SUPPLEMENT ARTICLE

# Molecular Characterization of Rotavirus Strains Circulating in Oman in 2005

**Said Al Baqlani,[1] Ina Peenze,[3] John Dewar,[3] Zainab Al Lawati,[1] Lindsey Pearson,[3] Varghese Rupa,[1] Charles Mothokoa,[3] Salah Al Awaidy,[2] Suleiman Al Busaidy,[1] and A. Duncan Steele[3,4]**

[1]Central Public Health Laboratories, and [2]Department of Surveillance and Disease Control, Ministry of Health, Muscat, Oman; [3]Medical Research Council Diarrhoeal Pathogens Research Unit, University of Limpopo, Medunsa Campus, Pretoria, South Africa; [4]Vaccines and Immunization, PATH, Seattle, Washington

**Limited genotyping data are available for rotavirus strains in the Middle East. In this study, we investigated the molecular epidemiology of human rotavirus strains circulating in the Sultanate of Oman during 2005. Rotavirus was detected in 178 (57.4%) of 310 of the diarrheal stools of young children <5 years admitted to hospitals and outpatients clinics. Polyacrylamide gel electrophoresis demonstrated the cocirculation of 8 strains, although 2 strains predominated across the Sultanate. Genotyping revealed the presence of human rotavirus strains of types G1P[8], G2P[4], and G3P[8]. Several strains exhibited unusual combinations of G and P genotypes and RNA electropherotypes, indicating the likelihood of natural reassortment events occurring with a high frequency. In addition, the unusual P[10] genotype was identified among the rotavirus strains, in combination with the G1 type.**

Globally, rotavirus remains a major cause of morbidity and mortality in developing countries and is associated with a mean of ~611,000 deaths annually (range, 454,000–705,000) in infants and young children <5 years of age [1]. Although the incidence of infection in children in industrialized and developing countries is similar, outcomes vary widely. The risk of dying from rotavirus disease before age 5 years is ~1 in 50,000 in industrialized countries, but in developing countries it can be as high as 1 in 200 [1]. Not surprisingly, the development of rotavirus vaccine is a priority of the World Health Organization and the Global Alliance for Vaccines and Immunization.

Rotaviruses form a genus of the family Reoviridae; they are nonenveloped viruses that are 75 nm in diameter. Their genome consists of 11 segments of double-stranded RNA (dsRNA) and encodes 6 structural proteins (VP1–VP4, VP6, and VP7) and 6 nonstructural proteins (NSP1–NSP6) [2]. Rotaviruses can be distinguished into 7 serogroups (A–G), on basis of the major inner capsid proteins. The most prevalent rotavirus group associated with infections in humans and animals is group A [3]. According to the antigenic characteristics of the VP7 outer capsid glycoprotein and the protease-sensitive spike protein VP4 of the outer layer, group A rotaviruses are further classified into different G and P types, respectively [2]. Although G serotypes and genotypes carry identical numbers, more P genotypes (designated by numbers in square brackets) than P serotypes have been described [2]. In addition, rotaviruses are further classified into different G and P genotypes based on differences in the nucleotide sequences of the genes encoding these antigenic proteins. To date, at least 15G and 27P genotypes have been characterized using molecular techniques [2, 4, 5].

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Reprint requests and correspondence: Dr. Duncan Steele, 1455 Leary Way NW, Seattle, WA 98107 (dsteele@path.org).

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These antigens are considered important for vaccine development, because they are immunogenic and elicit protective neutralizing antibodies [4].

Two live attenuated rotavirus vaccines have been developed and licensed [6, 7] and introduced into various countries, including the United States, many countries in Latin America [8], and some countries in Europe [9]. The 2 vaccines have been developed on the basis of different rationales for the mechanism of protection. The Rotarix vaccine (GSK Biologicals) has been developed on the basis of the expectation that repeated immunizations with the monovalent vaccine will elicit a broad, heterotypic cross-protection [6]. On the other hand, the RotaTeq vaccine (Merck) has been developed to elicit an immune response that has as broad a range of neutralizing antibodies as possible, including the major neutralizing antigens in a pentavalent reassortant vaccine [7].

