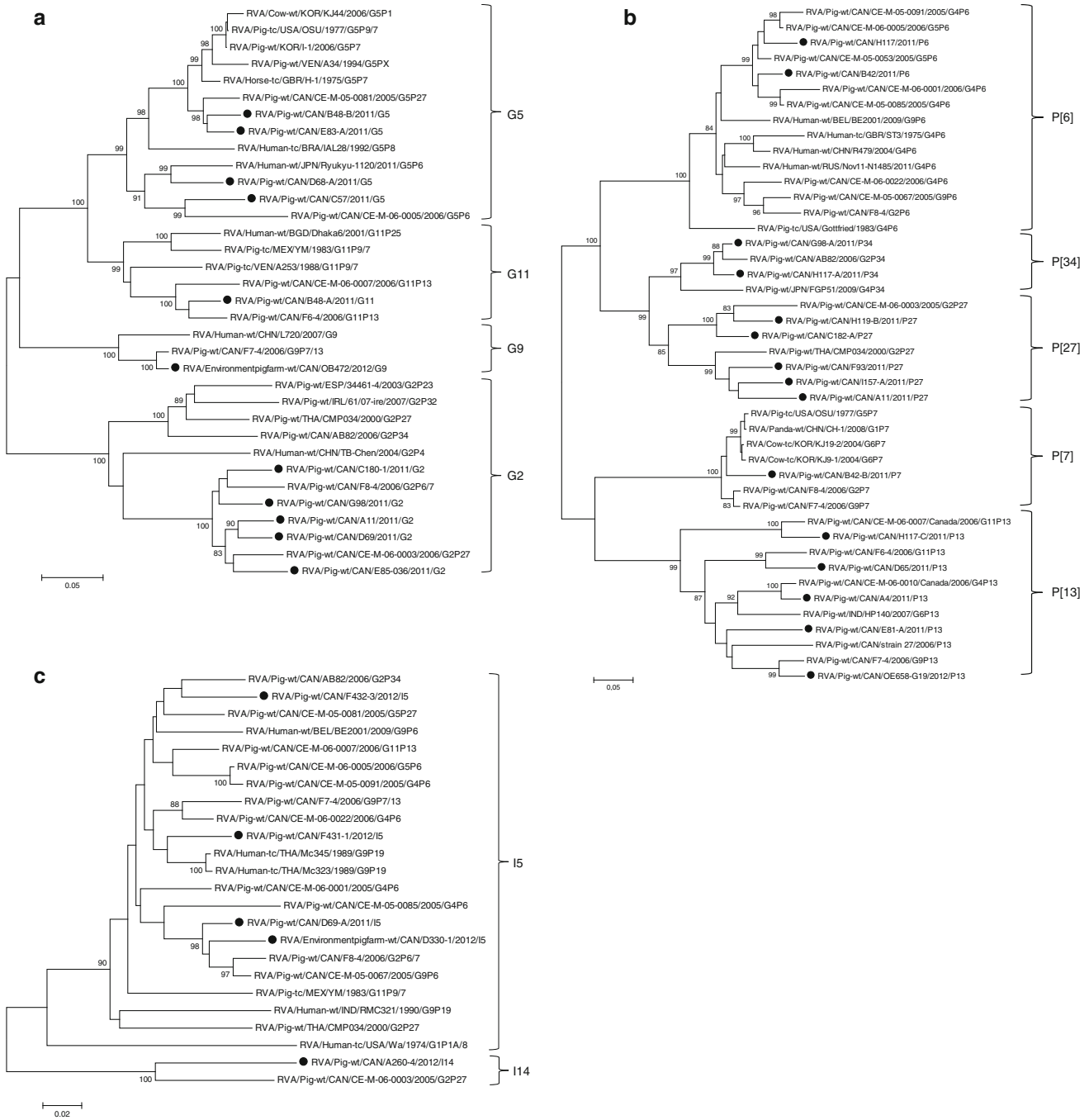

Fig. 1 Phylogenetic trees based on nucleotide sequence alignments of the complete VP7 gene (**a**), a partial VP4 gene (**b**), and the complete VP6 gene (**c**) of reference strains and strains from this study. VP7, VP4 and VP6 genotypes are shown on the right side of each tree. Strains identified in this study are indicated by a filled circle. Bootstrap values above 80 % are shown at the branch nodes

identity with porcine strain RVA/Pig-wt/CAN/CE-M-05-0081/2005/G5P[27] from Canada and strain RVA/Horse-tc/GBR/H-1/1975/G5P[7] detected in the stool of a young foal in 1975 [14, 25]. Interestingly, strain D68-A was most closely related to human strain RVA/Human-wt/JPN/Ryukyu-1120/2011/G5P[6], which was detected in the stool of a 4-year-old child and is suspected to be of porcine origin (91 % nt identity) [24]. Strains C57 and E83-A were most closely related to porcine strains RVA/Pig-wt/CAN/CE-M-06-0005/2006/G5P[6] (88 % nt identity) and CE-M-05-0081 (95 % nt identity), respectively [25]. The nucleotide sequence identity of the four G5 strains from this study to the canonical porcine G5 strain OSU (RVA/Pig-tc/USA/OSU/1977/G5P9[7]) ranged from 84-96 % [36].

