Antigenic and Molecular Characterization of Unusual Rotavirus Strains in Burkina Faso in 1999

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Thirty-six of 37 rotavirus strains recovered from the diarrheal stools of 166 children <3 years of age in Burkina Faso were characterized at both the antigenic and molecular levels. The rotavirus strains were confirmed by polyacrylamide gel electrophoresis; 30 displayed predominantly short electropherotype patterns, and 6 had a long RNA pattern. The strains were subgrouped by monoclonal antibody enzyme immunoassay for VP6 and were typed as subgroup I (29 of 30 short rotavirus strains) and subgroup II (5 of 6 long strains). The VP7 serotyping and genotyping showed that all 6 viruses with long electropherotype patterns were G1. The short strains were determined to be VP7 serotype G2 by reverse-transcription polymerase chain reaction (PCR) in 27 strains and nucleic acid sequencing of selected strains, although only 1 reacted with the G2-specific monoclonal antibodies. Finally, the short patterns were shown by the PCR genotyping method to be VP4 genotype P[6], and the long patterns were shown to be P[8]. The predominant strain found in Burkina Faso in this small study was an unusual G2P[6] strain that showed a short RNA electropherotype and VP6 subgroup I specificity and failed to react with a panel of G2-specific monoclonal antibodies.

Rotavirus is accepted to be a major cause of childhood diarrhea worldwide [1, 2]. In Africa, rotavirus is a major etiological agent for acute infantile gastroenteritis and is associated with 23%–24% of diarrhea cases in infants [2]. In developing countries, ~600,000 infants and young children annually are estimated to die before age 5 years because of rotavirus diarrhea [2, 3]. Many of these deaths occur in Africa, where ~150,000 deaths were estimated to occur annually in 2000 [4]. However, there have been limited studies in Africa investigating the epidemiology of rotavirus infection, and most existing studies have been concentrated in relatively few countries. Before the possible implementation of rotavirus immunization to reduce this burden, strains circulating in African countries where immunization is contemplated must be identified and characterized [5].

The World Health Organization has prioritized the development of rotavirus vaccine for developing countries and has recommended efficacy trials with the new rotavirus vaccines in developing countries of Africa and Asia [6]. In addition, ongoing surveillance has been considered important to understand the epidemiology of the circulating rotavirus serotypes in any region where rotavirus vaccination may be considered, to help define vaccine efficacy against circulating strains and document possible changes in the circulating strains after immunization. Global surveillance efforts have documented the hospital burden of disease due to rotavirus [7].

The rotavirus particle consists of an inner capsid made up of VP6, the major antigenic mass of the virion [1]. This inner capsid contains both the group antigen, which is the target of the current group A enzyme-linked immunosorbent assays (ELISAs), and the VP6
subgroup antigen, which is an important epidemiological marker [1]. The outer rotavirus capsid consists of 2 epidemiologically important proteins, which have been targeted in vaccine strategies. The VP7 serotype is the major neutralizing antigen of the virus, and 5 VP7 serotypes are commonly identified among human rotaviruses circulating in nature [8]. These are termed VP7 serotypes G1–G4 and G9; G1–G4 have been included in the bovine human reassortant vaccine developed by Merck Research Laboratories [9]. The VP4 is a protease-sensitive protein that protrudes from the virus particle and interacts with the cell receptor [1]. Three VP4 genotypes have been reported to occur commonly in human rotaviruses and are termed P[8], P[4], and P[6] [8]. The P[8] is considered the most common VP4 on human rotaviruses and has been included in the bovine-human reassortant pentavalent vaccine [9]. The most common strain globally—G1P[8]—forms the basis of the human monovalent vaccine produced by GlaxoSmithKline Biologicals [10].

Both VP7 and VP4 antigens elicit the production of neutralizing antibodies by the host and are considered important in vaccine development. In this study, rotavirus strains identified in Burkina Faso were characterized at the level of all 3 important antigens, the VP6 subgroup and the VP7 and VP4 genotypes [8], and complement the regional data on rotavirus strains from other surrounding countries [11–13]

METHODS

Stool samples. Stool specimens were collected from 166 young children <5 years of age presenting with diarrhea at Centre Hospitalier National Souro Sanou and Centre Hospitalier National Yalgado Ouédraogo in 1999. Ten percent suspensions of the diarrheal stool specimens were made in phosphate-buffered saline and then screened with a commercial ELISA (Rotavirus EIA; Trinity Biotech). The kit was used as described by the manufacturer.