Despite this global awareness of rotavirus infection and disease, little has been published on the impact of rotavirus on the health of Omani children. Scrimgeour et al [10] reported an epidemiological meta-analysis of infectious and tropical diseases in the Sultanate of Oman. These authors found a nearly 50% reduction in the incidence of gastroenteritis and diarrhea in Oman between 1992 and 1998 but did not report on the viral etiological agents involved in this study. However, there are some data regarding rotavirus epidemiology in the Sultanate of Oman; 31% of children admitted to the hospital for diarrhea treatment from November 1990 to October 1992 were infected with rotavirus [11]. In 2003, to address health needs in the Eastern Mediterranean region, the Eastern Mediterranean Regional Office (EMRO) of the World Health Organization organized a Genomics and Public Health Policy Executive Course in Muscat, Oman. The overall objective of the course was to explore collectively how to best harness genomics to improve health in the region. To assist in this process, EMRO requested that the Medical Research Council Diarrhoeal Pathogens Research Unit (DPRU), a rotavirus reference laboratory for southern, East and North Africa, train an Omani scientist in techniques involved in the molecular genotyping of rotaviruses. Subsequently, one of the authors (S.A.B.) was invited to receive such training at the DPRU in 2005. The current study was undertaken to describe rotavirus genotypes circulating in Oman in 2005 and enable informed decisions to be made about the introduction of a rotavirus vaccine in Oman.

## METHODS

***Specimen collection and preparation.*** Three hundred ten single stool specimens were collected from January to December 2005 from Omani children aged 1–72 months who presented with diarrhea at 10 selected hospitals and 3 primary health care clinics throughout Oman, which were nominated by the Ministry of Health. Demographic and clinical data were collected. The majority of the children (177 [57%] of 310) involved in this study were male; 191 [62%] were <12 months old 71 [23%] were 13–24 months old, and 48 [15%] were 25–72 months old.

Ten-percent suspensions of each stool specimen were prepared in phosphate-buffered saline buffer and screened with an enzyme-linked immunosorbent assay (ELISA) kit (IDEIA; Dako) for the presence of rotavirus antigen at the Department of Laboratories, Ministry of Health, Muscat, Oman.

***Rotavirus characterization.*** Of the ELISA-positive samples, 110 were available for further analysis; the remaining specimens did not contain sufficient viral material. Rotavirus dsRNA was extracted from the stool specimens using the phenol-chloroform method, as described elsewhere [12], and the rotavirus RNA electropherotype was determined by polyacrylamide gel electrophoresis (PAGE) and silver-staining of the rotavirus dsRNA [12]. These techniques have been widely reported from the DPRU in many studies from different countries [13].

***Rotavirus genotyping.*** One nundred ten rotavirus strains that showed the presence of dsRNA by PAGE and different RNA electropherotypes were selected for genotyping using the reverse-transcription polymerase chain reaction (RT-PCR), as described elsewhere [13–16].

## RESULTS

***Rotavirus-positive samples.*** Of the 310 samples collected, 178 (57.4%) tested positive for rotavirus antigen. The majority of the rotavirus-positive specimens identified (88.8%) were from children <2 years of age, mainly from those 6–12 months of age. Rotavirus was identified commonly during the course of the year, with a slight increase in the cooler months in the Sultanate of Oman, November to January.

***PAGE results.*** After electrophoresis of the rotavirus dsRNA, electrophoretic patterns were observed for 97 (93%) of 104 of the specimens. All of these showed the classic "long" RNA electrophoretic pattern. The majority of the rotavirus strains were grouped as L1 (27 strains) or L2 (62 strains), although 8 distinct electrophoretic patterns were identified. Seven ELISA-positive samples did not have sufficient RNA present in the stool to show an electrophoretic pattern but gave positive results when genotyped.

