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ETIOLOGY OF DIARRHEA IN CHILDREN LESS THAN FIVE YEARS OF AGE IN IFAKARA, TANZANIA

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Abstract. A total of 451 stool specimens were collected from children less than five years of age with acute diarrhea from Ifakara, Tanzania and processed to detect bacterial enteropathogens, parasites, and rotaviruses. These specimens were divided into 348 from the dry season and 103 from the rainy season. Overall, diarrheogenic *Escherichia coli* (35.7%) were the predominant enteropathogens, with enterotoxigenic *E. coli*, enteroaggregative *E. coli*, and enteropathogenic *E. coli* being the most prevalent. Moreover, enteroaggregative *E. coli* (63% versus 35.5%; $P < 0.05$), *Shigella* spp. (24% versus 12%; $P < 0.05$), and rotavirus (23% versus 4%; $P < 0.05$) were more prevalent in the dry season than in the rainy season and enterotoxigenic *E. coli* (51.6% versus 20%; $P < 0.05$) and *Giardia lamblia* (14% versus 1%; $P < 0.05$) were more prevalent in the rainy season.

INTRODUCTION

Diarrhea is an important cause of morbidity and mortality in children from developing countries. Children less than five years of age have 3.3 diarrheic episodes per year, and more than one-third of the deaths in this age group are associated with diarrhea. Therefore, every year there are approximately 1.5 billion diarrheic episodes and 4 million deaths in children less than five years of age (most from 6 months to 12 years) caused by this disease.[1–3]

The main etiology of the diarrhea is related to a wide range of bacteria (such as *Campylobacter jejuni*, *Escherichia coli*, *Salmonella spp.*, *Vibrio cholerae*, *Yersinia enterocolitica*, and *Aeromonas spp.*), enteroparasites (*Giardia spp.*, *Criptosporidium spp.*, and *Entamoeba histolytica*), and viruses (adenovirus, Norwalk virus, and rotavirus).

In many hospitals in developing countries lacking clinical microbiology laboratories, the cause of diarrhea in children is unknown. The seasonality of specific enteropathogens such as rotavirus or some parasites has been reported.[4–8]

The aim of this study was to determine the prevalence of enteropathogens, including bacteria, virus, and parasites, causing diarrhea among children less than five years old in Ifakara, Tanzania during the dry and rainy seasons.

MATERIALS AND METHODS

Subjects. The study was carried out in the town of Ifakara, in the Kilombero district, in southwestern Tanzania. The town has 40,000 inhabitants, the majority of whom are small-scale farmers. All children less than five years of age who were admitted to St. Francis Hospital in Ifakara, Tanzania because of diarrhea were recruited during the periods from July to September 1996 (dry season) and from February to May 1997 (rainy season). Informed consent was obtained from parents and/or close relatives. The study was reviewed and approved by the ethical committees of the Hospital Clinic (Barcelona, Spain).

Microorganisms. Whole stool specimens from 348 (172 boys and 176 girls) and 103 (51 boys and 52 girls) children less than five years old who were admitted to St. Francis Hospital in the dry and rainy seasons, respectively, were cultured for *E. coli* and other bacterial enteropathogens using conventional methods.[9] Diarrhea was defined as three or more watery or loose stools in a 24-hour period prior to admission to the hospital. Fresh specimens were examined directly to detect ova and vegetative forms of parasites. The visualized amebic cysts were confirmed by Hiedenhain staining. Stools were examined after concentration by the merthiolate-iodine-formalin technique and were stained with Kinyoun's carbol-fuchsine.[10] Rotavirus was detected using an agglutination test (Slidex Rotakit 2; BioMérieux, Marcy l'Etoile, France).

Detection of *E. coli* virulence factors. The virulence factors associated with diarrheogenic *E. coli* were detected by a polymerase chain reactgion (PCR) technique. Specific primers were used to detect enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and verotoxigenic *E. coli* (VTEC). The conditions used for the PCR technique were those described elsewhere.[11] Briefly, one colony of each isolate was suspended in 25 μL of sterile water and boiled for 10 minutes. A 25-μL of reaction mixture containing 20 mM Tris-HCl (pH 8.8), 100 mM KCl, 3.0 mM $MgCl_2$, 0.1% gelatin, 400 μM dNTPs, and 1 μM of each primer was added, together with 2.5 units of *Taq* polymerase. The reaction mixture was overlaid with a drop of mineral oil and subjected to the following program: 30 cycles at 95°C for 50 seconds, 55°C for 1.5 minutes, and 72°C for two minutes. The PCR product was detected by electrophoresis on a 2% agarose gel and stained with ethidium bromide.

