Short Report: Surveillance and Molecular Characterization of Group A Rotaviruses in Goroka, Papua New Guinea

Paul Francis Horwood,* Dagwin Luang-Suarkia, Sauli Bebes, Karen Boniface, Siddhartha Sankar Datta, Peter Max Siba, and Carl Dunn Kirkwood

Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea; Enteric Virus Research Group, Murdoch Childrens Research Institute, Royal Children's Hospital, Melbourne, Australia; World Health Organization, Port Moresby, Papua New Guinea

Abstract. In this study, we investigated the molecular epidemiology of group A rotaviruses in cases of acute gastroenteritis in Goroka, Papua New Guinea. From April 2008 through November 2010, 813 diarrheal stool samples were collected from children < 5 years of age hospitalized with acute gastroenteritis. Rotavirus antigen was detected in 31.2% of samples using a commercial enzyme-linked immunosorbent assay. Genotyping revealed the presence of the globally circulating strains G1P[8] (50.0%), G3P[8] (23.0%), and G2P[4] (8.2%). The globally emerging strains G9 and G12 were detected in 1.2% and 6.1% of samples, respectively. Mixed infections were detected in a high proportion of samples (11.9%), with 9.0% and 3.7% of samples displaying multiple G and P genotypes, respectively.

Group A rotaviruses are the most important cause of acute diarrhea in children throughout the world. Rotavirus infection causes acute watery diarrhea that may result in dehydration and death in young children. It has been estimated that rotavirus infections cause 2.4 million hospital admissions and more than 500,000 deaths throughout the world each year.¹ The recent introduction of rotavirus vaccines has greatly reduced the burden of this disease in many industrialized countries. However, rotavirus is still a major health problem in young children in all countries where the vaccine has not been introduced.

Rotavirus is a double-stranded RNA virus in the family *Reoviridae*. The group specificity of rotaviruses is determined by the internal structural protein VP6, with seven groups (A–G) identified to date. Group A rotaviruses are further characterized based on the surface proteins VP7 (G genotype) and VP4 (P genotype). To date, at least 27 G genotypes and 35 P genotypes have been described.² The most common genotypes of rotavirus detected around the world include the G types G1, G2, G3, G4, and G9, in association with the P types P[8], P[6], and P[4].³ However, many recent studies have described the detection of uncommon or untypable genotypes in developing countries.⁴

Two live attenuated vaccines have been licensed and proven efficacious for protection against rotavirus infections, namely RotaTeq (Merck & Co., Whitehouse Station, NJ) and Rotarix (GlaxoSmithKline Biologicals, Rixensart, Belgium).^{5,6} The enormous diversity of rotavirus serotypes has raised concerns about the possibility of vaccination leading to the selection of uncommon strains through vaccine selection pressure. Although it has been established that these vaccines provide effective protection against the most common genotypes of rotavirus, the ability to protect against uncommon rotavirus strains is not fully understood. Incomplete protection against uncommon strains may lead to the development of new globally important strains of rotavirus. Minor variations in the epidemiology of rotavirus strains have been reported since the introduction of widespread vaccination.⁷ However, it is uncertain if these observations are caused by natural genotype fluctuations

or viral population changes as a result of vaccine selection pressure. Therefore, it is important that baseline data are collected to determine which rotavirus genotypes are circulating in a population before vaccination programs are commenced. The continuation of surveillance following the introduction of a rotavirus vaccine is also important to assess the impact of the vaccination strategy and to monitor for the emergence of new or uncommon strains.

Papua New Guinea (PNG) is a large tropical island nation to the north of Australia with a population of ~7 million people. The country has a high under 5 mortality rate of 102 deaths per 1,000 live births, of which diarrheal illnesses contribute to 15% of deaths.⁸ Only limited studies have been conducted to determine the burden of rotavirus diarrhea in PNG.^{9–11} The aim of this study was to determine the burden and mortality associated with rotavirus disease in children under 5 years of age and to characterize the rotavirus strains circulating in the country with reference to the possible introduction of a rotavirus vaccine.

This study was approved by the Papua New Guinea Institute of Medical Research Institutional Review Board and the Papua New Guinea Medical Research Advisory Council. Fecal samples were collected from eligible children following informed consent from parents or guardians.

Fecal samples were collected from children that were hospitalized with acute gastroenteritis at Goroka General Hospital from April 2008 through November 2010. Goroka is the provincial capital of Eastern Highlands Province of Papua New Guinea. This region has a population of ~450,000 people and the Goroka General Hospital is the major referral hospital for this region. The inclusion criteria for these surveillance activities were children < 5 years of age, > 3 loose bowel movements in the last 24 hours, no blood in the feces, and collection of the sample within 48 hours of hospitalization. Fecal specimens were stored at 4°C and were transported to the laboratory within 4 hours of collection. Group A rotavirus antigen was detected from 10% fecal suspension using the ProSpecT Rotavirus Microplate Assay (Oxoid, UK) according to the manufacturer's instructions.

