An investigation of group and subtype diversity and distribution of porcine rotaviruses in Canadian suckling piglets with diarrhea, 2019-2023

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Summary

Objective: To determine the frequency of detection and group diversity of rotavirus (RV) A, B, and C, and G (glycoprotein antigen) serotype (based on viral protein 7 [VP7] gene analysis) infecting suckling piglets with diarrhea in Canadian farms.

Materials and methods: Canadian swine veterinarians submitted 1117 enteric samples from suckling piglets between July 2019 and December 2023 to the University of Guelph Animal Health Laboratory for RV group identification and VP7 sequencing for subtyping. Analysis of the VP7 sequence from 837 samples was performed using the Animal Health Sequivity Dashboard (Merck & Co, Inc) and descriptive statistics.

Results: Rotavirus A, B, and C were present in 40.7%, 12.5%, and 46.8% of samples, respectively. The most common RV identified was RVC G6, present in 296 samples, followed by RVA G9 in 205 samples. A single RV group was involved in 444 cases (72.3%), while in 170 cases (27.7%), more than one RV group/subtype was detected. Eighteen subtypes were identified by sequencing the VP7 protein (5 RVA, 9 RVB, and 4 RVC).

Implications: Rotavirus protection for suckling piglets comes from colostrum and milk. Knowing which RV group is causing diarrhea is important since vaccination does not generate cross-protection among groups. Using molecular diagnostic testing, it is possible to identify the specific group and subtype of RV circulating on the premises and decide the best treatment strategy for the disease.

Keywords: swine, rotavirus, diarrhea, VP7, vaccine.

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Resumen - Una investigación sobre la diversidad de grupos y subtipos y la distribución de rotavirus porcinos en lechones lactantes canadienses con diarrea, 2019-2023

Objetivo: Determinar la frecuencia de detección y la diversidad de grupos del serotipo A, B, y C de rotavirus (RV) y serotipo G (antígeno glicoprotéico) (basado en el análisis del gen de la proteína viral 7 [VP7]) que infectan lechones lactantes con diarrea en granjas canadienses.

Materiales y métodos: Los veterinarios porcinos canadienses enviaron 1117 muestras entéricas de lechones lactantes entre julio de 2019 y diciembre de 2023 al Laboratorio de Salud Animal de la Universidad de Guelph para la identificación del grupo de RV y la secuenciación de VP7 para su subtipificación. El análisis de la secuencia VP7 de 837 muestras se realizó utilizando el Tablero Sequivity de Salud Animal (Merck & Co, Inc) y estadística descriptiva.

Resultados: Los rotavirus A, B, y C estuvieron presentes en el 40.7%, 12.5%, y 46.8% de las muestras, respectivamente. El RV más común identificado fue el RVC G6, presente en 296 muestras, seguido del RVA G9 en 205 muestras. En 444 casos (72.3%) se detectó un solo grupo de RV (72.3%), mientras que en 170 casos (27.7%) se detectó más de un grupo/subtipo de RV. Se identificaron dieciocho subtipos mediante la secuenciación de la proteína VP7 (5 RVA, 9 RVB, y 4 RVC).

Implicaciones: La protección contra el rotavirus para los lechones lactantes proviene del calostro y la leche. Es importante saber qué grupo de RV está causando diarrea, ya que la vacunación no genera protección cruzada entre los grupos. Mediante el uso de pruebas de diagnóstico molecular, es posible identificar el grupo específico y el subtipo de RV que circula en las instalaciones y decidir la mejor estrategia de tratamiento para la enfermedad.

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Résumé - Étude sur la diversité des groupes et des sous-types et la distribution des rotavirus porcins chez des porcelets allaités canadiens atteints de diarrhée, 2019-2023

Objectif: Déterminer la fréquence de détection et la diversité des groupes de rotavirus (RV) A, B, et C et le sérotype G (antigène glycoprotéique) (sur la base de l'analyse du gène de la protéine virale 7 [VP7]) infectant les porcelets allaités atteints de diarrhée dans des fermes canadiennes.

Matériel et méthodes: Des vétérinaires porcins canadiens ont soumis 1117 échantillons entériques de porcelets allaités entre juillet 2019 et décembre 2023 au Laboratoire de santé animale de l'Université de Guelph pour l'identification du groupe RV et le séquençage VP7 pour le sous-typage. L'analyse de la séquence VP7 de 837 échantillons a été réalisée à l'aide de la plateforme Sequivity (Merck & Co, Inc) et de statistiques descriptives.