A total of 9 out of 41 (22 %) VP7 G11-type strains were detected in four out of the ten swine farms in this study. The nine strains shared over 95 % nucleotide sequence identity, and therefore, a single strain (B48-A) was selected as representative for this genotype. B48-A showed highest genetic identity to strain RVA/Pig-wt/CAN/F6-4/2006/G11P[13] from a Canadian pig (94 % nt identity) [30]. Nucleotide sequence identity to G11 porcine reference strains RVA/Pig-tc/MEX/YM/1983/G11P9[7] and RVA/Pig-tc/VEN/A253/1988/G11P9[7] was 86 % and 89 %, respectively [36, 38].

Sequence analysis of the VP4 segment

Using the 80 % nucleotide cutoff value established to distinguish 35 P genotypes [39], strains from this study were classified into five different VP4 types by the RotaC$^{2.0}$ online tool and Blastn analyses. A total of 48 strains from the present study were P-typed as P[6], P[7], P[13], P[27] and P[34] (Fig. 1b). As was done for the G types described above, representative strains were chosen within groups of strains showing $\geq$ 95 % nucleotide sequence identity for better clarity of the dendrograms.

A total of 5 out of 48 (10 %) P[6]-type strains were detected, and these formed two clusters, represented by strains B42 and H117. These two strains shared 93 % nucleotide sequence identity with each other and were genetically closest to a group of swine strains from Canada (94 % mean nt identity) (Fig. 1b) [25]. Interestingly, both strains had high nucleotide sequence identity (85 % and 88 %) to strain RVA/Human-wt/BEL/BE2001/2009/G9P[6] and strain RVA/Human-wt/RUS/Nov11-N1485/2011/G4P[6] from Belgium and Russia, respectively, both of which were recently described as porcine-like human strains [58, 59].

A single P[7]-type strain (B42-B) (2 %) was detected that shared 93 % nucleotide sequence identity with porcine/bovine reassortant RVA strains (RVA/Cow-tc/KOR/KJ9-1/2004/G6P[7] and RVA/Cow-tc/KOR/KJ19-2/2004/G6P[7]) isolated from cattle in Korea and a porcine-like RVA strain detected in a panda from China (RVA/Panda-wt/CHN/CH-1/2009/G1P[7]) [19, 46]. Strain B42-B was also related (93 % nt identity) to Canadian porcine strains F8-4 and RVA/Pig-wt/CAN/F7-4/2006/G9P[7/13] [30].

A total of 25/48 (52 %) strains were of the P[13] type. Five representative strains were selected, and these shared nucleotide sequence identity ranging from 75 % to 82 % (mean, 79 %). Strain A4 shared 96 % nucleotide sequence identity with strain RVA/Pig-wt/CAN/CE-M-06-0010/2006/G4P[13] [25]. Strains E81-A and OE658 shared 82 % and 93 % nucleotide sequence identity, respectively, with Canadian strain F7-4, whereas strain D65 was found to be genetically closest to porcine strain F6-4, also from Canada (91 % identity) [30]. Strain H117, which was the most genetically distant (76 % mean nt identity) to other P[13]-types from this study, shared 93 % nucleotide sequence identity with Canadian strain RVA/Pig-wt/CAN/CE-M-06-0007/2005/G11P[13] [25].

A total of 11 out of 48 (23 %) P[27]-type strains were detected, of which five were selected as representatives, and these shared a mean nucleotide sequence identity of 82 % (Fig. 1b). Three strains (I157-A, F93 and A11) were genetically closest to strain RVA/Pig-wt/THA/CMP034/2000/G2P[27] detected in a swine from Thailand (84-87 %) [23]. Two strains (H119-B and C182-A) shared 87 % nucleotide sequence identity with the Canadian porcine strain CE-M-06-0003 [25].

A total of 6 out of 48 strains (13 %) were classified as P[34] type. Strains H117-A and G98-A were selected as representatives, and they shared highest nucleotide sequence identity (93-96 %) with strain RVA/Pig-wt/CAN/AB82/2006/G2P[34] from a Canadian swine [30].

