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Molecular characterization of rotavirus strains detected during a clinical trial of a human rotavirus vaccine in Blantyre, Malawi

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Abstract

The human, G1P[8] rotavirus vaccine (Rotarix) significantly reduced severe rotavirus gastroenteritis episodes in a clinical trial in South Africa and Malawi, but vaccine efficacy was lower in Malawi (49.5%) than reported in South Africa (76.9%) and elsewhere. The aim of this study was to examine the molecular relationships of circulating wild-type rotaviruses detected during the clinical trial in Malawi to RIX4414 (the strain contained in Rotarix) and to common human rotavirus strains. Of 88 rotavirus-positive, diarrhoeal stool specimens, 43 rotaviruses exhibited identifiable RNA migration patterns when examined by polyacrylamide gel electrophoresis. The genes encoding VP7, VP4, VP6 and NSP4 of 5 representative strains possessing genotypes G12P[6], G1P[8], G9P[8], and G8P[4] were sequenced. While their VP7 (G) and VP4 (P) genotype designations were confirmed, the VP6 (I) and NSP4 (E) genotypes were either I1E1 or I2E2, indicating that they were of human rotavirus origin. RNA-RNA hybridization using 21 culture-adapted strains showed that Malawian rotaviruses had a genomic RNA constellation common to either the Wa-like or DS-1 like human rotaviruses. Overall, the Malawi strains appear similar in their genetic make-up to rotaviruses described in countries where vaccine efficacy is greater, suggesting that the lower efficacy in Malawi is unlikely to be explained by the diversity of circulating strains.

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Author Contributions

Toyoko Nakagomi, Osamu Nakagomi, Duncan Steele, Kathy Neuzil and Nigel Cunliffe conceived the study. Desiree Witte, Bagrey Ngwira and Stacy Todd were co-investigators on the primary study of rotavirus vaccine in Malawi. Winifred Dove and Yen Hai Doan conducted the laboratory and phylogenetic analyses. Toyoko Nakagomi drafted the paper with scientific input from all authors. All authors approved the final version of the manuscript.

Disclosures

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Keywords

Rotavirus; vaccine; genotype; strain diversity; genogroup

1. Introduction

Rotavirus is the single most important aetiological agent of severe, acute gastroenteritis in infants and young children worldwide, causing an estimated 527,000 deaths among children less than 5 years of age [1]. More than 65% of these deaths were estimated to occur in 11 countries in Asia and Africa [1, 2]. Since improvements in sanitation and hygiene will unlikely decrease the incidence of rotavirus infection, vaccination offers the main hope of reducing global rotavirus deaths [3]. After successful clinical trials of the rotavirus vaccines Rotarix (GSK Biologicals, Belgium) and RotaTeq (Merck & Co., USA) in Europe and the Americas [4, 5], the World Health Organization (WHO) recommended that rotavirus vaccines should be included into national immunization programmes in regions where efficacy data suggested that there would be a significant public health impact [6, 7]. The question remained as to how both rotavirus vaccines would perform in the world's poorest countries in Asia and Africa [3].

A randomized, placebo-controlled clinical trial of Rotarix conducted in Malawi and South Africa was completed in 2008, and demonstrated a vaccine efficacy against severe rotavirus gastroenteritis of 61.2% in the combined study populations [8]. While the efficacy in Malawi was 49.5%, 6.6 episodes of severe rotavirus gastroenteritis were prevented per 100 infant-years by vaccination, indicating a significant potential public health impact [8]. Thus, when considered together with other data from resource-poor settings, WHO recommended the inclusion of rotavirus vaccine into all national childhood immunization programmes, and the introduction of rotavirus vaccine was strongly recommended in countries where diarrhoea is responsible for 10% of mortality among children less than 5 years of age [9]. Nevertheless, the efficacy of Rotarix in Malawi (49.5%) was less than had been previously documented in other settings and below that observed in South Africa (76.9%). Rotavirus strain diversity is known to be greater in many developing countries than reported in industrialized countries and has been postulated as a factor that could adversely impact on vaccine performance [10, 11].

