

Human rotavirus associated diarrhea and strain diversity in Nepal

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ABSTRACT

Two new rotavirus vaccines were recently introduced to the market and have shown a tremendous impact in reducing severe diarrhea due to rotavirus in children. Diarrheal disease is a common cause of morbidity and mortality among Nepalese children. In anticipation of a rotavirus vaccine in Nepal, we systematically reviewed published articles on rotavirus infection in Nepal. Literatures were searched in health related national and international databases. Fifteen rotavirus related articles between 1978 and 2010 were identified. Of these, information from five articles were extracted and analyzed in the present study. Of 3671 participants, 864 were positive for rotavirus infection by ELISA. The prevalence of rotavirus infection ranged from 12 to 39%. G1P[8] (25%) was the most frequent combined G and P genotype, followed by G12P[6] (23%) and G2P[4] (16%) between 2003 and 2007. Nearly 9% of analyzed rotavirus positive samples (G and P genotype) were non-typable. The data demonstrated that rotavirus is the most identifiable cause of severe diarrhea in children less than 5 years old in Nepal. The wide variety of rotavirus strains circulating in Nepal; underscore the need for continued surveillance. This will be the key to understanding the epidemiological characteristics of rotavirus disease and the impact of vaccination after introduction.

Keywords: Rotavirus, epidemiology, genotypes, Nepal.

INTRODUCTION

Rotavirus is the single most important cause of life threatening severe diarrhea in children in developing countries. It is estimated that 611,000 childhood deaths occurs due to diarrhea-related rotavirus each year.¹ Although the incidence of rotavirus infection is similar in developed and developing countries, more than 80% of all rotavirus related deaths were estimated to occur in low income countries of sub-Saharan Africa and south Asia.¹

Rotavirus is a member of the family *Reoviridae* and is non-enveloped virus with a wheel like structure containing 11 segments of double stranded RNA.² Each segment encodes a single viral protein except 11th segment. Segment 9 and 4 codes for VP7 and VP4 respectively, and are major neutralizing antigens. VP7 defines the G serotype (glycoprotein) and VP4 defines the P serotype (protease-sensitive). Based on molecular characterization, 15 G genotypes and 26 P genotypes have been reported in humans.³ G1, G2, G3, G4 and G9 represent the most common G genotypes and commonly combine with P[8], P[4], or P[6] worldwide.⁴ Uncommon serotypes G5, G8, G10, G11 and G12 have been reported in humans from various parts of the world.⁵⁻⁸

The first licensed rhesus-human reassortant vaccine (RotaShield, Wyeth Laboratories, Marrieta, PA, USA) was withdrawn from the market due to its association

with cases of intussusceptions.⁹ The enormous global burden of rotavirus disease resulted in continued efforts to develop safe and effective rotavirus vaccines. Currently two oral, live attenuated vaccines, Rotarix (GlaxoSmithKline) and RotaTeq (Merck), have shown to be safe and efficacious against severe rotavirus diarrhea in clinical trials.^{10,11} Rotarix is a human G1P1A[8] monovalent vaccine, whereas RotaTeq is a pentavalent human-bovine rotavirus vaccine that contains G1-G4 combined with a bovine P serotype and a bovine G serotype combined with the human P1[8]. These vaccines primarily aim to provide protection against globally important serotypes G1-G4 and P[8]. Recently World Health Organization (WHO) has recommended universal use of rotavirus vaccine in all national childhood immunization programs.¹² Epidemiological profile and disease burden is crucial before vaccine introduction in a given place.

The present study reviews epidemiological profile of rotavirus infections and highlights the impact of rotavirus infections among diarrheal patients, which provide baseline data against which future vaccine efficacy can be measured in Nepal.