Sequence analysis of the VP6 segment

A total of 17 VP6 I types have been defined based on an 85 % nucleotide cutoff value [18, 39]. Thirty-two strains from this study were successfully I-typed, of which 17 were of the I5 type (53 %) and 15 strains were of the I14 type (47 %) (Fig. 1c). For clarity, three strains were chosen as representative for the I5-type group of sequences (based on $\geq$95 % nucleotide sequence identity within the same cluster): D69-A, F432-3 and F431-1. The three strains were related to Canadian swine strains (F8-4, RVA/Pig-wt/CAN/CE-M-05-0067/2005/G9P[6], AB82, CE-M-05-0081 and RVA/Pig-wt/CAN/CE-M-06-0022/2006/G4P[6]) with nucleotide sequence identities ranging from 93 to 96 % [25, 30]. Interestingly, strain F431-1 clustered on the same branch as human strains RVA/Human-tc/THA/Mc345/1989/G9P[19] and RVA/Human-tc/THA/Mc323/1989/G9P[19] from Thailand, both of which are believed to be of porcine origin (95 % nt identity) [15]. Strain A260-4 was the only representative strain for the newly characterized I14 type and shared 91 % identity at the nucleotide level with the canonical strain CE-M-06-0003 [25].

Analysis of environmental samples

Molecular detection revealed the presence of RVAs on 42 out of 80 fomites sampled. Of these, 27 were G and/or P typable. Interestingly, molecular characterization showed that on 6 out of 10 farms, different RVA genotypes were present in environmental samples compared to faecal

samples. Specifically, a total of 3 out of 24 environmental samples contained unique G-types, and 12 out of 35 environmental samples contained unique P-types that were not found in faecal samples from the corresponding farm. Further, strain OB472, which was detected on the landing stage of farm B and identified as a G9 type, is the only G9 type that was detected in all samples (fomites and faecal) from all 10 farms (Fig. 1a). Sequence analysis of this strain revealed 98 % nucleotide sequence identity to porcine strain F7-4 from Canada [30]. In many cases, however, the RVA strains detected in faecal samples and fomites within the same farm showed high nucleotide sequence identities (95 %), such as I5 type strain D69-A (feces) and D330-1 (fan blades), as would be expected (Fig. 1c).

Rotaviruses are known for their genetic diversity due to mechanisms such as point mutations, recombination and reassortment attributable to their segmented genomes [10]. With the advent of human RVA vaccination, coupled with large-scale surveillance studies of human and animal strains, there is now compelling evidence supporting zoonotic transmission of complete and reassorted RVA strains. Reassortment of RVA strains from different species can cause the emergence of new strains bearing properties derived from both parental lineages. Such transmissions from swine to humans are known to have occurred on frequent occasions [15, 34, 51, 55]. Since swine are considered a large reservoir for RVAs, the characterisation of circulating strains in the pig population is of pivotal importance, notably for public health issues, but also from an ecological and animal health perspective. Canada is an important swine-producing country, with over 21 million hogs slaughtered in 2012 (http://www.canadapork.com). However, very little is known about porcine RVA genetic diversity and zoonotic potential in this country. Hence, this study aimed at investigating the heterogeneity of RVA strains on ten finishing pig farms.

In this study, all farms harbored RVAs in faecal samples and fomites, and most had up to four different RVA genotypes based on G and P sequences. Similar diversity of RVA strains has been reported previously within single swine production sites [4, 5, 43]. Phylogenetic analysis revealed that most RVA strains characterized in the present study were related to porcine strains previously described in Canada and globally, and they were therefore considered typical swine strains (Fig. 1a, b and c). Nonetheless, a number of the RVA types detected are also considered epidemiologically relevant to infections of humans and other animal species. For instance, the G2, G5 and G11 VP7 types detected in swine in this study have also been detected in other animal species, including humans. In 2005, Martella and colleagues reported for the first time genetic relatedness between a porcine RVA strain from a diarrheic piglet and human G2 RVA strains [32]. Another similar strain was also detected in a symptomatic piglet from Thailand [23]. Furthermore, unusual G5 strains were detected in symptomatic children from Latin American and Asian countries, as well as in a young foal. Following sequence analysis, genome segments of these strains were suspected to be of porcine origin [1, 2, 7, 9, 28]. In addition, reassortment events between porcine and human G11 RVA strains have been identified recently [38, 55]. Swine VP4 types P[6] and P[7] are also known to be genetically related to human and bovine RVA strains, respectively [33, 45, 46, 58]. VP4 segments P[6] and P[7] from this study (B42, H117 and B42-B) shared a high level of sequence identity with human, bovine and panda strains, suggesting interspecies transmission events. Although the VP6 types from this study were mostly related to porcine strains, I5 VP6 strain F431-1 appeared genetically related to human strains Mc345 and Mc323 from Thailand, which are suspected to be of porcine origin [15]. Overall, these results reveal the existence of frequent spillover events between RVAs infecting pigs, humans, and other species and underpin their complex evolutionary paths.

