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Comparison of the genome ds RNA of human rotavirus strains shed in parts of Ibadan, Nigeria

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Abstract

The incidence and genome electropherotypes of human rotavirus detected in a hospital and in a community within Ibadan, Nigeria were compared by polyacrylamide gel electrophoresis. On the whole, 13% (31/239) rotavirus was detected; 14.7% (15/102) from the community and 11.7% (16/137) from the hospital. The incidence was significantly higher ($P < 0.01$) in the community than in the hospital. There were 11 (80.0%) long and 3 (20.0%) short forms observed in the community, whereas 14 (87.5%) long and 2 (12.5%) short forms were detected in the hospital. On co-electrophoresis, however, only 4 and 6 distinct electropherotypes were demonstrated in the hospital and the community respectively. Three of these were common to both places with 1 and 3 electropherotypes being unique to the hospital and the community, respectively.

Résumé

L'incidence et le génome électrophérotypes des rotavirus humains détectés à une hospital une agglomération d'Ibadan, Nigeria sont comparés à laide du gel polyecrylamine électrophorèse. Treize pour cent (31/239) rotavirus sont détectés; 14.7% (15/102) viennent de agglomération et 11.7% (16/137) viennent des hospital. L'incidence était plus élevée à de agglomeration ($p < 0.01$) qu'a hospital. Il y avait onze (80.0%) de longues variétés et trois (20.0%) de courtes variétés à de agglomeration, tandis que 14 longs (87.5%) et 2 courts (12.5%) sont détectés dans le hospital.

Après la co-electrophorèse, on détecte seulement quatre et six électrophérotypes distincts pour hospital et agglomeration respectivement. Trois d'entre eux se présentent également à hospital et agglomeration tandis que un et trois électrophérotypes se trouvent uniquement à de hospital et de agglomeration respectivement.

Introduction

Human rotavirus has been recognized as a major cause of infantile diarrhoea in both the developed and developing countries of the world [1,2]. Rotavirus infections spread via the faecal-oral route and are commonly associated with communities where low hygienic standards are maintained. Illiteracy and low socio-economic conditions enhance the outbreak and spread of infections in a community [3,4]. The virus consists of a core, an inner capsid or incomplete and an outer capsid or complete particle types. The virus belongs to the family Reoviridae, whose genome separates into [11] segments upon polyacrylamide gel electrophoresis (PAGE) to produce characteristics patterns called electropherotypes [3-5]. Currently, 11 serotypes of rotaviruses have been described, of which six (serotype 1,2,3,4,8 and 9) have been isolated from humans [5]. The methods of serotyping have been largely by the plaque reduction neutralization tests (PRNT), the fluorescent focus neutralization (FFN) assays and by special ELISAs using monoclonal antibodies or hyperimmune polyclonal monospecific antisera [6-9].

Since the dsRNA patterns usually remain constant for individual strains of rotavirus, analysis of the

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genome of rotaviruses has been useful for molecular epidemiological studies of human [5] and calf [10] rotaviruses. In this study, the rotaviruses shed in the stools of children with diarrhoea in Sabo community of Ibadan and from some other parts of Ibadan representing the elite sections, namely, Bodija, the University of Ibadan (UI), University College Hospital (UCH), International Institute of Tropical Agriculture (IITA) and Jericho (represented as children from elite communities EC) subsequently were detected by PAGE and their electropherotypes compared by the genome electropherotyping technique.

Materials and methods

The study areas, faecal samples

Cohen [13], described the people of Sabo community as "emigrants to Ibadan who stand out as the most exclusive, sharply delineated ethnic group to settle in the city.... The people remain distinct in their dresses, food customs and religious beliefs". That situation has not changed much today. The projected population of Sabo with 3.7% annual growth rate over the 1973 census could be between 20-30,000. The socio-economic levels of the inhabitants of this community is low and this has affected the living and hygienic conditions. The city of Ibadan is cosmopolitan with pockets of different communities dotted here and there. The different pockets can almost be sharply divided into three plan-oriented zones.

(i) The inner city core mostly unplanned, where the low socio-economic members of the society live, (ii) the semi-planned portions of the city corresponding with the residence of the low to middle class civil servants, white and blue collar workers and (iii) the planned residential areas where the top civil servants, businessmen and professionals live (EC). Sabo corresponds to the first group while Bodija, University of Ibadan, the University College Hospital, International Institute of Tropical Agriculture and Jericho correspond to the third group as listed above.