MATERIALS AND METHODS

The data used in this review were identified by searches of national and international journals, and

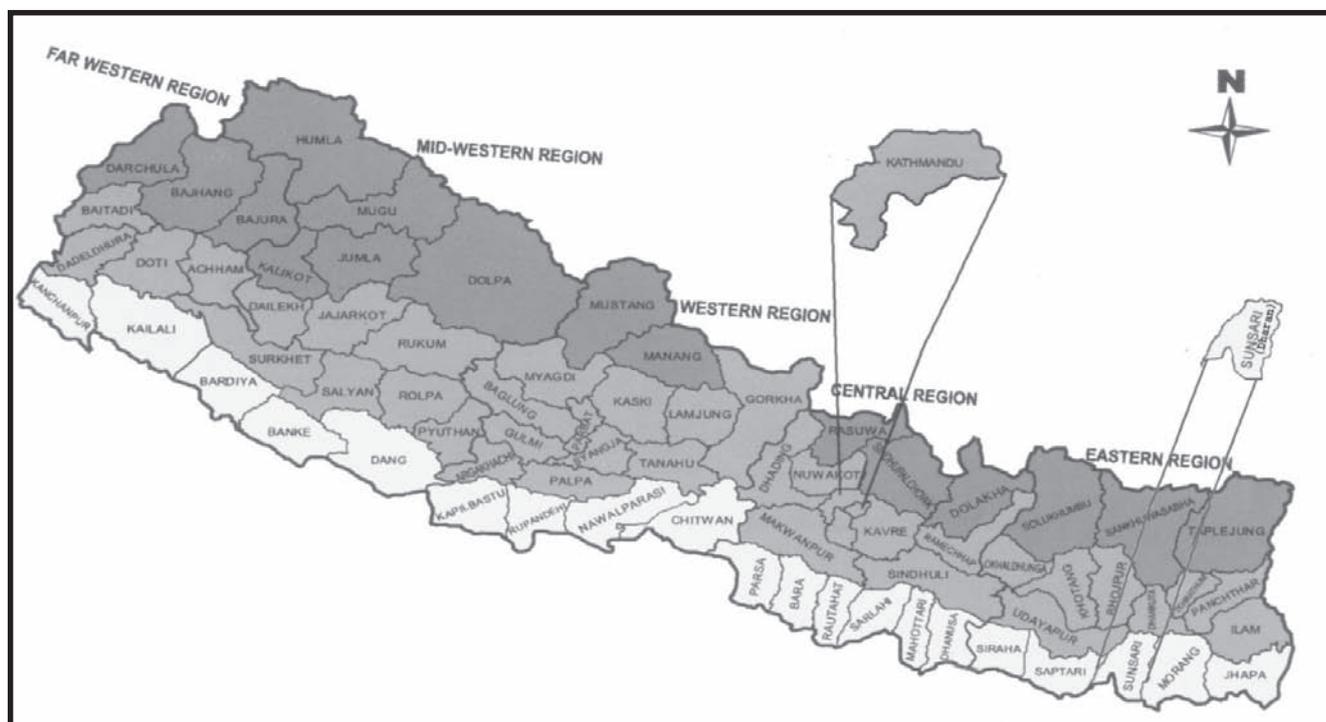


Fig. 1. Map showing the study sites of rotavirus diarrhea in Nepal.

Pubmed using the keywords of ‘rotavirus infection in Nepal’. In total, 15 publications on rotavirus were identified from the year 1978 until the present. Seven papers were identified in Pubmed, where eight were identified in national journals. Fourteen out of fifteen papers were published on Group A rotavirus. Papers were included in this article if the study period was at least not less than 12 consecutive months and that included more than 100 patients presenting to hospitals. Of fifteen rotavirus papers, five fulfilled the criteria for inclusion in this study. Data on rotavirus prevalence, serotype/genotype, detection rate, methods, duration, the location and the age of patients were retrieved. Tables and figures for the extraction of data were generated.