The predominant VP7, VP4 and VP6 genotypes from this study were G2, P[13] and I5. These results differ from a recent review by Papp and colleagues, who reported G5P[7] to be the most prevalent genotype combination for porcine RVA strains in America based on a total of 20 reports, of which three were from Canada [45]. These authors also noticed country-specific temporal changes in G-type and P-type predominance for Canada, Thailand and Spain. In Canada specifically, the predominant constellation type was reported to be G4P[6]I5 [25]. Those results contrast with the ones presented here and suggest either temporal and/or geographical variations in RVA genotype patterns, although sampling biases cannot be completely ruled out. Similarly, it is interesting to note that the I14 type has only been reported from Canadian swine so far. Previous studies have also detected differences in rotavirus prevalence as well as geographical and/or temporal changes in RVA genotypes [3, 8, 41]. These findings highlight the importance of continuous, thorough and local RVA strain surveillance to increase our knowledge about the evolution of RVAs at the population level.

Although all G, P and I genotypes reported here have been identified previously in swine, little is known about the P[34] type. Discovered for the first time in swine from Japan (AB571047), this P-type was subsequently detected in a single sample from Canada in 2006 [30]. To our knowledge, these are the only two countries where P[34] has been identified in swine. The prevalence of this particular type therefore appears low. This seemingly low prevalence of the P[34] type is intriguing. One possibility for the apparent low prevalence of this P type is low virulence or low transmissibility of P[34] strains. Another

explanation could be that this genotype is emerging in swine from a yet to be identified reservoir, although technical limitations such as primer efficiency cannot be ruled out. Nevertheless, the P[34] type represented 13 % (n=6) of the strains characterized on two farms from this study. It is tempting to speculate that these two farms were probably epidemiologically related, but this hypothesis could not be confirmed. The finding of the P[34] type underscores the importance of large-scale studies in bringing new knowledge about the diversity of RVAs and leaves open questions about the differences in virulence and environmental resilience of RVA strains.

Rotaviruses are known for their environmental resilience; they have been shown to maintain infectivity in feces for up to 9 months at room temperature (18-20 °C) and have also been detected in dust and dried feces on premises previously occupied by young pigs [57]. It is thus plausible that the farm environment contributes to the diversity of RVA strains found at each site by infecting incoming pigs. In this study, specific G and P types found in the farm environment were sometimes different from those detected in animal fecal samples of the same farm. These results suggest that "resident" strains might persist on premises and have the potential to contribute to viral diversity at farm sites. It would be interesting to investigate the potential of the environmental contamination to infect incoming naïve animals and/or to reassort with strains harbored by those animals, as has been suggested recently [42, 43]. Since finishing herds often include pigs from different nursery sites, it would be interesting to investigate the relative importance of these multiple sources of infection in terms of RVA diversity found in pigs.

Although extensive, the genetic diversity of the RVAs reported here is most likely underestimated. For instance, 30/48 (63 %) of RVA-positive samples did not yield amplicons for all three segments investigated. Of these samples, 63 % (19/30) lacked VP6 genotype information, while VP7 and VP4 were missing in 40 % and 37 % of samples, respectively. Suboptimal PCR primers are the most likely explanation for such failures. As more RVA sequences become available, alternative primers can be designed that could possibly reveal yet unforeseen diversity. Another important limitation of this study is the sampling design, in which 10 farms situated in the same geographical region were sampled. This clearly represents a small fraction of the swine industry of this country and, again, most likely underestimated the true RVA variability present in Canadian swine.

RVAs were detected in all seasons investigated in this study: summer (June-August), fall (September-November) and winter (December-February). Seasonal RVA shedding in swine has been poorly documented, although differences related to age groups has been observed in piglets, which showed higher rates of RV prevalence during the summer (June-August), whereas infection of adult pigs showed peaks during the winter season (December-March) [11]. In humans, most studies report higher rotavirus prevalence in children during the winter season [56]. Seasonality in rotavirus prevalence in adults showed discordance with either a winter or summer peak, or the total absence of a seasonal trend [6, 44, 56]. A recent review has suggested that seasonality of rotavirus disease in humans depends not only on the proprieties of the viral agent but also on many other factors such as geographic location or country income [48]. Further studies are warranted to understand seasonal RVA patterns of infections in adult pigs.

In summary, the results reported here reveal extensive genetic diversity of swine RVAs both within and between premises, some of which are epidemiologically relevant to humans and to other mammalian species. The results also suggest either temporal or regional fluctuations in RVA genotype distribution in addition to the consistent presence of RVAs in pigs over three consecutive seasons. Continued surveillance of livestock and human RVAs is warranted to better understand the ecology of these viruses, especially from a public health perspective.

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