Diarrhoeic faecal samples were collected from children 0-5 years of age from Sabo community and from children of the same age group who attended the out-patients department clinics or hospitalized in the University College Hospital (UCH), Ibadan who came from the elite third group and excluded any patients from the Sabo community. A total of 239 diarrhoeic faecal samples were collected (102 from Sabo and 137 from EC) into sterile plastic or

screw-capped glass containers between May and December, 1990. Samples were stored at -20°C and analysed within one week of collection.

Extraction and electrophoresis of viral dsRNA

Rotavirus was extracted directly from the stool samples by the method of Herring *et al* [14]. Briefly, about 0.25g or 25% w/v faeces was treated with extraction buffer (0.1M sodium acetate pH 5.0 containing 1% v/v sodium dodecyl sulphate (SDS). An equal volume of phenol-chloroform mixture was added to the faecal samples suspension, vortexed and clarified at 16,000 x g for 10 min in a Mighty-Fast micro-centrifuge (Hoefer Scientific Instruments, San Francisco, C.A. USA). The resultant supernatant (40U) was mixed with 10ul tracker dye for electrophoresis.

For the initial screening, 50ul of each sample was loaded in single wells of 7.5% preformed polyacrylamide gel and electrophoresis carried out using the Loening buffer system [15]. For comparison of the electropherotypes, extracted samples were further precipitated with chilled 100% ethanol and coelectrophoresed in 10% resolving and 3% stacking gel using the Laemmli discontinuous buffer system [16]. Electrophoresis was at a constant current of 10mA for 18-20 hrs at room temperature. All gels were stained by the silver staining technique of Herring *et al* [14].

Results

Detection of rotavirus

Of 239 faecal samples from Sabo and EC, 31 or 13% were positive for rotavirus; made up of 15 or 14.7% of 102 sample's from Sabo and 16 or 11.7% of samples from UCH. The incidence of rotavirus infection was significantly higher ($P < 0.01$) in Sabo than in EC (table 1).

Table 1: Incidence of human Rotavirus in Sabo community and some elite communities in Ibadan.

Location	No. of samples tested	No. positive %	Significant level
Sabo com.	102	15 (14.7)	$P < 0.01^*$
EC Ibadan	137	16 (11.7)	
Total	239	31 (13.0)	

* Determined by χ^2 analysis.

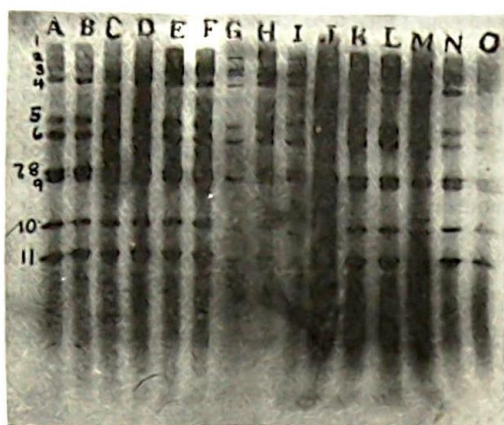


Fig. 1a

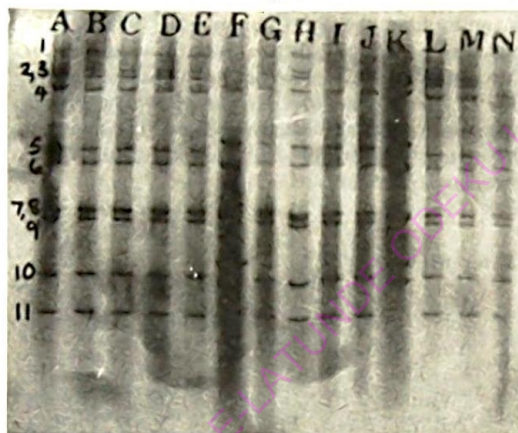


Fig. 1b

Fig.1: The different electropherotypes from Sabo (1a) and from EC (1b). Two of the predominant electropherotypes in UCH were left out in this electrophoresis so as to accommodate the number of slots the gel system can handle. Polyacrylamide gel electrophoresis was on 10% resolving and 3% stacking gels using the discontinuous buffer system. Electrophoresis was from top to bottom and numbers to the left indicate the RNA segments.

Note: Lanes (A-F, KLN), (G), (H), (O) fig. (1a) (A-E, JLM), (GN), (H) Fig. (1b) for long electropherotypes and Lanes (I) (J, M) and (F,K). Figs. 1a and 1b, respectively for short electropherotypes.