Rotavirus is a segmented double-stranded RNA virus that belongs to the family *Reoviridae*, and its G and P serotypes are defined by the antigenicity of the outer capsid neutralisation proteins, VP7 and VP4, respectively. These serotypes are often referred to as G and P genotypes, respectively, for molecular assays are more commonly used for their determination than are serologic assays. Recently, genotype classification has been expanded to include all 11 genome segments; for example, the genotypes of the middle capsid protein (VP6) and the viral enterotoxin (NSP4) are now referred to as I genotype and E genotype, respectively [12].

In Malawi, an extensive diversity of G and P genotypes was identified during the clinical trial; three-quarters of strains belonged to G12P[6] (27%), G8P[4] (24%) and G9P[8] (24%), with only 13% of strains being G1P[8], the homotypic genotype with respect to the RIX4414 strain that is contained in Rotarix [8]. This extensive diversity of G and P genotypes noted during the clinical trial was not exceptional since diverse rotavirus strains were known to have circulated during 10 years of surveillance in Malawi [13-17]. While it was reported that there was no statistically significant difference in vaccine efficacy against G1 and non-G1 genotypes in the clinical trial [8], we considered it important to examine whether the strain variation observed for the two surface protein genes extended to the other

genome segments. Of note, there is a considerable lack of overall genomic RNA homology between human rotavirus strains with long RNA patterns (as represented by the Wa strain; hence called the Wa genogroup to which RIX4414 belongs), and human rotavirus strains with short RNA patterns (as represented by the DS-1 strain; hence called the DS-1 genogroup to which strains including genotype G2P[4] belong) [18-20]. The aim of this study was to compare by RNA-RNA hybridization the whole genomic RNA constellation of circulating wild-type rotaviruses detected during the clinical trial in Malawi with RIX4414 (the strain contained in Rotarix). This study also aimed to determine the nucleotide sequence similarities between RIX4414 and circulating wild-type rotaviruses in Malawi, as compared with RIX4414 and other globally circulating strains, in the genome segments coding for the neutralisation proteins VP7 (G genotype) and VP4 (P genotype), the middle capsid protein (VP6: I genotype), and the viral enterotoxin (NSP4: E genotype).

2. Materials and Methods

Samples used for characterization

Rotavirus-positive specimens (N=147) collected from vaccine and placebo recipients in the clinical trial in Blantyre, Malawi, were previously examined for G and P types at DDL Diagnostic Laboratory (Voorburg, the Netherlands) by a testing algorithm using RT-PCR followed by a reverse hybridization assay [21]. Of those, only specimens containing a minimum volume of 500 µl as 10% suspension in Earl's Balanced Salt Solution (N=88) were utilized in this study. Rotavirus specimens examined comprised G12P[6] (N=25), G8P[4] (N=28), G1P[8] (N=11), G9P[8] (N=9), G12P[8] (N=5), G2P[4] (N=3), G8P[8] (N=2), G12P[4] (N=1), G1P[6] (N=1), G8P[6] (N=1), G12P[6]/P[8] (N=1) and G8P[4]/P[6] (N=1).

The vaccine strain (RIX4414) used in this study was recovered following inoculation into MA104 cell culture of a portion of the Rotarix commercial vaccine.

RNA electropherotyping

Rotavirus RNA was extracted using an RNAeasy kit (Qiagen Ltd., Sussex, UK) according to the manufacturer's instructions, and separated by electrophoresis on a 10% polyacrylamide gel followed by staining with silver nitrate as described previously [22]. Electropherotypes were assigned for those strains that showed 11 segments of double-stranded (ds) RNA, and were determined by comparison of the individual RNA migration patterns of genome segments on the gel. Short and long RNA patterns were defined by the relative migration of genome segments 10 and 11, with slower-moving genome segments 10 and 11 indicating a short RNA pattern [23]. Representative strains possessing distinct electropherotypes were examined further by nucleotide sequencing and RNA-RNA hybridization following cell-culture adaptation.

RT-PCR and nucleotide sequencing

Partial or full-length genes encoding VP7, VP4, VP6, and NSP4 were amplified by RT-PCR and the products were used directly for nucleotide sequencing (Cogenics, Essex, UK). Primers Beg9 and End9 were used to amplify a 1062 bp VP7 fragment [24]; primers con2 and con3 were used to amplify a 877 bp VP4 fragment [25]; primers GEN_VP6F and GEN_VP6R were used to amplify a 1356 bp VP6 fragment [26]; and primers BegG10 and EndG10 were used to amplify a 750 bp NSP4 fragment [27]. Genotype assignment was undertaken according to the criteria established by the Rotavirus Classification Working Group [12].