The 37 rotavirus-positive stools were recovered from children between 8 months and 3 years of age. The rotavirus-positive stools were shipped on ice to the Medical Research Council of South Africa Diarrhoeal Pathogens Research Laboratory, University of Limpopo, Pretoria, for further analysis.

Polyacrylamide gel electrophoresis of double-stranded RNA. The rotavirus RNA was analysed by polyacrylamide gel electrophoresis (PAGE). First, the RNA was extracted from the stool suspensions by phenol-chloroform treatment and ethanol precipitation, as described elsewhere [14]. The extracted rotavirus RNA was loaded onto 10% polyacrylamide gels and electrophoresed at 100V overnight at room temperature. The RNA bands were visualized by silver staining [14].

VP6 subgroup ELISA. Determination of the VP6 subgroup was performed using subgroup-specific monoclonal antibodies, as described elsewhere [15, 16]. The VP6 subgroup–specific direct sandwich ELISA uses monoclonal antibodies that are specific for subgroup I rotavirus (NIH hybridoma 255/60/125/14) and subgroup II rotavirus (NIH hybridoma 631/9/104/56). These monoclonal antibodies have been described in detail elsewhere [15] and have been extensively used in this laboratory [16]. In brief, microtiter plates were coated with a rabbit antirotavirus serum in a carbonate buffer and incubated with the rotavirus-positive stools. The monoclonal antibodies were added after overnight incubation and washing of the microtiter plates, bound to the appropriate antigens, and detected by a horseradish peroxidase conjugate (tetramethylbenzidine enzymatic kit; Roche), as described elsewhere [16].

VP7 serotype ELISA. Two panels of VP7 serotype–specific monoclonal antibodies were used in this study. These monoclonal antibodies consisted of monoclonal KL-4 (specific for G1), S2–2G10 (G2), YO-1E2 (G3), and ST-2G7 (G4) and were used as described elsewhere [17]. A second panel of VP7 serotype–specific monoclonal antibodies included monoclonal antibodies specific for serotype G1 (5E8), G2 (1C10), and G3 (159). An additional antibody (M60) was included in the assay and is targeted to cross-reactive epitopes on the VP7 outer capsid, as a positive control that the VP7 capsid was intact for the ELISA. The panel of monoclonal antibodies was supplied by Dr Dennis Lang, National Institutes of Health (NIH), and used as described in detail elsewhere [18].

Reverse-transcription polymerase chain reaction genotyping. All of the rotavirus strains were genotyped using methods described for the VP7 [19] and VP4 [20] genotypes. These techniques have also been widely used in this laboratory.

Initially, the viral RNA was extracted by a phenol-chloroform method, followed by ethanol precipitation [21] and purification by RNaid (BIO 101; Southern Cross Biotechnology). The purified RNA was reverse transcribed with avian myeloblastosis virus reverse transcriptase at 43°C for 25 min, in the presence of primers to the terminal sequences of the VP7 gene. For G typing, the primer pair sBeg 9–End 9 was used as described by the manufacturer. The complementary DNA was amplified in a PerkinElmer Thermal Cycler (4800) at 95°C for 1 min to denature the complementary DNA, then at 48°C for 2 min to anneal the primers, and finally at 72°C for 3 min for extension of the strands. A nested, multiplex polymerase chain reaction (PCR) analysis was then performed using different primers specific for serotype-specific regions of the VP7 gene. The seminested second round of genotyping amplification used the consensus primer sBeg9 with primers 9T1–1(G1), 9T1–2(G2), 9T-3P(G3), 9T-4(G4), 9T-9B(G9), and MW-8(G8) [19]. The VP4 genotype was confirmed in a similar manner using a reverse-transcription (RT) PCR system devised by Gentsch et al [20]. Specific primers were used to amplify the VP8* gene, which was then differentiated into VP4 genotypes by a cocktail
Characterization of Unusual Rotavirus Strains in Burkina Faso