RESULTS

Rotavirus epidemiological studies have been conducted between 1999 and 2007 in central and eastern part of Nepal¹³⁻¹⁷ (Fig.1). 3671 individuals in the 5 studies were tested for rotavirus by ELISA method and 864 samples were positive (Table-1). Three studies reported G and P types, and two reported only the presence of antibodies. Prevalence rate of rotavirus infection was ranged from 12 to 39%. Out of 5 studies 3 included only <5 years old children, whereas other 2 studies included <15 years and all age groups respectively (Table-2). Children <5 years old are mostly affected group, although rotavirus infection was detected in all age groups. Rotavirus infection rate was found ranged from 17 to 39 % in children <5 years old, while 5% in 15 years and above

Table-1: Epidemiology of rotavirus infection among patients with diarrhea attending to hospitals in Nepal from 1999 to 2007.

Study characteristics				Samples			
Study area	Year	Study Period (month)	Method	Season	Tested	Positive (%)	Ref.
Dharan	1999-2000	12	ELISA/Ridascreen (IP&OP)	winter	160	62(39)	[13]
NM	2001-2002	12	ELISA (IP&CB)	winter	326	100(31)	[14]
Kathmandu	2003-2004	12	ELISA/RT-PCR (IP & OP)	winter	1,315	153(12)	[15]
Kathmandu	2004-2005	12	ELISA/RT-PCR (IP)	winter	731	170(23)	[16]
Kathmandu	2005-2006	12	ELISA/RT-PCR (IP)	winter	666	223(34)	[17]
Kathmandu	2006-2007	12	ELISA/RT-PCR (IP)	winter	473	156(33)	[17]

Ref, Reference; ELISA, Enzyme -linked immuno sorbent assay; NM, Not mentioned; IP, Inpatient; OP, Out-patient; CB, Community-based.

(Table-2). In children, between 5 to 14 years, rotavirus detection rate was found ranged from 7 to 10%. The rotavirus was peaked in the winter season in children <5 years of age (Table-1). Similar seasonal pattern, however, was not marked in 15 years and older, where rotavirus infection was mostly observed in rainy season from May to July.¹⁵

Molecular characterization of rotavirus strains have shown that globally common serotypes G1, G2, G3 except G4 were circulating in Nepal. Uncommon serotype G12 was accounted for a significant proportion among Nepalese children with diarrhea. G12 was found to be stable in two consecutive study periods 2003-2004 (20%) and 2004-2005 (23%). However, it was increased in the following year 2005-2006 (50%). G12 was associated with P[8], P[6] or P[4] VP4 genotypes during the 4-year study period. VP4 P[8], P[6], and P[4] circulated throughout the study period, while P[25] was detected in the year 2003-2004. P[25] was found to be along with G11 strain.¹⁵ However, it did not appear in the following years. G3 and G9 were continued to appear since 2004-2005 until recent years. G9 strains were found to be associated with P[4], P[8] or P[6] VP4 genotypes, but neither G3 nor G9 occurred in 2003-2004 study period. Of combined G and P genotypes, G1P[8] was the most prevalent strain type, accounting for 25% followed by G12P[6] (23%) and G2P[4] (16%) between 2003 and 2007 (Fig.3). However, the combination of G and P genotype varied with time period. G1P[8] was the most prevalent strains, representing 65% of rotavirus positive specimens in 2003-2004, while G2P[4] in 2004-2005 representing 33% of analyzed rotavirus specimens. G12P[6] was the most frequent strain type in 2005-2006 and 2006-2007 study period representing 34% and 24%