Phylogenetic analysis of the genome segments of the strains representing each of the major genotype combination was carried out using MEGA ver 4.0 [28] by drawing trees using the neighbour-joining method [29]. Bootstrap analysis of 2,000 replicates was conducted to identify the significance of branching of the constructed tree.

Cell culture adaptation of strains

Rotavirus strains subjected to RNA-RNA hybridization assays were adapted to cell culture according to the method of Kutsuzawa et al [30].

Genogrouping by RNA-RNA hybridization

RNA-RNA hybridization was carried out as previously described [18]. Briefly, the genomic RNA was transcribed into 11 positive-sense RNAs (i.e., transcription probes) in the presence of [³²P]-labelled GTP by using endogenous viral RNA polymerase present in purified double-layered particles. Thus, three different probes were prepared from RIX4414 (G1P[8], long RNA pattern), MAL60 (G8P[4], short RNA pattern) and MAL88 (G12P[6], short RNA pattern). Hybridization was allowed to occur at high stringency conditions (at 65 °C, for 16 h) between the genomic RNAs from various Malawian strains as well as Wa (G1P[8], long RNA pattern) and KUN (G2P[4], short RNA pattern), and each of the three probes. Hybrids were then separated by electrophoresis on a 10% polyacrylamide gel, and the dried gels were exposed to imaging plates and read with BAS5000 (Fuji film, Tokyo, Japan).

3. Results

RNA electropherotype and G and P genotype designations

Of 88 rotavirus-positive faecal specimens, 43 (49%) showed identifiable RNA migration patterns upon polyacrylamide gel electrophoresis. These comprised genotypes G8P[4] (N=19), G12P[6] (N=11), G9P[8] (N=4), G1P[8] (N=3), G12P[8] (N=2), G1P[6] (N=1), G8P[6] (N=1), G8P[8] (N=1), and G2P[4] (N=1). All G8P[4], G8P[6] and G2P[4] strains showed short RNA patterns with slower-moving genome segments 10 and 11, while all G9P[8], G1P[8], G12P[8], G8P[8] and G1P[6] strains showed long RNA patterns (Fig. 1). Among 11 G12P[6] strains with identifiable electropherotypes, 8 showed short RNA patterns whereas 3 showed long RNA patterns. From the above strains carrying diverse combinations of G and P genotypes, cell-culture adaptation was attempted for those rotaviruses (n = 29) that represented different G/P type combinations and different electropherotypes; of these, 8 rotaviruses could not be culture-adapted. As a consequence, a total of 21 rotaviruses were available for further studies (Fig. 1): these comprised genotypes G8P[4] (MAL01, MAL02, MAL33, MAL47, MAL55, MAL60, MAL70, and MAL81); G8P[6] (MAL43); G12P[6] (2 short pattern viruses MAL39 and MAL88, and 2 long RNA pattern viruses MAL12 and MAL40); G9P[8] (MAL80 and MAL82); G1P[8] (MAL23, MAL38, and MAL50); G1P[6] (MAL63); G12P[8] (MAL65); and G2P[4] (MAL66).

Since strains carrying G8P[4], G12P[6], G9P[8] and G1P[8] previously accounted for 89% of the strains identified among placebo recipients, single representative strains from each of these major genotype combinations (two in the case of G12P[6] representing both short and long RNA patterns) were subjected to nucleotide sequencing of the genome segment coding for VP7, VP4, VP6, and NSP4. These included MAL81 for G8P[4], MAL88 for G12P[6] short RNA pattern, MAL12 for G12P[6] long RNA pattern, MAL82 for G9P[8], and MAL23 for G1P[8].

Genotype assignment by nucleotide sequence analysis

Nucleotide sequence analysis confirmed the G and P genotypes previously ascribed by RT-PCR, and assigned genotypes to VP6 and NSP4 (Table 1). All long RNA pattern viruses

possessed VP6 and NSP4 genotypes of I1 and E1, respectively, whereas all short RNA pattern viruses had VP6 and NSP4 genotypes of I2 and E2 respectively.