Rotavirus RNA Electropherotypes

The diverse genomic migration patterns of rotaviruses from Sabo and EC are shown in figs. 1a and 1b respectively. There were 12 (80.0%) long and 3 (20.0%) short electropherotypes from Sabo whereas in EC there were 14 (87.5%) long and 2 (12.5%) short forms. In the EC, all the short forms were of the same electropherotypes (Lanes (F & K)) while the long forms were distributed among 3 distinct electropherotypes (Lanes (A-E, JLM), (G,N) and (H)). On the other hand, the short forms from Sabo were distributed into 2 distinct electropherotypes (Lanes (I) and (J,M)) while the long forms occurred in 4 distinct patterns (Lanes (A-F, KLN), (G), (H) and (O)).

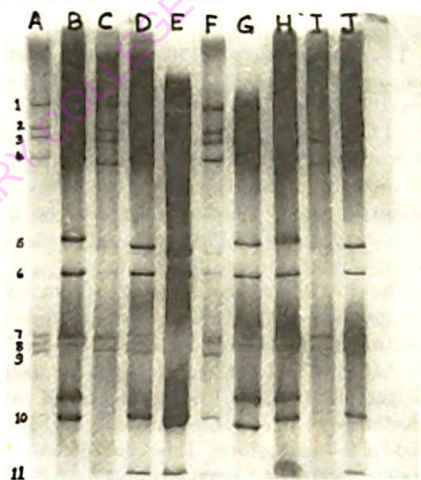


Fig. 2: The distinct electropherotypes from Sabo (6) (Lanes E-J) and EC (4) (A-D). The numbers to the left indicate RNA segments. All other conditions were the same as for the gels in fig. 1. Short forms are indicated by lanes B(EC) and G, H (Sabo).

Coelectrophoresis of the different electropherotypes revealed six distinct strains in the Sabo community (fig. 2 Lanes E-J) with one predominant electropherotype detected in 7 (46.7%) children; whereas 4 distinct patterns were observed in the EC (fig. 2 Lanes A-D) and the predominant strain was detected in 11 (68.8%) children. The predominant strains in both Sabo and EC were the same. Three of the 4 strains seen in EC were also observed in Sabo and the remaining one strain left was unique to the

EC. On the other hand, there were three unique electropherotypes to Sabo which were not detected in the EC. Fig. 2 shows the distinct electropherotypes seen in Sabo and the EC.

Discussion

Several hospital and community-based studies have been undertaken by a few workers in an effort to elucidate the incidence of human rotavirus in Nigerian infants [17-20] but none had studied the epidemiology of these rotaviruses from the genome (molecular) stand point. We have established in this study the different electropherotypes circulating within Sabo community as distinct from those circulating in the other parts of Ibadan (the elite group).

The incidence of 13% in our study was comparable to the 11-17% incidence reported by some [8-20] but lower than the 23-62% incidence found by others [17, 21]. The reasons for this could be attributed to the number of samples examined and the methods of investigation. Extraneous proteins present in faecal samples could have bound non-specifically to the ELISA employed by these other workers giving false positive results.

The PAGE on the contrary, is not prone to non-specific results. However, some workers who have compared the sensitivities and the specificities of the PAGE and ELISA techniques found them to be comparable [12,22]. The incidence of rotavirus infection was, however, significantly higher in Sabo than in the elite group. This could be due to poor hygienic and living standards obtaining in the Sabo community. Some investigators had associated high incidence of rotavirus infection to poor socio-economic conditions [18, 23].

It was remarkable to observe that all but one strain (electropherotype) observed at the EC were also present in the Sabo community and that included the predominant strains in both places. However there were 3 of the 6 strains present in Sabo that were unique to the community and not found in the EC. The explanation to these observations could be the fact that there are no restrictions of movements in and out of Sabo. These common strains could have been transferred from one community to the other. In addition, there could have been more children in Ibadan that were more susceptible to these strains. On the other hand, the unique strains (1 in EC and 3 in Sabo) could have been less virulent and incapable

of establishing epidemics, or the appropriate conditions for their transfer to the opposite community were lacking. Continued investigation of samples from the EC beyond 1990 and into 1991 (data not shown) has yet to reveal any of the 3 unique strains found in Sabo. The higher occurrence of the long electropherotypes over the short forms observed in this study irrespective of the study areas, corroborate the findings of other workers [24-26]. It therefore seems that the small numbers of the short electropherotypes appear to be the norm globally.

It would be interesting to compare the strains of rotaviruses circulating in Ibadan with those circulating in the rest of the country as it would be useful information in composing an effective vaccine for the near future.

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