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Characterization of human rotavirus strains causing gastroenteritis in Kenya

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SUMMARY

Human rotavirus strains from Kenya, from children with gastroenteritis in an urban area (Nairobi) and three rural areas were characterized by antigenic and genomic analysis. While in all areas strains with subgroups II and G serotype 1 antigens were most common, two unusual strains were detected. One strain (NK59: subgroup II, G serotype 4) possessed an additional RNA band on polyacrylamide gel electrophoresis, the other (D202) which had antigenic specificity of subgroup II and G serotype 1 showed a 'short' RNA pattern. The latter strain was adapted to growth in cell culture.

Group A human rotavirus (HRV) has been recognized as the major cause of gastroenteritis worldwide. The rotavirus virion contains 11 double-stranded RNA segments enclosed within a double-shelled capsid [1]. Most group A HRVs can be placed into subgroups I or II, according to the antigenicity of inner capsid protein VP6 encoded by RNA segment 6. The outer capsid of the infectious particle contains two neutralization antigens, VP4 and VP7. VP7, a product of RNA segment 7, 8 or 9, defines G serotype specificity. Seven G serotypes have been detected in HRVs [2–5]. On the other hand, VP4, a gene 4 product, defines another serotype specificity, P (or VP4) serotype, which is separate from G serotype specificity. At least four P serotypes and one subtype have been reported in HRV [6, 7]. Surveys of P and G serotypes of HRV in developing countries are important in the preparation of rotavirus vaccines, because both VP4 and VP7 of the vaccine strains can induce protective immunity, and because efficacy of the vaccine depends on identity between vaccine strain and prevailing wild type HRV strains.

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It has been reported that subgroup specificity of HRV is intimately related to migration pattern of viral RNA in polyacrylamide gel electrophoresis (PAGE): HRVs with subgroup I specificity mostly have 'short' RNA patterns characterized by slow-migrating segments 10 and 11, while those with subgroup II specificity have 'long' RNA patterns characterized by rapidly-migrating segments 10 and 11. However, exceptional strains have also been detected and characterization of such strains have provided useful information on the diversity of HRVs [8, 9].

Using the antigenic and genetic markers described above, HRVs from urban and rural area in Kenya were analysed. A total of 278 stool specimens from urban area were collected from children with diarrhoea admitted to the Infectious Diseases Hospital (I.D.H.), a branch of the Kenyatta National Hospital in Nairobi, during the period February–September 1989. The other 144 stool specimens were obtained from three rural district hospitals, Nanyuki, Kitui and Narok in March 1991. The three rural towns are located about 200 km to the north, east and west of Nairobi, respectively.

Approximately 15% stool suspension was made in phosphate-buffered saline, treated with fluorocarbon and clarified by low-speed centrifugation. Group A rotavirus antigen in stool specimen was detected using enzyme-linked immunosorbent assay (ELISA) kit commercially available (Dakopatts, a/s, Denmark). The HRV positive specimens were then tested for their subgroups and serotypes by ELISA using subgroup I and II specific monoclonal antibodies (MAbs) [10] provided by Dr Taniguchi of Sapporo Medical College, Japan, and commercial MAbs specific for G serotypes 1, 2, 3 and 4 (ROTA-MA, Serotec, Japan) [11].

Rotavirus positivity rates were 33·8% (94/278) in Nairobi, 35% (21/60) in Nanyuki, 13·8% (4/29) in Narok, and 1·8% (1/56) in Kitui. The rotavirus-positive rate was highest in children less than 12 months of age. Sufficient materials were available from 79·2% (95/120) of specimens to allow subgrouping and serotyping by ELISA. Subgroups of 81 (85·3%) HRV strains in stool specimens were determined, and 49 strains (51·6%) could be serotyped. Serotype 1-subgroup II strains were most common (28·4%) followed by serotype 2-subgroup I strains (13·7%) and serotype 4-subgroup II strains (7·4%).

Genomic RNAs were extracted from 0·5 ml of the crude suspension and electrophoresed in a slab gel consisting 10% acrylamide. The gel was stained with silver nitrate using commercial reagent (Ag-STAIN 'DAIICHI', Daiichi Pure Chemicals, Japan) as described previously [9]. RNA patterns were detectable in 98·9% (94/95) of all rotavirus-positive specimens. Long RNA pattern and short RNA pattern were observed in 77·7% (73/94) and 22·3% (21/94) of the specimens, respectively.

Although most of all the specimens with short and long RNA pattern belonged to subgroup I and II, respectively, two unusual HRVs were detected. A virus contained in specimen NK59 from Nanyuki, which was determined as subgroup II-serotype 4, showed an unusual long RNA pattern with an additional band associated with RNA segments 5 and 6 (Fig. 1, lane 3). One possible explanation for this phenomenon is that the additional band represents a rearranged genome which consists of a normal RNA segment combining with a short fragment of the identical RNA segment [12]. The other possible explanation for the unusual RNA profile of NK59 is that the specimen may have been a mixture of two HRV strains