Genogrouping by RNA-RNA hybridization assays

The results of RNA-RNA hybridization assays are shown in Figures 2-4. When the RIX4414 probe was allowed to hybridize with the panel of dsRNAs from representative Malawian strains carrying various genotype combinations, the probe produced 6-8 hybrid bands with genomic RNAs from MAL23 (G1P[8]), MAL38 (G1P[8]), MAL80 (G9P[8]), and MAL12 (G12P[6]), all of which had long RNA patterns. A caveat in interpretation of the result of liquid-phase RNA-RNA hybridization is that there often occurs aberrant migration of a hybrid band in which a lesser degree of sequence homology exists between the probe segment and its corresponding minus strand of the genomic RNA because in such a case the hybrid band becomes much less compact than the homoduplex band, resulting in slower migration upon gel electrophoresis. However, judging from the level of aberrant migration of the hybrid bands on the autoradiograph, the homology of the RIX4414 probe appeared higher with the genomic RNAs from the prototype Wa strain compared with Malawian long RNA pattern viruses (Fig. 2). By sharp contrast, the probe produced almost no hybrid band with genomic RNAs from MAL88 (G12P[6]), MAL43 (G8P[6]), MAL60 (G8P[4]), MAL70 (G8P[4]) and MAL66 (G2P[4]), all of which had short RNA patterns (Fig. 2). When the MAL60 (G8P[4]) probe was allowed to hybridize with the panel of dsRNAs from representative Malawian strains carrying various genotype combinations, the probe produced 7 or more hybrid bands with genomic RNAs from MAL88 (G12P[6]), MAL43 (G8P[6]), MAL70 (G8P[4]), MAL66 (G2P[4]) and KUN (G2P[4]). By contrast, the MAL60 (G8P[4]) probe produced almost no bands with Wa (G1P[8]), RIX4414 (G1P[8]), MAL23 (G1P[8]), MAL38 (G1P[8]), MAL50 (G1P[8]), MAL63 (G1P[6]), MAL82 (G9P[8]), MAL65 (G12P[8]) and MAL40 (G12P[6]) (Fig. 3). These results, when taken together, indicate that Malawian long RNA pattern viruses belonged to the Wa genogroup and Malawian short RNA pattern viruses belonged to the DS-1 genogroup.

For the two distinct G12P[6] strains having either short or long RNA pattern, the probe made from MAL88, a short pattern G12P[6] virus, produced 11 hybrid bands with MAL39, another short RNA pattern virus, that were very similar to the homologous bands, but produced with MAL12 and MAL40, long RNA pattern G12P[6] viruses, only one strong hybrid band around the area of segments 7-9 and two weak bands around the area of segments 1-4 (Fig. 4). The intense hybrid band noted in the region of genome segments 7, 8 and 9 in each of the lanes containing genomic RNAs from MAL12, MAL40, and MAL65, was interpreted as the G12 VP7 gene.

Phylogenetic analysis of VP7, VP4, VP6 and NSP4 genes

Phylogenetic trees were constructed in order to better understand the genetic relationships of representative Malawian strains with RIX4414 and with globally circulating rotaviruses with respect to each of their VP7, VP4, VP6, and NSP4 genes.

The VP7 gene—The G1 VP7 phylogenetic tree using sequences available in the DNA databases identified the presence of 6 lineages including 2 lineages that apparently consisted of mostly bovine and porcine strains (lineage V and VI) (Fig. 5a). The other 4 lineages contained only viruses of human origin. RIX4414 belonged to lineage I, whereas MAL23 (G1P[8]) belonged to lineage III (Fig. 5a). These two sequences were divergent by 5.4% at the nucleotide sequence level. In the G8 VP7 phylogenetic tree there were 3 lineages (Fig. 5b). MAL81(G8P[4]) belonged to lineage II which contained primarily strains of African origin, and its sequence clustered closely with Malawian strains which were detected between 1997 and 2001. In the G9 VP7 phylogenetic tree there were 4 lineages (Fig. 5c).

MAL82 (G9P[8]) belonged to lineage III, which comprised most of the recently-emerged global G9 strains.

In the G12 VP7 phylogenetic tree there were 4 lineages (Fig. 5d). Both MAL12 (G12P[6], long RNA pattern) and MAL88 (G12P[6], short RNA pattern) belonged to lineage III. These two sequences had a very high sequence identity of 99.4%, and supported the intense hybrid band observed between MAL12 and MAL88. However, it appeared that the VP7 sequences of G12P[6] strains were very closely related to each other irrespective of their electropherotype designation or geographical origin.

