Rotavirus Prevalence and Relationships With Climatological Factors in Gabon, Africa

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A one-year study of rotavirus prevalence was carried out using electron microscopy in Gabon, equatorial Africa. One hundred fifty-six nonhospitalized diarrheic children up to 10 years of age and 115 age-matched controls were investigated together with diarrheic adults and children over 10 years of age. Rotaviruses were observed only in diarrheic children under 10 years of age (10.2%). Rotaviruses were more prevalent in the dry-season months (19.2% versus 1.3%, $P < .001$). Rotaviruses were the most commonly found among all the viral, bacterial, and parasitic agents we detected, with the exception of the coronaviruslike particles, which are the subject of another paper [Sitbon, 1985].

Key words: diarrhea, rotavirus, human, seasonal prevalence, Gabon

INTRODUCTION

Diarrheal diseases are a major cause of morbidity and mortality in developing countries [Murphy, 1981] and the rotavirus seems to be the most frequently encountered agent [Holmes, 1982]. However, long-term surveys concerning African countries remain scarce and most of the reports have concerned hospitalized patients who present a high risk of nosocomial infections [Middleton, 1982; Champsaur et al, 1984]. We report here a one-year survey of rotavirus infection in Gabon, Africa, and its relationship with climatological factors. Our study concerned only nonhospitalized patients and depicted for the first time the natural prevalence of rotavirus in this area over a long period.

MATERIALS AND METHODS

Stool Samples

This study was carried out from December 1980 to December 1981 in the southeastern province of Haut-Ogooué and included stools from 211 nonhospitalized

Accepted for publication December 6, 1984.

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diarrheic patients (group I) and 115 healthy donors (group II). Diarrhea was defined when patients had three or more loose stools in 24 hours. Both diarrheic and control groups were composed of subjects living in urban and rural areas. Group I included 156 children under 10 years of age, 12 children between 10 and 15 years, and 43 adults. Group II included only children under 10 years of age who had no gastroenteritis or respiratory signs. Within a day after collection, samples of some stools were used for bacteriological and parasitological studies. All stools were frozen at -20°C or immediately processed for electron microscopy.

Electron Microscopy (EM)

Stool samples were diluted to about 20% (w/v) in sterile double-distilled water and clarified at 4,000g for 20 minutes. Supernatants were stored at -70°C or immediately used for preparation of EM grids. A drop of fresh or thawed supernatant was used to prepare two carbon-collodion-coated copper grids. Four percent (w/v) phospho-tungstic acid, pH 6.5 was used as negative stain. Preparations were examined in an Elmiskop 101 (Siemens) at 80 kV.

Bacteriology

One hundred thirty of the 156 stools collected from diarrheic children under 10 years of age and 39 of the 55 stools from diarrheic patients over 10 years of age were cultured for enteropathic *Escherichia coli* (EPEC), salmonella, and shigella. Isolation and characterization steps included cultures in selenite and Muller-Kauffmann broths (Institut Pasteur Production, France), and plating on salmonella-shigella agar and Bromcresol purple-containing media (IPP, France). Biochemical identification and serotyping were performed on API strips (API System, France) and by seroagglutination with immune sera (IPP, France).

Parasitology

Seventy-nine of the 156 diarrheic children under 10 years of age and 24 stools of the 55 diarrheic patients over 10 years of age were examined for presence of parasites by direct examination and after concentration procedures [Garin et al, 1978].

Meteorological Data

Meteorological data were kindly provided by the Service de la Météorologie Nationale (Libreville, Gabon) from surveys carried out at the International Airport of Franceville.

Statistical Analysis

Statistical analyses were performed by using the chi-square test (χ^2) with 1 degree of freedom. A difference was considered significant when $\chi^2 \geq 3.84$ ($P < .05$).

RESULTS

Rotavirus in the Different Age Groups

We observed rotavirus particles only in diarrheic stools of children under 10 years of age, and more frequently among patients under 2 years of age (12.2% versus

2.9%; $P < .02$) (Table I). The highest prevalence was observed in the under-6-month age group (23.5%) (Table I).

Seasonal Prevalence of Rotavirus

Seasons in Gabon alternate as follows: a short dry season (December and January), a long rainy season (February to May), a long dry season (June to September), and a short rainy season (October and November). Monthly distribution showed a higher prevalence of rotavirus during the dry-season months (Fig. 1). Since rainfall was unusually high in December 1981 (Fig. 2A), we included this month in the rainy-season months. The frequency of rotavirus detection was significantly different between the rainy and dry months (1.3% versus 19.2%, $P < .001$) (Table II). Moreover, by using the deviation between the daily maximum and minimum relative humidity (ΔRH), we were able to show that hygrometric fluctuations closely followed the classical monthly division of seasons in Gabon (Fig. 2C). Rotavirus prevalence varied inversely with the monthly rainfall (Fig. 2A), and the monthly average of the

TABLE I. Frequency of Rotavirus Detection in Relation to Age Among Diarrheic Subjects

Age group	No. of patients with rotavirus	
	No. of patients	% positive
≤ 6 mo	8/34	23.5
7–12 mo	2/43	4.7
13–18 mo	2/17	11.8
19–24 mo	1/13	7.7
25 mo–5 yr	2/25	8
6–10 yr	1/24	4.2
11–15 yr	0/12	0
> 15 yr	0/43	0
Total ≤ 2 yr	13/107	12.2
Total > 2 yr	3/104	2.9

Fig. 1. Monthly prevalence of rotavirus in diarrheic children up to 10 years of age.