Résultats: Les rotavirus A, B, et C étaient présents dans 40.7%, 12.5%, et 46.8% des échantillons, respectivement. Le RV le plus fréquemment identifié était le RVC G6, présent dans 296 échantillons, suivi du RVA G9 dans 205 échantillons. Un seul groupe de RV était impliqué dans 444 cas (72.3%), tandis que dans 170 cas (27.7%), plusieurs groupes/sous-types de RV ont été détectés. Dix-huit sous-types ont été identifiés par séquençage de la protéine VP7 (5 RVA, 9 RVB, et 4 RVC).

Implications: La protection contre le rotavirus pour les porcelets allaités provient du colostrum et du lait. Il est important de savoir quel groupe de RV est à l'origine de la diarrhée, car la vaccination ne génère pas de protection croisée entre les groupes. À l'aide de tests de diagnostic moléculaire, il est possible d'identifier le groupe et le sous-type spécifiques de RV circulant dans les bâtiments et de décider de la meilleure stratégie de traitement pour la maladie.

Rotavirus (RV) is a ubiquitous pathogen able to cause diarrhea in pigs of all ages, although suckling piglets are the most susceptible.[1-4] As animals age, most become protected from the disease by developing post-exposure immunity to RV coupled with maturation of the gut physiology and overall immunity.[1,5] Rotavirus groups A, B, and C are the most common in pigs, although E and H have also been demonstrated to cause disease in swine.[1] The RV groups are identified by the antigenicity of viral protein (VP) 6.[1] Sequencing of other structural viral proteins, such as VP7 and VP4, are employed to further type the virus into G (glycoprotein antigen) or P (protease-sensitive antigen) serotypes based on their antibody neutralization properties.[1,2,6]

Group A was the first RV to be identified in pig production and has been considered the most critical and prevalent RV causing diarrhea in suckling piglets.[6] Although RV groups B and C have been detected since the 1980s, the difficulty in growing these in cell culture did not allow for extensive investigation and analyses until recently.[2] Rotavirus C relevance as a diarrhea-causing pathogen in the pork industry was first thought sporadic. However, it has recently been recognized as endemic in most pig herds causing both subclinical disease and severe gastroenteritis in young piglets (78%, < 3 days of age).[2] Group B appears as a less prevalent RV and is sporadically found in pig herds and has been shown to have the ability to cause disease in piglets.[6,7] Due to the difficulty in culturing groups B and C, the only commercial vaccine available in Canada is based on the RV A G5 and A G9 subtypes. The prescription RNA particle vaccines are available for all three RV groups.

We aimed to understand the genetic diversity and geographical distribution of RV groups A, B, C, and G subtypes (VP7) infecting suckling piglets in Canadian farms. The determination of RV as cause of disease is not within the scope of this study, as not enough diagnostic data was collected, and the detection of RV does not imply infection and disease.

Animal care and use

This study used laboratory submission data from diagnostic veterinary submissions. Institutional animal use approval was not required.

Materials and methods

The animals were adequately housed and cared for in 290 commercial swine herds located in Alberta (AB), British Columbia (BC), Manitoba (MB), New Brunswick (NB), Ontario (ON), Quebec (QC), and Saskatchewan (SK). Fifty-eight swine veterinarians from 30 clinics submitted targeted (not random) enteric samples (fecal swab, intestinal content, or intestinal tissue) from suckling piglets presenting with rotaviral diarrhea between July 1, 2019 and December 31, 2023. Each sample collection was a result of the veterinarian investigating the cause of diarrhea in suckling piglets on their client's farms. As they had previously eliminated other sources of pathogen-induced diarrhea, they submitted samples for RV sequencing to produce a prescription RV vaccine for each farm under the Sequivity RNA particle vaccine program (Merck & Co, Inc). Thus, samples, number of samples, and sample collection methods were not standardized among veterinarians and farms.

Samples received by the Animal Health Lab (AHL) at the University of Guelph were tested upon arrival for the RV group by polymerase chain reaction (qPCR), as previously described,[8] followed by Sanger sequencing of the G type (VP7). If multiple samples within the same submission (case) were positive, only the sample with the lowest cycle threshold (Ct) on qPCR for each group (if more than one detected) was sequenced. Results were recorded matching the sequence to the clinic, farm, and veterinarian name (which remained confidential), province, date of collection, and age of pigs presenting clinical signs (only samples identified as suckling piglets were included). Sequencing results were analyzed using the Animal Health Sequivity Dashboard (Merck & Co, Inc), an RNA vaccine platform database and tool for sequence storage and analysis, as previously described by Sebo[9] and followed by descriptive analysis.