The VP4 gene—In the P[8] VP4 phylogenetic tree there were 4 lineages (Fig. 6a). MAL23 (G1P[8]) and MAL82 (G9P[8]) belonged to lineage IV, whereas RIX4414 belonged to lineage II. The P[8] VP4 genes carried by Malawian strains reported previously belonged to lineages I, III and IV [15], and thus despite the same geographical origin, Malawi P[8] VP4 genes were noted to be highly divergent. In the P[6] VP4 phylogenetic tree there were 5 lineages (Fig. 6b). Both MAL12 (G12P[6], long RNA pattern) and MAL88 (G12P[6], short RNA pattern) belonged to lineage I, sublineage 1a. Unlike the P[8] VP4 gene, all P[6] VP4 genes detected in Malawi belonged to the same sublineage within the same lineage, suggesting much smaller sequence diversity than within the P[8] VP4 gene. In the P[4] VP4 phylogenetic tree there were 3 lineages, and MAL81 (G8P[4]) belonged to lineage II (Fig. 6c). This P[4] VP4 sequence was very closely related to G8P[4] strains detected previously in Kenya, Brazil and Malawi.

The VP6 gene—While there are more than 10 I types in the VP6 genes, phylogenetic analysis clearly clustered three I1 sequences from MAL12 (G12P[6]), MAL23 (G1P[8]) and MAL82 (G9P[8]) together into the same lineage within the I1 genotype but distinct from the lineage to which RIX4414 belonged (Fig. 7). Similarly, two I2 sequences from MAL81 (G8P[4]) and MAL88 (G12P[6], short RNA pattern) clearly clustered into the same lineage within I2.

The NSP4 gene—While there are more than 11 E types in the NSP4 genes, phylogenetic analysis clearly clustered three E1 sequences from MAL12 (G12P[6]), MAL23 (G1P[8]) and MAL82 (G9P[8]) with the E1 genotype to which RIX4414 belonged (Fig. 8). Similarly, two E2 sequences from MAL81 (G8P[4]) and MAL88 (G12P[6], short RNA pattern) were clearly clustered within the E2 genotype.

4. Discussion

The diversity of the rotavirus genome, particularly the variety of G and P genotype combinations, is one of several factors that have been proposed to be a theoretical obstacle to the successful control of rotavirus disease by rotavirus vaccines. Such genetic diversity is recognised to be generally greater in developing countries including African countries than in industrialised countries [10, 11, 31]. Malawi, which has historically harboured a rich diversity of circulating rotaviruses [15, 16] was selected as a site for a pivotal clinical trial of a human, monovalent G1P[8] rotavirus vaccine, Rotarix [8]. In the trial in Malawi, the diversity of circulating rotavirus strains was greater [8] than in any previously published rotavirus vaccine trial, in which the globally most common G1P[8] strain has predominated [32]. Thus, in Malawi, only 13% of the rotavirus strains were of genotype G1P[8], the strain on which Rotarix is based and the most common strain among children globally [10, 11].

The observed lower vaccine efficacy in Malawi (49.5% against severe rotavirus gastroenteritis) was not attributed by the authors to this striking strain diversity of G and P genotypes, on the grounds that the efficacy of Rotarix against severe gastroenteritis caused

by G1 and non-G1 rotaviruses was similar [8]. However, because the G8 VP7 gene of Malawian rotavirus strains was previously suggested to be derived from bovine rotaviruses [14], we further investigated rotavirus strains detected during the clinical trial in Malawi by nucleotide sequencing of selected genome segments including those that code for VP7 and VP4, and by RNA-RNA hybridization, in order to assess their divergence from common human rotavirus strains reported from elsewhere in the world.

As to the VP7 gene which is considered the most important in inducing serotype-specific neutralizing antibodies [23], Malawian G8, G9 and G12 genes clustered into lineages that contained rotavirus strains exclusively or almost exclusively of human origin. This includes the G8 VP7 gene, which was previously suspected to be derived from bovine rotaviruses [14]. Furthermore, the observation that the G8 VP7 gene from the current study belonged to the same lineage (lineage II) as the G8 VP7 genes from strains detected in Malawi in the late 1990s and early 2000s suggests that strains with very similar G8 VP7 gene sequences have continuously circulated in Malawi. As to G9 and G12 VP7 sequences from Malawi, they belong to the most common, recently-emerging lineages of human rotavirus origin. Thus, despite the diversity in circulating G types, Malawian rotavirus VP7 sequences were not unusual when compared with strains from elsewhere bearing the same genotypes.