Table 1. Molecular and antigenic characterization of rotavirus strains in Burkina Faso

<table>
<thead>
<tr>
<th>VP6 subgroup and RNA pattern</th>
<th>No. of specimens</th>
<th>VP7 serotype antibody (no. of specimens)</th>
<th>Genotype (no. of specimens)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subgroup I, short pattern 1</td>
<td>20</td>
<td>M60 (11); 1C10/S2–2G10 (1)</td>
<td>G2 (17) P[6] (18); P[6/8] (2)</td>
</tr>
<tr>
<td>Subgroup II, long pattern</td>
<td>6</td>
<td>5E8 (4)</td>
<td>G1 (6) P[8] (5)</td>
</tr>
<tr>
<td>Subgroup I/II, short pattern</td>
<td>1</td>
<td>Nonreactive</td>
<td>G2 (1) P[6] (1)</td>
</tr>
</tbody>
</table>

NOTE. RNA patterns were determined by means of polyacrylamide gel electrophoresis.

a One strain was excluded from analysis.

b M60 is a crossreactive monoclonal antibody to VP7 capsid; 1C10/S2–2G10 are monoclonal antibodies specific for G2 epitopes; 5E8 is a monoclonal antibody specific for G1 epitopes.

The single rotavirus-positive stool for which PAGE did not reveal an RNA electropherotype also showed a lack of reactivity with subgroup I or II monoclonal antibodies, presumably owing to low concentrations of virus in the stool sample. This sample was excluded from further analysis.

VP7 serotypes. Five specimens were typed by the monoclonal antibody panels that were used in this study; 4 were typed as G1 by the 5E8 monoclonal antibody, and 1 strain reacted with both the 1C10 and S2–2G10 monoclonal antibodies that are specific for G2 epitopes. Despite the predominance of strains with a short electropherotype, none of these other strains were identified by the panel of antibodies including the G2 monoclonal antibodies. The M60 antibody that served as a control for the presence of VP7 antigen was reactive with 16 of the short strains, indicating that the VP7 outer capsid was present. The serotype-specific controls that were used with each microtiter plate worked well.

PCR genotyping. The VP7 genotyping assay included primers designed to detect G1–G4, G8, and G9. All 6 strains that were shown to be subgroup II and have a long RNA electropherotype were determined to carry a VP7 G1 genotype and VP4 P[8] genotype (Table 1). On the other hand, the strains that had the VP6 subgroup I antigens and exhibited 1 of 2 short RNA patterns were mostly typed as G2 and P[6] (Table 1). However, 6 of these strains were shown with PCR to have both G1- and G2-specific amplicons, although the G1 amplicon was far weaker in the gels. The conditions of the multiplex assay were adjusted (the annealing temperature was reduced, and the magnesium chloride concentration was varied) to type these strains as G2 strains. The VP4 genotypes of these short RNA profile strains were P[6].

VP7 sequence analysis and phylogenetic analysis. Because the combination of a VP7 genotype G2 and VP4 genotype P[6] strain is rare, we sequenced 2 of these strains to determine the VP7 serotype. The complete VP7 gene sequence analysis of both strains confirmed that these strains were VP7 serotype G2. A phylogenetic tree was constructed using the deduced amino
acid sequences of the VP7 gene of the G2 strains and compared with the original DS-1 G2 strain and others from Africa (Figure 1). The West African strains cluster together and can be seen to diverge from strains isolated from other regions of Africa.

**DISCUSSION**

In this study, we report the molecular and antigenic characterization of rotavirus strains from Burkina Faso. Most of the rotavirus strains, collected during a single season, displayed a short RNA electropherotype pattern, which correlated with a VP6 subgroup I specificity, a well-documented combination for human rotaviruses [1, 8, 16]. Furthermore, the strains were shown to carry the VP7 genotype for G2 rotaviruses. This combination of VP7, VP6, and RNA pattern is also well established in the literature [1]. However, these strains were all demonstrated to carry a VP4 genotype of P[6], an unusual VP4 type for these strains, which normally bear VP4P[4]; this indicates that these strains may have arisen by a reassortant event be-

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**Figure 1.** Phylogenetic analysis of the VP7 nucleotide sequences of serotype G2 strains from Burkina Faso. DS-1 has been included as the G2 standard and as the outgroup strain. The phylogenetic tree was constructed using the TreeView program, and genetic distances were generated with DNAMan computer software. The length of the abscissa to the connecting node is proportional to the genetic distance between sequences and is indicated by the scale bar. Bootstrap values of >90% are indicated at the appropriate nodes.
tween 2 human rotaviruses, as has been documented in other studies worldwide [8].

