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SHORT COMMUNICATION

Rotavirus group C infections in neonatal and grower pigs in Australia

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Abstract

Background: Rotavirus infections of neonatal and older pigs are widely reported. Analysis of rotavirus group C prevalence and diversity has not previously been reported for Australian pig farms.

Methods: Twenty-seven farms with or without diarrhoea present among neonatal or older pigs were enrolled across eastern Australia. Fresh faecal samples were analysed by ELISA for rotavirus and RNA extractions by polyacrylamide gel electrophoresis and RT-PCR for rotavirus. Rotavirus group C samples were genotyped via sequencing.

Results and conclusions: Rotavirus infection was diagnosed in pigs on 10 of 19 farms investigated for neonatal diarrhoea, four with group A and six with group C; also among post-weaned (5- to 11-week-old) diarrhoeic pigs on two farms. Neonatal rotavirus group C infections were exclusively noted in piglets less than 1-week-old, consisting of farm infections with a single VP7 genotype (G5 or G6). Infections in post-weaned pigs were associated with multiple VP7 genotypes (G1, G3). This first report of rotavirus group C infections of Australian pigs suggests they may form a limited population of VP7 genotypes.

INTRODUCTION

Rotavirus is a non-enveloped, triple-layered, double-stranded RNA virus with 11 genomic segments; with genetic classification into nine distinct groups (RVA to RVJ) based on the intermediate capsid layer protein (VP6).[1,2] Further binomial characterisation of rotavirus strains into G and P genotypes, via analysis of the outer capsid proteins VP7 (which induces neutralising antibody) and VP4 respectively, indicates that various genotypes occur in natural infections in neonatal pigs.[3,4]

Neonatal rotavirus infections are a well-characterised cause of intestinal epithelial damage and resultant diarrhoea in the pig and other animal hosts. Diarrhoea in older pigs due to rotaviral infections has also previously been recognised occasionally.[5]

Rotavirus diversity is often generated by reassortment events, sometimes involving zoonotic transmission. While the analysis of genetic shifts and epidemiological features among porcine rotavirus infections has been explored extensively in Europe and America, little such information exists for Australian herds. The long isolation of Australian pig herds from foreign live pig (and porcine rotavirus) imports may have resulted in long-term, endemic circulation of rotavirus. However, the diversity and epidemiology of porcine rotavirus in Australia has not been described to date. This study describes the first detection and characterisation of group C (RVC) rotavirus in Australian pig herds.

MATERIALS AND METHODS

Nineteen pig farms (*# 1–19*) were enrolled across eastern Australia with cases of neonatal diarrhoea, defined as more than 10% of piglets 3-week-old or less, with watery faeces. Two further farms (*# 20,21*) with clinical diarrhoea evident in post-weaned 5- to 11-week-old pigs were also enrolled. Two sets of three case-control farms with no such history of diarrhoea in neonatal (*# 22, 23, 24*) or older (*# 25, 26, 27*) pigs respectively were also enrolled. Three of the farms were connected by supply of either breeding females (farm *# 1* to *# 5*) or 3-week-old piglets (farm *# 1* to *# 20*). While pregnant breeding females were group-housed prior to entering the farrowing area, neither rotavirus vaccination nor the farming practice of exposure of young breeding

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females to faecal materials from older sows/piglets, aimed at inducing passive immunity, was conducted in the enrolled herds.

The Animal Ethics Care Committee of the State Government of Victoria approved the sampling protocols used in this study.

At least five fresh faecal samples were collected from affected or case-control pigs on each farm. Samples were immediately transported on ice to the Murdoch Children's Research Institute and stored at −80°C. Portions of intestine obtained at autopsy from diarrhoeic piglets at farm sites *# 1* through *19* were also subjected to routine histopathology.

The presence of rotavirus group A (RVA) antigen was investigated in each faecal sample using a commercial ELISA (Rota-Corona-k99 ELISA, Idexx Laboratories Inc., One Idexx drive, Westbrook, Maine 04092, USA), as per the manufacturer's instructions.

Viral RNA was extracted from 10%−20% (w/v) faecal extracts using the QIAamp viral RNA extraction kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Viral RNA samples were analysed by polyacrylamide gel electrophoresis (PAGE) and silver stained to allow visualisation of rotavirus genome segments identified by electrophoretic pattern, as described previously.[6] PAGE was not performed on all specimens. Samples exhibiting an electropherotype indicative of RVC were analysed further.

Initial characterisation of RVC was performed using a one-step RT-PCR assay incorporating the specific oligonucleotide VP6 primers GCRV-VP6-C1 and GCRV-VP6-C4, and VP7 primers GrC VP7-20F and GrC VP7-106R, as described previously.[7,8] Positive VP6 and VP7 amplicons (356 and 1043 bp, respectively) were purified from gel slices, using a commercial kit (QIAquick Gel Extraction kit, QIAGEN, Germany), according to the manufacturer's instructions. Purified DNA and primers were submitted for unidirectional sequencing using the ABI PRISM BigDye terminator cycle sequencing reaction kit (Applied Biosystems, Foster City, USA). Labelled products were loaded to an automated sequence analyzer (Applied Biosystems 3730xl DNA analyser). Sequences were edited with Sequencher version 4.10.1, and VP7 genotype assignment was determined using BLAST web-based programs (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

RESULTS

The results of rotavirus testing of pig faecal samples from each farm site is summarised in Table 1. Rotavirus infections were diagnosed on 10 of 19 farms investigated for neonatal diarrhoea, four with rotavirus group A (RVA) and six with rotavirus group C (RVC). Mixed rotavirus group infections were not detected on any of the farms. Both rotavirus group A and group C infections of neonatal piglets were noted in piglets less than 1-week-old. VP7 sequencing of RVC samples derived from neonatal piglets only showed farm infections with single genotypes evident (either G5 or G6). Whereas the two farms with RVC detected in 5- and 11-week-old pigs showed multiple genotypes evident on each farm (G1 and G3, Table 1).