Results

A total of 1117 samples from 614 cases of diarrhea were submitted to the AHL, where the samples with the lowest Cts (837 samples) were identified by sequencing the VP7 gene. Ontario had the highest representation in sequenced samples, with 22.6% (189 of 837) of the total samples, followed by AB with 21.8% (183 of 837), MB with 21.3% (178 of 837), SK with 17.7% (148 of 837), QC with 14.6% (122 of 837), BC with 1.2% (10 of 837), and

NB with 0.8% (7 of 837). From all samples sequenced, RVA was present in 40.7% (341 of 837) of samples, RVB in 12.5% (105 of 837), and RVC in 46.8% (391 of 837) (Table 1). In most provinces (AB, BC, MB, NB, QC, and SK), RVC was the most detected group followed by RVA and RVB, while ON observed a higher presence of RVA followed by RVC and RVB. The number of farms and cases from each province are detailed in Table 1.

Single RV detections (only one group or subtype involved) represented 72.3% (444 of 614) of the cases, while 170 of the 614 cases (27.7%) had more than one RV group and subtype detected. The 170 RV codetection cases were represented by 393 sequences, from which RVC was present in 40.5% (159 of 393), followed by RVA in 38.4% (151 of 393), and RVB in 21.1% (83 of 393). Thirty-two cases had all three groups (RV A, B, and C) detected, while 90 codetection cases had groups A and C present; other combinations of groups or subtypes were also identified (Table 2).

Eighteen RV subtypes were identified within all cases and included 5 RVA, 9 RVB, and 4 RVC (Figure 1). The most common RV was RVC G6 detected in 296 samples, with a mean homology of 90.8% (range: 69.06%-100%) among samples. Rotavirus A G9 was found in 205 samples with a mean homology of 94.5% (range: 86.76%-100%). Similar mean homology was found within provinces (Table 3). Some less common RV sequences were present only in a specific region or province, for example, RVB G8 was only detected in SK.

Discussion

Rotavirus-related diarrhea in suckling piglets is a concern for the pork industry due to its high prevalence and impact on preweaning mortality and piglet performance.[10] Like other studies, we found that suckling piglet samples were mainly positive with only one RV, although multigroup/subtype RV codetections were present.[2,5,11] In this study, RVC was

Table 1: Number of sequenced rotavirus (RV) groups, number of farms, and number of cases by Canadian provinces

	Canadian province							
	Alberta	**British Columbia**	**Manitoba**	**New Brunswick**	**Ontario**	**Quebec**	**Saskatchewan**	**Total**
No. of farms	43	2	66	1	81	59	38	290
No. of cases	121	9	126	6	145	112	95	614
RVA sequences	73	1	74	2	106	29	56	341
RVB sequences	32	1	27	0	7	3	35	105
RVC sequences	78	8	77	5	76	90	57	391
Total RV Sequences	183	10	178	7	189	122	148	837

Table 2: Number of cases with rotavirus (RV) group or sub-type codetection by combinations

	RV group/sub-type combinations						
	A + B	**A + C**	**C + B**	**B + B***	**C + C†**	**A + B + C**	**Total**
No. of cases	20	90	26	1	1	32	170

* B G8 and B G14.

† C G1 and C G6.

Figure 1: Distribution of rotavirus groups and types by Canadian province.