Fecal specimens, which were positive for rotavirus A antigen using the enzyme-linked immunosorbent assay (ELISA), were further characterized for rotavirus G and P types. Rotavirus RNA was extracted from 10% to 20% FE using the

* Address correspondence to Paul Francis Horwood, Papua New Guinea Institute of Medical Research, PO Box 60, Goroka EHP441, Papua New Guinea. E-mail: paul.horwood@pngimr.org.pg

TABLE 1

Age distribution of rotavirus infection and mortality among children hospitalized for acute gastroenteritis between April 2008–November 2012 in Goroka, Papua New Guinea ($N = 813$)

Age (months)	Acute diarrhea hospitalizations	Tested positive for rotavirus no. (%)	Rotavirus mortality no. (%)
0 to 5	185	51 (27.6)	1 (2.0)
6 to 11	314	114 (36.3)	2 (1.8)
12 to 23	207	66 (31.9)	2 (3.0)
24 to 59	83	21 (25.3)	1 (4.8)
Unknown	24	2 (8.3)	0 (0)
All < 59	813	254 (31.2)	6 (2.4)

QIAamp Viral RNA Kit (Qiagen, Hilden, Germany). The VP7 and VP4 genotypes of rotavirus strains were determined by 2 step multiplex, hemi-nested reverse transcription-polymerase chain reaction (RT-PCR) assays. The G genotypes were determined using G-specific primers and P genotypes were determined using P-specific primers.^{12–14}

A total of 813 diarrheal fecal samples were collected from children < 5 years of age admitted to Goroka General Hospital in-patients with acute diarrhea. Rotavirus antigen was detected in 31.2% ($N = 254$) of specimens collected from April 2008 through November 2010. The majority of diarrheal hospitalizations (86.8%) and rotavirus infections (90.9%) occurred in children < 2 years of age (Table 1). Mortality associated with rotavirus infection was recorded across all of the age groups in this study, with 2.4% ($N = 6$) of rotavirus infections resulting in death.

Rotavirus was detected throughout the year at Goroka General Hospital. However, a peak in infection was observed from May through July (data not shown), which corresponds with the beginning of the dry season in Eastern Highlands Province. Although seasonality is less pronounced in tropical climates, rotavirus disease is usually more common during the drier and cooler months.¹⁵

A total of 244 specimens were characterized to determine G and P genotypes (Table 2). The most commonly detected strains were G1P[8] (50.0%), G3P[8] (23.0%), and G2P[4] (8.2%), which are among the most commonly detected genotypes throughout the world. The globally emerging strains G9 and G12 were detected in 1.2% and 6.1% of samples, respectively.

Mixed infections were detected in 11.9% (G-type mixed infections, 9.0%; P-type mixed infections, 3.7%) of samples, which is comparable to reports from other developing countries where high rates of mixed infections are commonly reported.¹⁶ No particular combination of mixed G or P types was observed at a high frequency. Untypeable G genotypes (2.0%) and untypeable P genotypes (1.2%) were also detected in this study and may reflect uncommon genotypes or RNA mutations at primer binding sites.

The detection of rotavirus in 31.2% of children hospitalized with acute gastroenteritis is similar to results from other countries in the region such as Thailand¹⁷ and Malaysia¹⁸ where rotavirus was associated with 39% and 38% of diarrheal hospitalizations, respectively. A study in Fiji found a similar disease burden (39%) and age distribution of rotavirus infections; however, G3P[8] was the predominant genotype, constituting 85% of the rotavirus strains detected in that study.¹⁹ In previous studies conducted at Goroka General Hospital, Shann and others (1980)⁹ detected rotavirus in 70% of 77 children by electron microscopy; Pratt and others (1992)¹⁰ detected rotavirus in 68% of 38 children by ELISA; and Howard and others (2000)¹¹ detected rotavirus in 23% of 1,526 children by ELISA.

A high proportion (27.6%) of rotavirus hospitalizations occurred in infants < 6 months of age and 65.0% occurred in the first year of life. The early age of rotavirus acquisition observed in this study indicates that rotavirus vaccination strategies in PNG would need to focus on infants early in life to maximize protection during the peak period of acquisition. These figures are consistent with other developing countries where rotavirus infections typically occur earlier than for developed countries.^{20,21} The relatively high mortality rate (2.4%) observed during the study period indicates that rotavirus mortalities may be an important cause of childhood deaths throughout the country. Unfortunately, these figures give no indication to the mortality rates associated with rotavirus infections for the majority of Papua New Guinean children who live in rural areas with poor access to healthcare facilities.