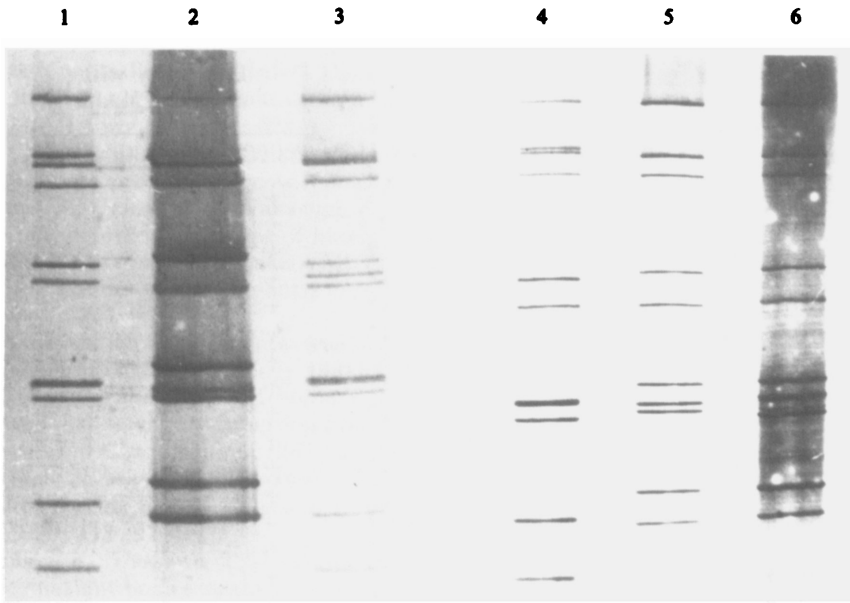

Fig. 1. Electrophoretic migration patterns of genomic RNA of selected HRVs in specimens from rural (lanes 1–3) and urban areas (lanes 4–6) of Kenya. Lane 1, NR22 (long); Lane 2, NR29 (short); Lane 3, NK59 (long pattern with an additional segment); Lane 4, D191 (long); Lane 5, D192 (short); Lane 6, D202 (short).

having RNA pattern similar to each other. We could not verify either of these hypotheses because isolation of this strain in cell culture was unsuccessful. The other unusual strain, D202, detected in specimens from I.D.H. was found to belong to subgroup II but showed short RNA pattern (Fig. 1, lane 6).

Attempts were made to isolate all the rotavirus positive specimens in MA-104 cells as described previously [13]. Twenty HRV strains including strain D202 were isolated. Results of characterization of these strains are shown in Table 1. Strain D202 was determined as serotype 1 in ELISA using the propagated strain, which was confirmed by a PCR method for rotavirus serotype determination described previously [14]. Although several HRVs with uncommon combinations of subgroup and RNA pattern have been reported [8, 9], all of them are of subgroup I with long RNA pattern; subgroup II strains with short RNA pattern have yet to be detected. Detection of strain D202 may strengthen the hypothesis that the relationship between subgroup specificity and RNA pattern has no molecular basis. Moreover, the virulence of D202 seems not to be different from ordinary HRV strains, because the strain was isolated from male patient 7 months of age with overt symptoms.

Genetically and antigenically, VP4s of human rotaviruses have been classified by Gorziglia and colleagues [6] into three different types with type 1 further subdivided, i.e. 1A, 1B, 2 and 3. Furthermore, a new P serotype of HRV may be represented by strain 69M [7]. Reactivities of isolated strains with anti-VP4 MAbs S3-2C, KU-10C, and K8-2C12 were tested by ELISA for characterization of VP4 antigen [15, 16]. While one subgroup I-serotype 2 strain (D205) reacted only with MAb S3-2C, 16 other strains reacted with both MAbs S3-2C and KU-10C. No

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Table 1. *Characterization of cell culture-adapted rotavirus strains*

Specimen code (place and time of isolation)	Subgroup	Serotype	Reactivity with anti-VP4 MAbs			RNA pattern
			S3-2C	KU-10C	K8-2C12	
D38 (Nairobi, Apr. 1989)	II	1	+	+	−	Long
D58 (Nairobi, Apr. 1989)	I	2	−	−	−	Short
D65 (Nairobi, May 1989)	II*	1*	+	+	−	Long
D73 (Nairobi, May 1989)	II*	1*	+	+	−	Long
D98 (Nairobi, Jun. 1989)	II*	1*	+	+	−	Long
D114 (Nairobi, Jun. 1989)	II*	1*	+	+	−	Long
D119 (Nairobi, Jun. 1989)	II	1	+	+	−	Long
D135 (Nairobi, Jul. 1989)	II	1	+	+	−	Long
D139 (Nairobi, Jul. 1989)	II*	1*	+	+	−	Long
D184 (Nairobi, Aug. 1989)	II	1	+	+	−	Long
D202 (Nairobi, Aug. 1989)	II	1*	+	+	−	Short
D205 (Nairobi, Aug. 1989)	I	2*	+	−	−	Short
D206 (Nairobi, Aug. 1989)	I	N.I.†	−	−	−	Short
D232 (Nairobi, Aug. 1989)	II	1*	+	+	−	Long
D233 (Nairobi, Aug. 1989)	II	1*	+	+	−	Long
D235 (Nairobi, Aug. 1989)	II	1*	+	+	−	Long
NK11(Nanyuki, Mar. 1991)	II	1*	+	+	−	Long
NK13(Nanyuki, Mar. 1991)	II	1	−	−	−	Long
NK24(Nanyuki, Mar. 1991)	II	4*	+	+	−	Long
NR22(Narok, Mar. 1991)	II	1	+	+	−	Long

* These subgroups and serotypes were not determined using fecal specimens.
† Not identified.

isolate reacted with MAb K8-2C12 which is directed at the unique VP4 of strain K8 and represents the P(VP4) serotype 3 [6]. Previous results obtained with these MAbs showed that while MAb S3-2C reacts with HRV strains belonging to P serotypes 1A, 1B, and 2, KU-10C reacts with HRVs of P serotypes 1A and 2 [6, 15]. Hence, 16 isolated strains including D202 which are reactive with S3-2C and KU-10C may be classified into VP4 serotype 1A or 2, which suggests that VP4 of D202 may be antigenically different from that of serotype 2 HRVs with short RNA pattern (VP4 serotype 1B). Further genetic analysis may elucidate derivation of this unusual strain.

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