Recent studies from the Indian subcontinent and Africa have indicated that VP4 genotype P[6] strains are frequently detected in symptomatically infected neonates and young children [11–13, 22–25]. The rotavirus P[6] genotype appears to be widely distributed in the developing countries of Africa and Asia but is distributed much less widely in the developed world [8, 22]. In Africa particularly, the P[6] genotype has commonly been reported in West Africa, and the results from the current study support the distribution of this genotype in Ghana [12] and Nigeria [22]. Furthermore, P[6] strains have been detected in Malawi [23] and South Africa [26].

Recent reports from studies investigating the VP7 and VP4 genotypes of circulating rotavirus strains have described a wide diversity of strains and an unusually large number of possible recombinations of these antigen markers [22–25]. However, in 2 recent studies of strains from Nigeria conducted in this laboratory, no G2 strains could be detected by using various monoclonal antibodies against the G2 antigenic epitopes [27, 28]. The inability to type any G2 strains from these studies is probably due to a lack of the G2-specific antigenic epitopes, as described from the United Kingdom [29]. In the current study, an attempt was made to serotype the G2 strains with the available G2 monoclonal antibodies, to no avail. However, the panel of monoclonal antibodies was working well, as evidenced by the controls in the test, and several of the “untypeable” strains did react with an antibody directed to the VP7 outer capsid as a control, indicating that the capsid was present and was just not reacting with the monoclonal antibodies used. A single strain reacted with both G2-specific antibodies and was characterized further, as described elsewhere [30]. This strain was shown to have a common VP7 gene sequence and phylogenetic similarity compared with other strains in West Africa.

Almost all of the rotavirus strains with a short RNA electrophoretic pattern carried the same serotypic or genotypic makeup. These included VP6 subgroup I, VP7 genotype G2, and VP4 genotype P[6]. In 2 other studies these rotavirus strains have been reported commonly. The first in Ghana examined rotavirus strains from young children in Navrongo, a rural area in northwest Ghana, bordering Burkina Faso [12]. The second study was conducted in northeastern Nigeria, which is also a rural area [31] and not far from Burkina Faso. It is interesting that these studies were all conducted in the same region of Africa and the samples were all obtained in 1999–2000, showing the regional distribution of these unusual strains. This finding is further supported by the phylogenetic analysis, which shows the clustering of these strains together, compared with G2P[4] strains from the continent as a whole [30]. The phylogenetic analysis of the West African sublineage (bootstrap value, 95.8%) indicates that the epidemiology and transmission of rotavirus strains in this region is geographically limited and distinct [30].

Previous reports have indicated that reassortant G2 rotaviruses were responsible for epidemics of gastroenteritis in China [32], Japan [33], and Brazil [34]. Furthermore, symptomatic reinfection with rotavirus, which is unusual in the natural history of the virus, has been reported with G2 strains [35]. Finally, the cyclic nature of G2 rotavirus infections is observed in long-term surveillance studies in which G2 strains occur every 3–4 years [36]. These factors highlight the need to monitor rotavirus strains circulating in the community, especially as rotavirus vaccines become available and are introduced more widely.

Recent reports from Brazil, where the vaccine is being used in routine childhood immunization, indicate the particular importance of monitoring rotavirus strains, particularly G2 strains [37]. It would be interesting to see whether these G2 strains in Brazil have the neutralization epitopes that have been determined to be commonly present in G2 serotype strains or whether there has been an antigenic drift in their epitopes, making them less recognizable by antigenic criteria and possibly neutralizing escape mutants. The findings of this study highlight the need to examine strains by means of neutralization epitopes, as well as by the more convenient and widespread method of PCR genotyping.

Acknowledgments

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References

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