Statistical analysis. Proportions were compared using the chi-square test.

RESULTS

Patients presented mainly with watery diarrhea in both the dry (70.68%) and the rainy seasons (72.81%), while only 18.10% and 5.82%, respectively, presented with dysentery. Dehydration was detected in 11 patients (10.68%) in the rainy season and in 40 patients (11.49%) in the dry season.

Two hundred forty (71.83%) stool samples collected during the dry season and 65 (63.1%) collected during the rainy season showed at least one enteropathogen. The prevalence of the different enteropathogens in each season is shown in Table 1.

TABLE 1

Enteropathogens isolated from children with diarrhea from Ifakara, Tanzania in the dry season (July–September 1996) and the rainy season (February–May 1997)

	Dry season (348 patients) No. of cases (%)	Rainy season (103 patients) No. of cases (%)	P*
Bacteria			
Diarrheaogenic *Escherichia coli*	130 (37.36)	31 (30.1)	
Shigella spp.	84 (24.14)	13 (12.6)	0.021
Campylobacter spp.	9 (2.56)	0	
Salmonella spp.	5 (1.44)	0	
Aeromonas spp.	3 (0.86)	0	
Plesiomonas shigelloides	3 (0.86)	0	
Hafnia alvei	2 (0.59)	0	
Edwarsiella tarda	2 (0.59)	0	
Protozoa			
Entamoeba histolytica	10 (2.87)	2	
Giardia lamblia	4 (1.15)	15 (14.5)	<0.001
Cryptosporidium	0	1	
Helminths			
Necator/Ancylostoma spp.	7 (2.01)	0	
Ascaris lumbricoides	2 (0.59)	0	
Viruses			
Rotavirus	82 (23.56)	4 (3.9)	0.001
Total children with at least one enteropathogen	240 (71.83)	65 (63.1)	

* By chi-square test.

The most commonly isolated enteropathogens during the dry season were diarrheogenic *E. coli* (37.36%), *Shigella* spp. (24.14%), and rotavirus (23.56%). Other enteropathogens such as *Campylobacter* spp., *Salmonella* spp., *Aeromonas hydrophila, Plesiomonas shigelloides*, *Hafnia alvei*, and *Edwarsiella tarda* detected during this season showed an incidence less than 3%. During the rainy season, the most frequently isolated enteropathogens were also diarrheogenic *E. coli* (30.1%), followed by *Giardia lamblia* (14.5%) and *Shigella* spp. (12.6%).

Seventy-three (56.2%) of 130 diarrheogenic *E. coli* isolated in the dry season and 13 (41.8%) of 31 isolated in the rainy season were associated with other enteropathogens. Among diarrheogenic *E. coli* (Table 2), ETEC were isolated more often during the rainy season (16 of 31, 51.6%) than during the dry season (26 of 130, 20%) ($P < 0.0001$). Moreover, ETEC with heat-stable toxin (ETEC-ST) was detected more often in comparison with ETEC with heat-labile toxin (ETEC-LT) and ETEC-LT/ST.

A significantly higher proportion of EAEC (63% of the diarrheogenic *E. coli*) was observed during dry season compared with the rainy season (35.5%; $P = 0.005$). The EPEC were isolated at similar frequencies in both seasons. Meanwhile, only one strain of EIEC and one strain of VTEC producing verotoxin 2 (VT2) were detected during the dry season.

The proportion of *Shigella* spp. isolated during the dry season was significantly higher (24.14%) than that isolated during the rainy season ($P = 0.012$). The high proportion of *G. lamblia* isolated during the rainy season (14.5%) was significantly higher than that isolated during the dry season (1.15%) ($P < 0.00001$), while the proportion of rotavirus (23.56%) isolated during the dry season was significantly higher than that isolated during the rainy season ($P < 0.00001$).