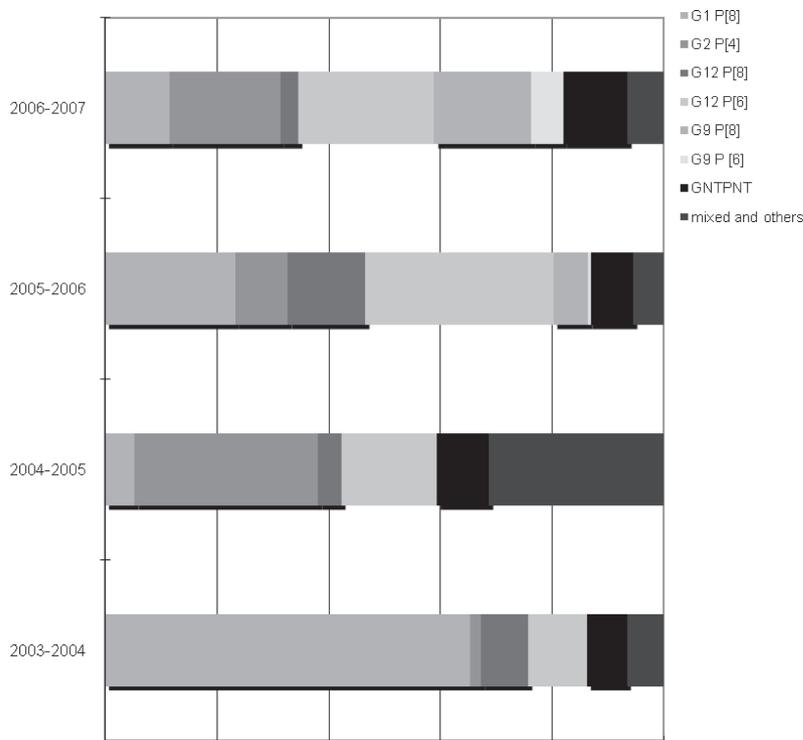


Fig. 2. Yearly distribution of common combinations of G and P genotypes among rotavirus positive specimens collected from diarrheal patients presenting to hospitals from 2003 to 2007 in Kathmandu, Nepal.

of rotavirus strains respectively (Fig.2). Nearly 9% of G and P genotype remained untypable among rotavirus positive specimens.

DISCUSSION

Diarrheal diseases remain a serious public health problem in Nepal. However, comprehensive knowledge of rotavirus infection among diarrheal patients is limited. The present study showed that rotavirus positivity rate was ranged from 12 to 39 % among patients with acute diarrhea presenting to hospitals. In children aged under 5 years, 17 to 39% [median 32%] of rotavirus infection was associated with diarrhea (Table-2). The median detection rate is comparable to rates from Hong Kong [18-35%, median 28%],¹⁸ but reported higher than in India [6-45%, median 20.8%] and Pakistan [6-26%, median 14%].^{19,20} Although, improvements in nutrition hygiene, increased awareness among caretakers and the use of oral rehydration therapy contributed to decline the incidence of diarrheal diseases over the past years in Nepal,²¹ this result revealed that rotavirus infection remained high among diarrheal children.

Children less than 5 years old with rotavirus diarrhea show marked seasonal variations, with peaks in winter and drier months of the year. This finding is in accordance with other studies reported elsewhere.^{22, 23} In aged 15 and above, rotavirus

Table-2: Age distribution of rotavirus infection.

Year study	Age (year)	Age group			Ref.
		<5y	5-14y	>15y	
1999-2000	<5	39%			[13]
2001-2002	<5	31%			[14]
2003-2004	All	17%	7%	5%	[15]
2004-2005	<15	27%	10%		[16]
2005-2006	<5	34%			[17]
2006-2007	<5	33%			[17]

Ref, Reference; y, Year

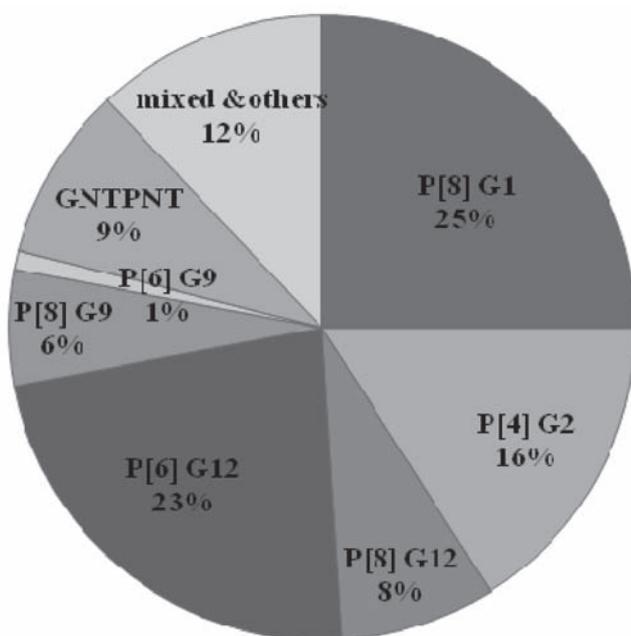


Fig. 3. Distribution of rotavirus G and P combinations between 2003 and 2007.

diarrhea occurred during rainy season, with peaks in July (May to July), indicating that adult rotavirus infection is not as season-specific (winter disease) as childhood disease.¹⁵ Therefore, rotavirus infection should consider in the differential diagnosis in adults with diarrhea attending to hospitals regardless of season.