Since the mid-1990s G9 genotypes have been recognized as globally important strains. Indeed, the G9P[8] strains are now regarded as amongst the five most common genotypes of rotavirus worldwide, in conjunction with G1P[8], G3P[8],

TABLE 2

G and P genotypes of group A rotaviruses recovered from young children hospitalized with acute gastroenteritis in Goroka, Papua New Guinea ($N = 244$)

G genotype	No. of samples, P genotype						Total (%)
	P[8]	P[4]	P[4/8]	P[6]	P[6/8]	Pnt	
G1	122	1	3	0	0	0	126 (51.6)
G3	56	0	0	0	0	3	59 (24.2)
G2	0	20	3	0	0	0	23 (9.4)
G12	6	0	0	1	0	0	7 (2.9)
G9	2	0	0	0	0	0	2 (0.8)
G1/G3	4	0	0	0	0	0	4 (1.6)
G1/G2	1	4	0	0	0	0	5 (2.0)
G1/G4	1	0	0	0	0	0	1 (0.4)
G1/G12	2	0	0	0	0	0	2 (0.8)
G1/G2/G3	1	0	0	0	0	0	1 (0.4)
G2/G3	0	1	1	0	0	0	2 (0.8)
G3/G12	4	0	0	1	0	0	5 (2.0)
G3/G4	1	0	0	0	0	0	1 (0.4)
G4/G9/G12	0	0	0	0	1	0	1 (0.4)
Gnt	3	1	1	0	0	0	5 (2.0)
Total (%)	203 (83.2)	27 (11.1)	8 (3.3)	2 (0.8)	1 (0.4)	3 (1.2)	244 (100)

G4P[8], and G2P[4].^{4,22} Although the G9 genotype was only detected in 1.2% of samples, it further shows the global distribution of the G9 strains. Rotavirus G12 strains were first detected in the Philippines in 1987,²³ and in recent years they have been reported from Thailand,²⁴ India,²⁵ Indonesia,²⁶ Bangladesh,²⁷ and many other countries. In this study G12P[6] was detected in one sample, whereas G12P[8] was detected in six samples. A previous study suggested that detection of the G12P[8] genotype may be a result of reassortment between G1P[8] and G12P[6] strains.²⁸ There is evidence that putative reassortants can spread through a population and become clinically important, as reported in Bangladesh,²⁹ Italy,³⁰ and Brazil.³¹

The high rate of mixed infections detected in this study (11.9%) may be epidemiologically significant as these cases may lead to reassortment of rotavirus genes.^{32,33} High rates of mixed infections have been reported in many developing countries and may have a potential impact on the efficacy of rotavirus vaccine interventions in these settings.¹⁶ In contrast, the rate of mixed infections in developed countries is typically < 5%.³⁴

Although our findings need to be confirmed by similar studies in different locations throughout PNG, we have established that rotavirus is an important cause of morbidity and mortality in children. Our findings also suggest that there is a high level of mixed infections with the increased possibility of reassortment between strains. Further studies are required to determine the burden and impact of rotavirus gastroenteritis throughout PNG. If a rotavirus vaccine is introduced into the vaccination schedule in PNG it is important that surveillance continues, to ensure that vaccine pressure does not select for G9, G12 and other emerging strains.

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Authors' addresses: Paul Francis Horwood, Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea, E-mail: paul.horwood@pngimr.org.pg. Dagwin Luang-Suarkia and Sauli Bebes, Papua New Guinea Institute of Medical Research, Infection and Immunity, Goroka, Papua New Guinea, E-mails: dagwins@gmail.com and sauli.bebes@pngimr.org.pg. Karen Boniface, Murdoch Childrens Research Institute, Royal Children's Hospital, Enteric Virus Research Group, Melbourne, Australia, E-mail: Karen.boniface@mcri.edu.au. Siddhartha Sankar Datta, World Health Organization, Western Pacific Regional Office, Port Moresby, Papua New Guinea, E-mail: DattaS@wpro.who.int. Peter Max Siba, Papua New Guinea Institute of Medical Research, Directorate, Goroka, Eastern Highlands Province, Papua New Guinea, E-mail: Peter.Siba@pngimr.org.pg. Carl Dunn Kirkwood, Enteric Virus Research Group, Murdoch Childrens Research Institute, Royal Children's Hospital, Melbourne, Australia, E-mail: carl.kirkwood@mcri.edu.au.

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