DISCUSSION

The prevalence of cases of diarrhea in Ifakara, Tanzania with a known etiology was 71.83% in the dry season and 63.1% in the rainy season. Diarrhea in children in developing countries other than Tanzania has been reported in 50–60% of the diagnosed cases.[7,12] The main difference between these reports and the present work is the inclusion of diarrheogenic *E. coli*, which increased the frequency of cases of diarrhea with known etiology to 34.6% and 28% in the dry and the rainy seasons, respectively.

Overall, the proportion of diarrheogenic *E. coli* in both seasons was high, which is consistent with previous reports from developing countries.[2,13] However, in some cases, differences were found in comparison with reports in the literature.[14–16] Our results differ with those analyzing the prevalence of ETEC in children from Bangladesh in whom a high prevalence of ETEC was found, although these infections peaked during the dry, warm months.[14] Similar results have been reported for ETEC infections in children from Egypt.[15] In contrast, in another study in children and adults in Tanzania, a seasonal difference in the prevalence of ETEC was not observed.[16]

Many reports have demonstrated the association of EAEC with diarrhea in children in developing countries.[13,17] Similarly, our results show a high proportion of EAEC found in children with diarrhea in Tanzania, especially in the dry season.

Only one isolate of EIEC and another of VTEC were recovered during the study. The low levels of these pathogens is consistent with previous reports that showed the low prevalence of EIEC as a cause of diarrhea in children less than five years old.[18] It has been reported that VTEC mainly affects developed countries.[19]

Our results obtained show a low prevalence of *Campylobacter* spp. Although a higher prevalence (2.9%) of *Campylobacter* was found during the dry season than in the rainy

TABLE 2

Prevalence of virulence factors associated with diarrheogenic *Escherichia coli* of children from Ifakara, Tanzania in the dry season versus the rainy season*

	Dry season (130 *E. coli*) No. of cases (%)	Rainy season (31 *E. coli*) No. of cases (%)	P†
Enteroaggregative *E. coli* (EAEC)	82 (63)	11 (35.5)	0.005
Enterotoxigenic *E. coli* (ETEC)	26 (20)	16 (51.6)	<0.001
Heat-stable toxin (ST)	13	9	
Heat-labile toxin (LT)	9	5	
ST plus LT	4	2	
Enteropathogenic *E. coli* (EPEC)	20 (15.4)	4 (12.9)	
EPEC attaching-effacing (eae)	12	3	
eae plus bundle-forming factor (bfp)	4	0	
eae plus EPEC adherence factor (EAF)	2	0	
eae plus bfp plus EAF	1	1	
bfp	1	0	
Enteroinvasive *E. coli* (EIEC)	1	0	
Verotoxin-producing *E. coli* (VTEC)-VT2$^+$	1	0	

* VT2 = verotoxin 2.
† By chi-square test.

season, this difference was not significant. A high prevalence of *Campylobacter* has been reported during the wet season in Zaire,[20] although other investigators did not find seasonal differences.[16]

Shigella spp. were isolated at a higher frequency during the dry season. This result is consistent with a previous report that showed that shigellosis was more prevalent during dry months.[6] Although, it should be mentioned that the results obtained in our study might be biased by the superimposition of a *Shigella flexneri* outbreak during the dry season,[21] this outbreak might also explain the differences found among dysentery cases in both seasons.

Rotavirus has been reported as the main virus associated with diarrhea in young African children.[22–24] The mortality associated with this pathogen in sub-Saharan areas of Africa has been estimated to be approximately 145,000 deaths/year. In our study, a high frequency of rotavirus was detected during the dry season. This is consistent with previous observations of a peak in the incidence of rotavirus during the dry season in children with diarrhea from different areas of Africa.[12,24,25]

Giardia lamblia was another microorganism in which a significant difference in its frequency was found between the dry and the rainy seasons. A seasonal difference in the prevalence of *G. lamblia* has also been reported in a study in children from Jordan.[4]

In summary, the present study shows that diarrheogenic *E. coli* are the predominant enteropathogen causing diarrhea in children less than five years old in Ifakara, Tanzania in both the dry and the rainy seasons. Moreover, ETEC, *Shigella* spp., and rotavirus were more prevalent in the dry season, whereas EAEC and *G. lamblia* were more prevalent in the rainy season.

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