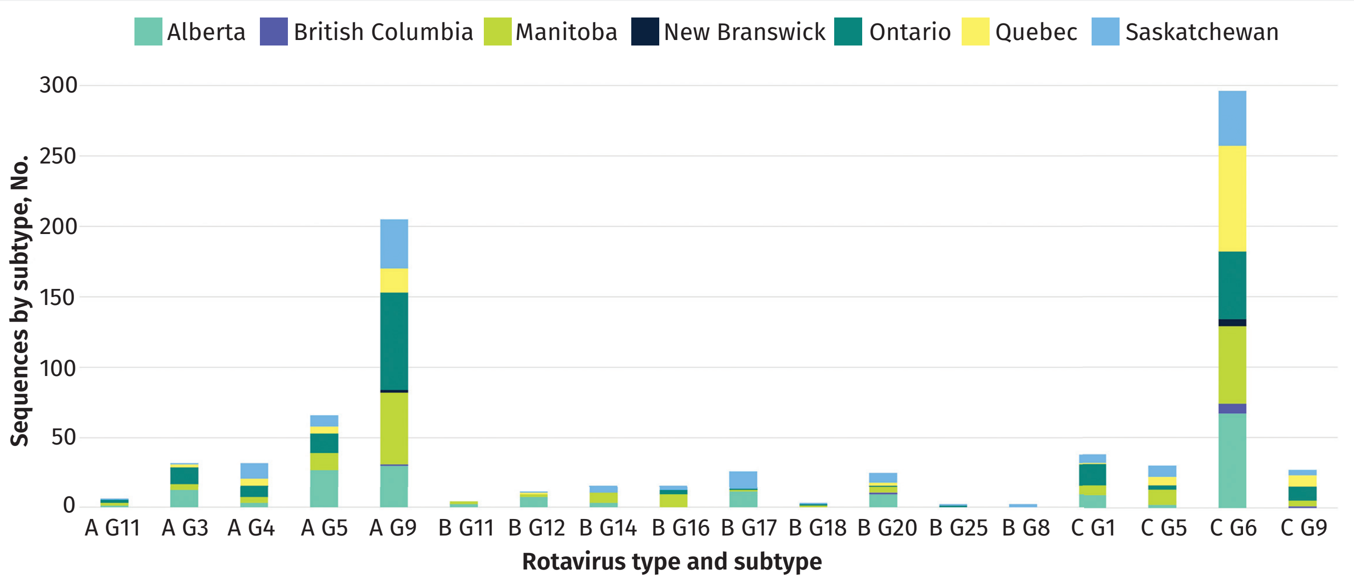

Table 3: Number and homology percentage of rotavirus (RV) sub-type detections by province

	Canadian province						
	Alberta	**British Columbia**	**Manitoba**	**New Brunswick**	**Ontario**	**Quebec**	**Saskatchewan**
RVA G9							
No. of detections	31	1	54	2	71	16	31
% homology	94.17	NA	94.76	99.23	95.26	95.27	95.38
RVC G6							
No. of detections	73	7	61	5	50	61	39
% homology	91.33	97.60	92.85	99.35	90.46	92.22	92.49

NA = not applicable.

the most detected RV in Canadian provinces (except in ON), followed by RVA, which was similar to previous results from the United States where RVC has been detected in 76% of suckling piglets. As previously observed, RVB was the least detected yet most diverse group.[6] Our results indicated that RVA was the most detected RV in ON, which is similar to past studies conducted in this province.[11,8] Buchan and colleagues[11] summarized three years of diagnostic reports involving diarrhea presentations in ON during the lactation and nursery phases. Rotavirus A was detected in 69% of diarrhea cases in suckling piglets, RVC in 37%, and RVB in 13%. Similarly, Tran et al[8] found RVA in 56.4% of samples from suckling pigs, 10% of RVB, and 34.4% of RVC (93% of all samples were from Ontario and Quebec).

Marthaler et al[12] tested 7508 samples from pigs with diarrhea in Canada, the United States, and Mexico. They found that 83% of samples were qPCR positive for RVA, RVB, or RVC. Group A was detected at the highest percentage (62%). While RVB and RVC were seen at a lower frequency (33% and 53%, respectively), both were considered epidemiologically relevant. The study also reported that RV detection can be related to the age of the pig sampled. Rotavirus C was more frequently detected in pigs within the first 21 days of age, while RVA and RVB were suggested as the cause of diarrhea in pigs over 21 days of age,[12] which may explain the higher detection of RVC observed in our study which targeted samples from suckling piglets.

The reason why the prominent group detected differed in ON from other provinces is not apparent. However, RV group detection has been shown to vary geographically.[1] Furthermore, sow vaccination programs, age, diet, genetics, and farrowing room management can vary from province to province, potentially influencing RV distribution. A diversity of subtypes within groups were observed demonstrating the diversity of RV. The VP7 sequences can vary within the same group as was shown in the homology analyses within the two most detected RV subtypes (A G9 and C G6). It is unknown what percentage of homology of VP7 would offer cross-protective immunity within the same subtype, although different subtypes within the same group are known to have small to no cross-protection.[13,14] Higher mean homology was observed among the A G9 sequences than the C G6, which had lower mean homology both within the country as well as within provinces.

The results presented here were not paired and analyzed with qPCR Ct results, clinical signs, or specific diagnostic tests to confirm RV-related disease. However, samples were collected from farms presenting with diarrhea in suckling piglets, where the veterinarian had previously tested for other pathogens and eliminated them as the cause of disease. Our observations suggest two different primary RV groups in Canada, RVA in ON and RVC in the western provinces and QC, indicating the relevance of RVC and the classic RVA in the Canadian swine industry.

Implications

Under the conditions of this study:

- Rotavirus C was the most detected RV in Canadian suckling piglets.
- Most cases were single RV detections, although RV codetections were common.
- Knowledge of RV subtypes inform veterinarians on prevention programs.

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Conflict of interest

Malgarin and de Grau are both employed by Merck Animal Health, which provided the funding for all diagnostic tests used in this study. All diagnostic tests were conducted by the AHL at the University of Guelph.

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