***G and P genotyping.*** Of the 110 specimens that were typed by RT-PCR for the VP7 G type, the majority were G1 (41 specimens), G3 (20), or G2 (12). Single G9 and G4 specimens were also noted. Mixed strains were observed, including G1G3 (8 specimens), G1G4 (1), G1G2G8 (1), G2G8 (1), G2G3 (1), and G3G9 (1).

The majority of the rotavirus strains were associated with the VP4 genotypes P[8] (54 strains) and P[4] (28), and only 3 were P[6] genotype. Interestingly, we identified 6 strains that carried the P[10] genotype, which is extremely rare in nature.

G-P combinations of individual strains were assigned as follows (Table 1): G1P[8] (21 strains), G2P[4] (9), G3P[8] (15), G2P[8] (1), G1G2P[8] (1), G1G3P[8] (4), G1G4P[8] (1), G1P[6] (2), and G4P[6] (1). Sixteen of the strains could not be assigned a P genotype: 6 G1 strains, 2 G2 strains, 4 G3 strains, 3 G1G3 strains, and 1 G9 strain.

## DISCUSSION

There are limited data regarding the epidemiology of rotavirus infection in the Sultanate of Oman. A small study conducted from November 1990 to October 1992 found that 31% of young children <24 months of age with gastroenteritis were infected with rotavirus, compared with 6% of nondiarrheal control subjects [11]. The children with rotavirus disease were observed to have more vomiting and fever and, interestingly, a higher frequency of respiratory symptoms than those infected with bacterial agents [11]. In the current study, 57% of children investigated across the Sultanate of Oman tested positive for rotavirus antigen. These results together indicate that a significant number (from one-third to more than half) of all young Omani children with acute gastroenteritis may be infected with rotavirus. This finding has a significant impact on their health and on the Omani health care system. The results of the present study provide strong support for the establishment of a hospital-based study of the rotavirus-associated burden of disease that would provide insights into the impact of rotavirus infection on the health and nutrition of Omani children and on the Omani health care system.

Most of the children involved in the current study were <2 years old. This is consistent with published reports on the epidemiology of rotavirus infection in countries with a temperate climate [17, 18] and another report from Oman [11]. Rotavirus infection was detected in all age groups in the current study, although a disproportionate rate of infection was observed in infants <12 months of age (61%); only 10% of the toddlers aged >25 months with diarrhea had detectable rotavirus in their stools. The early age of acquisition of severe rotavirus infection seen in this study indicates that vaccination would need to be given early in life and should be based on the Expanded Program on Immunization schedule.

In addition, we report for the first time on the molecular characterization of rotaviruses collected from Omani children. First, multiple rotavirus electropherotypes of the rotavirus genome were observed, reflecting the genetic diversity of human rotaviruses throughout Oman. All of these were observed to have the well-described long RNA electropherotype. This represents an unusual occurrence, in that some strains were genotyped as belonging to the group of rotaviruses that is classically associated with "short" RNA patterns [2, 17].

The G2P[4] strains are characteristically associated with a short RNA electropherotype [2] but were all observed in human rotavirus strains with a long RNA pattern. This is an unusual observation and may indicate naturally occurring reassortment between human rotaviruses from the 2 classic genogroups [2]. This hypothesis is further supported by several G1P[4] strains and a single strain typed as G2P[8], which are reassortant viruses between the different genogroups. Why this high frequency of reassortment occurs in Oman is unknown and warrants further epidemiological study.