Fig. 2. Rotavirus frequency among diarrheic subjects up to 10 years of age compared with (A) monthly rainfall; (B) monthly average of daily maximum temperatures; (C) monthly average deviation between the daily maximum and minimum relative humidity (ΔRH).

TABLE II. Seasonal Prevalence of Rotavirus Among Diarrheic Children*

Seasonal group	No. of patients with rotavirus	
	No. of patients	% positive
Rainy season months (Feb, Mar, Apr, May, Oct, Nov, 1980, Dec, 1981)	1/78	1.3
Dry season months (Dec, Jan, June, July, Aug, Sept, 1980)	15/78	19.2

*Only children up to 10 years of age are considered since rotavirus was never observed in older subjects (Table I). $\chi^2 = 13.65$; $P < .001$

daily maximum temperature (Fig. 2B), as well as with the monthly average of ΔRH (Fig. 2C).

Other Viruses, Bacteriology, and Parasitology

We observed coronaviruslike particles very frequently in diarrheic and control stools [Sitbon, 1985]. Adenoviruses, parvoviruses, and small round viruses were observed only in the diarrheics in 7/156 (4%) of the children under 10 years of age; 5/55 (9%) of the diarrheics over 10 years of age excreted the two latter viruses. EPEC were isolated in 9/130 (7%) of the diarrheic children under 10 years of age and in 2/39 (5%) of the diarrheic patients over 10 years of age; in this latter group, both EPEC strains were isolated from adults. Salmonella serotypes were isolated in 2/130 (1.5%) of the patients under 10 years of age and in 2/39 (5%) of the patients over 10 years of age. Shigellas (*S. boydii*) were found only in one diarrheic adult. Giardia lamblia, Entamoeba histolytica, and Schistosoma intercalatum have been observed in 6/79 (7.6%) of the diarrheic children under 10 years of age; no strongyloides were observed in their feces. Feces of 6/24 (25%) of the patients over 10 years of age contained one or more of these four parasites. No obvious seasonal variations have been observed in the prevalence of these viral, bacterial, and parasitic agents with the exception of the coronaviruslike particles, which are the subject of another paper [Sitbon, 1985].

DISCUSSION

This study was performed in order to document the natural prevalence of different viral agents involved in childhood gastroenteritis in sub-Saharan Africa. For this purpose, we collected stools from nonhospitalized individuals in urban and rural areas of Haut-Ogooué, Gabon.

To our surprise, we found an extremely high prevalence of coronaviruslike particles in both control and diarrheic groups [Sitbon, 1985]. Rotaviruses were the next-most-commonly found agents in diarrheic children under 10 years of age. Moreover, no rotavirus was observed in controls or in diarrheic patients over 10 years of age. Other viruses including adenoviruses, parvoviruses, and unidentified small round viruses were observed in a few cases. Bacterial and parasitic agents were observed in both age groups of diarrheic patients.

In the population of our study, infants were usually breast-fed until 1 year old, after which, mixed feeding was introduced and continued until about 2 years of age. Since breast-feeding may protect infants against viral infections [Welsh and May, 1979; Flewett, 1982; Feachem and Koblinsky, 1984], we were surprised to find that rotavirus infection was most prevalent among children under 6 months of age. This was also reported in Australian aboriginals [Schnagl et al, 1978] as opposed to what was generally observed in other studies [Flewett, 1982; Middleton, 1982]. However, it is still possible that our observation was due to a bias through which newborns with a "rotaviral diarrhea" were more frequently brought to medical consultations than older children.

Despite the low number of stools positive for rotavirus, we were able to observe a statistically significant seasonal prevalence of rotavirus infection. As already reported for sub-Saharan Africa [de Mol et al, 1982; Paul and Erinle, 1982], the highest prevalence of rotavirus occurred during the dryer months. However, in our area of

study, temperature and relative humidity (RH) also presented seasonal variation although the amplitudes of variation of these parameters were low. Interestingly, an obvious cyclical variation of RH was observed only when the daily deviations between maximum and minimum RH (Δ RH) were plotted. It was shown that in vitro low and high extremes of RH could also affect the survival of rotaviruses [Moe and Shirley, 1982]. However, we were unable to determine which of the climatological factors was primarily responsible for the cyclicity of rotavirus gastroenteritis.

Rotavirus infections followed a seasonal cycle in this region where climatic conditions were relatively stable. We think that this cyclicity was detected because of the long term of this study. Future studies on rotavirus prevalences in tropical countries will have to exclude a possible bias due to a short time of study during which samples are collected. This might explain in part some discrepancies [De Mol et al, 1982; Paul and Erinle, 1982] between different reports on rotavirus prevalence in Africa.

ACKNOWLEDGMENTS

M. Sitbon wishes to thank Dr. T.H. Flewett, Regional Virus Laboratory, East Birmingham Hospital, United Kingdom, for his continuing support. This work could not have been done without the collaboration of G. Béville, and A. Olouo-Koumba, Centre International de Recherches Médicales, Franceville, Gabon. Sincere thanks to H. Blahnik, G. Hettrick, and R. Evans, of Rocky Mountain Laboratories, for their skillful help in the elaboration of the manuscript.

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