As compared to P[8] and P[4], which are regarded as indigenous to human rotaviruses, the origin of P[6] is more diverse; yet the P[6] VP4 genes of current and previously detected Malawian strains belong to the same sublineage of lineage I, the most common human lineage. Although the VP8* portion of the VP4 protein contains much variability among different P types in the amino acid sequence (corresponding to the globular domain of the viral spike) [23], interpretation of these findings needs to be undertaken cautiously since our analysis was only based on the VP8* gene.

As to the VP6 gene that codes for the middle-layer capsid protein, our study has demonstrated that the VP6 gene of Malawian strains belonged to either the I1 or I2 genotype, the genotypes common to human rotaviruses of the DS-1 genogroup and the Wa genogroup, respectively [12]. Similarly, as to the NSP4 gene that codes for an enterotoxin, the NSP4 gene of Malawian strains belonged to genotype E1 or E2 which are common to human rotavirus strains [12].

Furthermore, RNA-RNA hybridization showed that all Malawian rotavirus strains that had a long RNA pattern belonged to the Wa genogroup and that strains which had a short RNA pattern belonged to the DS-1 genogroup. Thus, while there was great diversity in the genes that code for the outer capsid proteins VP7 and VP4, rotavirus strains circulating in Malawi at the time of the vaccine trial were no more different than rotavirus strains circulating elsewhere in the world where Rotarix had previously demonstrated a higher level of efficacy.

There is now increasing evidence that Rotarix offers protection against fully heterotypic strains with respect to VP7 and VP4 [33]. This has to date been most clearly demonstrated with respect to G2P[4] strains, with vaccine efficacy against severe rotavirus gastroenteritis caused by this strain estimated to be 71% in a meta-analysis of clinical trials [34], 85% in a clinical trial in Europe [35], and 77-85% in the first year of life in a vaccine effectiveness study conducted in Recife, Brazil [36]. Thus, Rotarix provides protection against severe disease caused by human rotaviruses irrespective of their outermost surface proteins, VP7 and VP4, and therefore does not solely rely on serotype-specific immunity. The mechanism responsible for this apparent cross-protection afforded by Rotarix is unknown, but could involve the internal or non-structural proteins shared by human rotavirus strains i.e., homologous immunity [37-40].

Taken together, the cause of the lower efficacy of Rotarix in Malawi is likely to be explained by factors other than the observed strain diversity. Thus, the sharing of the VP6 and NSP4 genotypes as well as the whole genomic RNA constellation with either of the two common human rotavirus genogroups may provide the molecular basis for the protection conferred by Rotarix against heterotypic strains that has been demonstrated in Malawi and elsewhere. Further work is therefore necessary to explore other possible causes of the lower efficacy of Rotarix in Malawi and to elucidate the mechanisms of protection conferred by rotavirus vaccine against severe rotavirus gastroenteritis.

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Figure 1.

Electropherotypes on 10% polyacrylamide gels of cell-culture adapted rotavirus strains used in this study. The control RNAs were obtained from strains Wa (G1P[8]), KUN (G2P[4]), and RIX4414 (G1P[8]). Gels were stained with ethidium bromide and illuminated under UV light. The name of the strain is indicated on the top of each lane. G8P[4] (MAL01, MAL02, MAL33, MAL47, MAL55, MAL60, MAL70, and MAL81); G8P[6] (MAL43); G12P[6] (2 short pattern viruses MAL39 and MAL88, and 2 long RNA pattern viruses MAL12 and MAL40); G9P[8] (MAL80 and MAL82); G1P[8] (MAL23, MAL38, and MAL50); G1P[6] (MAL63); G12P[8] (MAL65); and G2P[4] (MAL66).

Probe: RIX4414

Figure 2.