Perhaps significant were the number of rotavirus strains with a mixed genotype, more than half of which were typed as G1G3 types. Interestingly, this pattern has been seen in Nigeria, West Africa [19, 20]. The strains in these West African studies were investigated with monoclonal antibodies to the G1 and G3

**Table 1. Genotypes of Rotavirus Strains Isolated from Children Aged 1–72 Months in Oman in 2005, as Determined by Reverse-Transcription Polymerase Chain Reaction**

| | No. (%) of specimens, by P genotype, | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| G genotype | P[8] | P[6] | P[6]P[8] | P[10] | P[8]P[4] | P[4] | Untyped | Total |
| G1 | 21 | 2 | 0 | 5 | 0 | 7 | 6 | 41 (37.3) |
| G2 | 1 | 0 | 0 | 0 | 0 | 9 | 2 | 12 (10.9) |
| G3 | 15 | 0 | 1 | 0 | 0 | 0 | 4 | 20 (18.2) |
| G9 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 (0.9) |
| G4 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 (0.9) |
| G1G3 | 4 | 0 | 0 | 0 | 1 | 0 | 3 | 8 (7.3) |
| G2G8 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 (0.9) |
| G2G3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 (0.9) |
| G1G2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 (0.9) |
| G3G9 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 (0.9) |
| G1G2G8 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 (0.9) |
| G1G4 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 (0.9) |
| Untyped | 8 | 0 | 0 | 1 | 0 | 12 | 0 | 21 (19.1) |
| Total | 54 (49.1) | 3 (2.7) | 2 (1.8) | 6 (5.5) | 1 (0.9) | 28 (25.5) | 16 (14.5) | 110 (100) |

epitopes and found to react with both, indicating that they were indeed viral strains that carried G1 and G3 antigenic markers.

This fact is important epidemiologically, because mixed rotavirus infections are a prerequisite for reassortment in vivo or in vitro. The number of potential natural reassortants observed in this study is indeed considerable: 5 viruses with common G types but combined with P[10] viruses, 21 untyped G viruses (of which 12 were P[4] and 9 had long electropherotypes), 16 untyped P viruses (mainly G1 and G3), adding up to 33 of 110 (30%). In this context the identification of 9 G2P[4] viruses with a long RNA electropherotype is also important and contributes to the speculation that cocirculating human rotaviruses reassort frequently in nature, either in humans or in other mammalian hosts. Researchers in the United Kingdom found that only ~2% of human rotavirus strains were natural reassortants [21]. Furthermore, the importance of animal rotaviruses as a reservoir for human infections and with the potential for genetic reassortment has also been demonstrated with domestic livestock [22, 23].

It was interesting to note that several strains were identified as carrying the P[10] VP4 genotype, which is also an unusual human strain and was reported elsewhere with the prototype human G8 strain 69M and observed in very low frequencies in global studies [24]. In this study, all the G1P[10] strains were identified in patients who required admission to a hospital for severe rotavirus infection and who were <12 months of age. These unusual strains also warrant further investigation.

Global data from 1994 through 2003 indicate that the 4 most prevalent human rotavirus genotypes worldwide were G1P[8] (52%), G2P[4] (11%), G4P[8] (8%), and G3P[8] (3%) [25]. However, most recent data suggest that G9 genotype has gained global importance during the past 10 years or so. Large global surveys have also shown that the incidence of "unusual" rotavirus strains in tropical and subtropical countries is much higher than in countries with temperate climates [13, 26, 27]. The rotavirus strains in the Sultanate of Oman thus reflect the globally most important human rotavirus strains, although they seem to occur in unusual combinations. This is similar to findings of other studies in sub-Saharan Africa, where many untyped and novel human rotaviruses have been detected [13].

A large number of samples (25%) could not be typed by the multiplex PCR methods used. This has been reported elsewhere for studies in Africa and in Asia [13, 16, 25, 26] and is probably due to a combination of factors, including novel strains, mismatched primer sets, and inhibitory factors in the stool [24, 25]. Focused analysis of these strains in a specialist laboratory can help type many samples that are untypeable at PCR, as reported elsewhere in this supplement [28].

The current findings emphasize the need for further epidemiology and surveillance of rotavirus infection in Oman, and we are planning a wider study that will also involve molecular characterization and sequencing of the mixed and the untypeable G genotypes. The results of this study may be used to help advocate for the establishment of routine rotavirus surveillance in the Sultanate of Oman and to predict the impact of introducing a rotavirus vaccine there.

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