Histopathological assessment of portions of diarrhoeic intestines, from neonatal piglets sampled on farms *# 1* to *10*, indicated epithelial cell damage and villous stunting consistent with pathogenic rotavirus infections. No other specific causes of diarrhoea were recognised during the study period on these or other enrolled farms.

The diarrhoea observed in typically-affected 5- to 11-week-old pigs on farms # 20 and 21 was characterised as sloppy, green-grey, with no blood or mucus evident. Affected pigs' appetite and body condition remained normal. Further testing of faecal samples from these previously-sampled, affected pigs on farms *# 20* and *21* at 15-week-old was negative for rotavirus by all assays. Concurrent testing for the presence of *Lawsonia intracellularis*, *Brachyspira* spp., *Salmonella* spp, and enterotoxigenic *Escherichia coli* (ETEC) in all these post-weaning samples was negative.

DISCUSSION

This study confirmed that intestinal damage and diarrhoea in neonatal piglets can be caused commonly by rotavirus infections, of either group A or group C. Piglets affected by RVC infections were less than 1-week-old, confirming previous studies of its likely signalment.[9,10] Given the environmental persistence and incubation period of rotavirus infections, these youngster piglet outbreaks are presumably acquired from infected farrowing areas. This first report of RVC infections among Australian pig herds confirms surveys elsewhere indicating that perhaps 20% or more of farm outbreaks of neonatal diarrhoea have a significant component of RVC infections.[3,4,7,10]

This study also confirmed that occasional outbreaks of moderate and self-limiting diarrhoea in post-weaned pigs can occur due to RVC. While our survey, and previous ones showing pathogenic RVC infections among post-weaned pigs,[5] did not establish the actual prevalence of this syndrome in diarrhoeic post-weaned pigs, it is likely to be low. In this study, we only report the detection of RVC in two selected farms with diarrhoeic post-weaned pigs; numerous other farms with diarrhoea among post-weaned pigs were investigated, and diarrhoea was due to more common pathogens, such as *Lawsonia intracellularis*.

We only detected a single VP7 genotype (G5 or G6) among neonatal piglets on each affected farm. The only modern-era importations of live breeding pigs (and presumably their possible rotavirus carriage) into Australia occurred in the period 1982–1986, from Canada and the British Isles. The predominant VP7 genotypes in pigs from those locations in past decades were reportedly G1, G3, G5, or G6,[4] as also detected in this study. We did not detect novel genotypes, and we also failed to detect co-infection of RVA and RVC

TABLE 1 Features of porcine rotavirus infections in Australia

Farm #	Number of breeding females	Age of pigs examined* (weeks-old)	Clinical diarrhoea Present	RNA gel PAGE Rotavirus	Rotavirus group A ELISA	Rotavirus group C VP6 PCR assay number positive/number tested	Rotavirus group C VP7 sequence group analysis
1	1200	1	+	–	+	0/8	–
2	600	1	+	–	+	0/7	–
3	1200	1	+	ND	+	0/5	–
4	2000	1	+	–	+	0/9	–
5	1200	1	+	+	–	1/5	G6
6	1500	1	+	+	–	5/5	G6
7	480	1	+	+	–	5/5	G6
8	2000	1	+	ND	–	12/12	G6
9	1400	1	+	+	–	17/21	G6
10	1000	1	+	+	–	2/5	G5
11	600	1	+	–	–	0/6	–
12	500	1	+	–	–	0/5	–
13	1400	1	+	ND	–	0/9	–
14	800	1	+	ND	–	0/9	–
15	2000	1	+	ND	–	0/5	–
16	800	2	+	ND	–	0/5	–
17	400	2	+	ND	–	0/6	–
18	1200	3	+	ND	–	0/5	–
19	800	3	+	ND	–	0/5	–
20	600	11	+	ND	–	4/7	G1, G3
21	1400	5	+	ND	–	10/11	G1, G3
22	1200	1	–	ND	–	0/5	–
23	1200	1	–	ND	–	0/6	–
24	800	1	–	ND	–	0/10	–
25	800	8	–	ND	–	0/5	–
26	500	6	–	ND	–	0/5	–
27	600	11	–	ND	–	0/6	–

Abbreviation: ND, not done.
*Neonatal pigs with or without diarrhoea are those between 0- and 3-week-old (farms 1–19 and 22–24).

among the farms investigated. This may suggest that a limited number of porcine RVC genotypes circulate among the isolated Australian herds. In the pig industries of America and Europe, where greater movement of pigs occurs, the emergence of novel rotavirus genotypes and strains has been reported more frequently, and is considered more likely, under the established principle that in situations with a high rate of co-infections, the occurrence of reassortment and atypical rotavirus types is higher.[3,11] Some RVA genotypes that have become dominant in humans, such as G9 or G12, are thought to have porcine origins.[12] While reassortment between animal and human rotavirus may contribute to increased diversity of rotavirus among humans, the reverse may be less common. The interspecies transmission of rotavirus from a human (or other host) back to pigs would presumably require an on-farm faeces soiling event.[4] Rotavirus infections appear to be common on Australian pig farms, and therefore some benefit may be derived from rotavirus vaccination.

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CONFLICT OF INTEREST

The authors have no competing interests to declare.

AUTHOR CONTRIBUTIONS

RH Dunlop and S McOrist planned the study and conducted pig farm investigations. S Roczo-Farkas, CM Donato and CD Kirkwood conducted virus analysis. All authors contributed to the reporting of the work. RH Dunlop is the responsible guarantor of the work.

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