Four common G types (G1, G2, G3 and G4) in conjunction with P[8] or P[4] represent 68% of rotavirus infections in Asia.⁴ In the present study, two genotypes, G1P[8] and G2P[4] represented 41%, while G12 strains along with P[8] or P[6] accounted for 31% of analyzed rotavirus specimens during the 4-year study period. These combinations together accounted for 72% of the rotavirus positive samples. G12P[6] has replaced globally common G and P combinations and became the most frequent genotype combination during two consecutive years 2005-2006 and 2006-2007. It has been shown that emergence of G12 strain in human populations could be the result of reassortment between human and animal rotaviruses,²⁴ suggesting that similar event may be readily taking place in Nepal. However, animal rotavirus study is needed to confirm or refute this hypothesis. G4, one of the globally common G types, was not detected, while a few G3 strain has been observed since 2004-2005. The frequency of G3 detection in Nepal contrasted with the studies in China and Vietnam, where G3 strain was the most prevalent genotypes in recent years.^{25,26} G9 strain was first appeared in 2004-2005 along with P[4] specificity in Nepal and its combination with VP4 has been extended to P[8] and P[6] genotypes in the following years. Multiple VP4 genotypes associated

with G9 strain was in accordance with findings reported elsewhere.⁴ However, it was not clear whether G9 with multiple VP4 genotypes represented a recent emergence or an established combination in Nepal, since it was not detected in 2003-2004. G9 was first detected in 1983²⁷ and reemerged with multiple VP4 genotypes after a decade. It is now considered the fifth most common genotype in the world.⁴ Currently licensed rotavirus vaccines, Rotarix and RotaTeq, bear neither G12 nor P[6] genotypes in their components.^{10,11} It is, thus, important to determine whether currently available vaccines will provide protection against G12 infections, particularly in resource-poor settings, although these vaccines have demonstrated highly efficacious in preventing most prevalent serotypes G1-G4, including G9 rotavirus diarrhea in high and middle income countries.^{10,11}

Approximately 9% of rotavirus positive samples remained G and P untypable in each epidemiological study in Nepal. A failure to genotype has been reported from various parts of the world. Accumulation of point mutations or genetic reassortment or intragenic recombination contribute to the evolution of viruses resulting in unusual or new rotavirus strains²⁸⁻³⁰ that led to mismatch at the primer binding region(s) of gene segment by existing primers. Unusual serotypes such as G5, G10 or G11 have been reported in humans from neighboring countries China, India or Bangladesh in recent years.^{6,8,31} Thus, non-typable samples could represent similar uncommon serotypes in Nepal. For example, P [25] G11 strain, detected in 2003-2004, was initially untypable by available primers. Untypable rotavirus strains have been constantly reported from developing countries as well as developed countries; despite new methodologies have been employed.⁴ Therefore, it is critical to develop specific primers to detect unusual strains that are becoming apparent in humans among non-typeable samples in epidemiological studies.

The present study clearly demonstrated that rotavirus infection is the most identifiable cause of severe diarrhea, particularly in children less than 5 years old in Nepal. This review profile should provide an important tool for policy makers in understanding the epidemiological characteristics of rotavirus infections and the potential impact of rotavirus vaccination after introduction. Nevertheless, rotavirus strains circulating in Kathmandu city may not necessarily represent those of strains circulating in other parts of the country. Multi-centre rotavirus surveillance, therefore, will be required to get a more accurate picture on rotavirus strains circulating in Nepal.

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