Hybridization of RIX4414 probe with genomic RNAs from various Malawian rotavirus strains. 1: Wa; 2: RIX4414; 3: RIX4414; 4: MAL23, G1P[8]; 5: MAL38, G1P[8]; 6: KUN, G2P[4]; 7: MAL80, G9P[8]; 8: MAL12, G12P[6]; 9: RIX4414; 10: MAL88, G12P[6]; 11: MAL43, G8P[6]; 12: MAL60, G8P[4]; 13: MAL70, G8P[4]; 14: MAL66, G2P[4]. Top panel: ethidium bromide staining followed by ultraviolet illumination. Bottom panel: corresponding autoradiograph.

Probe: MAL60

Figure 3.

Hybridization of MAL60 probe (G8P[4]) with genomic RNAs from various Malawian rotavirus strains. 1: Wa; 2: RIX4414; 3: MAL23, G1P[8]; 4: MAL38, G1P[8]; 5: MAL50, G1P[8]; 6: MAL63, G1P[6]; 7: MAL82, G9P[8]; 8: MAL65, G12P[8]; 9: MAL40, G12P[6]; 10: MAL88, G12P[6]; 11: MAL43, G8P[6]; 12: MAL60, G8P[4]; 13: MAL70, G8P[4]; 14: MAL66, G2P[4]. 15: KUN, G2P[4]. Top panel: ethidium bromide staining followed by ultraviolet illumination. Bottom panel: corresponding autoradiograph.

Probe: MAL88

Figure 4.

Hybridization of MAL88 probe (G12P[6], short RNA pattern) with genomic RNAs from various Malawian rotavirus strains. 1: MAL88, G12P[6]; 2: MAL39, G12P[6]; 3: MAL88, G12P[6]; 4: MAL12, G12P[6]; 5: MAL40, G12P[6]; 6: MAL65, G12P[8]. Top panel: ethidium bromide staining followed by ultraviolet illumination. Bottom panel: corresponding autoradiograph.

a
G1- VP7 genes

Figure 5.

Phylogenetic trees for the G1 VP7 genes (a), the G8 VP7 genes (b), the G9 VP7 genes (c), and the G12 VP7 genes (d). The phylogenetic trees were constructed using the neighbour-joining method and Kimura's 2-parameter model. Percentage bootstrap values are given at branch nodes except values <70 which are not shown. The nucleotide sequences of the VP7 genes of the Malawi strains used in this study have been deposited in GenBank as follows: MAL12, MAL23, MAL81, MAL82, MAL88 have been assigned accession numbers JN591403-7 respectively.

Figure 6.

Phylogenetic trees for the P[8] VP4 genes (a), the P[6] VP4 genes, and the P[4] VP4 genes (c). The phylogenetic trees were constructed using the neighbour-joining method and Kimura's 2-parameter model. Percentage bootstrap values are given at branch nodes except values <70 which are not shown. The nucleotide sequences of the VP4 genes of the Malawi strains used in this study have been deposited in GenBank as follows: MAL12, MAL23, MAL81, MAL82, MAL88 have been assigned accession numbers JN591398-402 respectively.

Figure 7.

Phylogenetic tree for the VP6 genes. The phylogenetic tree was constructed using the neighbour-joining method and Kimura's 2-parameter model. Percentage bootstrap values are given at branch nodes except values <70 which are not shown. The nucleotide sequences of the VP6 genes of the Malawi strains used in this study have been deposited in GenBank as follows: MAL12, MAL23, MAL81, MAL82, MAL88 have been assigned accession numbers JN591408-12 respectively.

Figure 8.

Phylogenetic tree for the NSP4 genes. The phylogenetic tree was constructed using the neighbour-joining method and Kimura's 2-parameter model. Percentage bootstrap values are given at branch nodes except values <70 which are not shown. The nucleotide sequences of the NSP4 genes of the Malawi strains used in this study have been deposited in GenBank as follows: MAL12, MAL23, MAL81, MAL82, MAL88 have been assigned accession numbers JN591393-7 respectively.

Table 1
Genotype classification of representative Malawian strains regarding VP7, VP4, VP6 and NSP4

Strain	VP7 (G)	VP4 (P)	VP6 (I)	NSP4 (E)
MAL12	G12	P[6]	I1	E1
MAL23	G1	P[8]	I1	E1
MAL82	G9	P[8]	I1	E1
MAL81	G8	P[4]	I2	E2
MAL88	G12	P[6]	I2	E2