A study of the aetiological agents of childhood diarrhoea in Lagos, Nigeria

T. I. OGUNSANYA, V. O. ROTIMI* and A. ADENUGA†

Departments of Medical Microbiology and Parasitology, and †Paediatrics, College of Medicine of the University of Lagos, Idi Araba, PMB 120003, Lagos, Nigeria and * Division of Clinical Microbiology, King Fahd Armed Forces Hospital, PO Box 9862, Jeddah 21159, Kingdom of Saudi Arabia

Summary. From December 1989 to May 1990, 315 faecal samples from children under 5 years old with diarrhoea (215) and without diarrhoea (100) seen at paediatric clinics were investigated for bacterial, viral and parasitic enteropathogens. Standard and recently described methods were used for the investigations, which revealed that 74.9% of children with diarrhoea were infected with enteropathogens compared with 28% of controls. In the diarrhoeal group, 59.1% had a bacterial, 26.5% a viral and 2.3% a parasitic aetiology. Rotavirus was the pathogen most frequently detected, accounting for 22.3% of positive findings in the group with diarrhoea versus 9% in the control group. Other important agents were: enterotoxigenic *Escherichia coli* (ETEC) (14.4 versus 6%), enteropathogenic *E. coli* (EPEC) (10.7 versus 5%), enteroadherent *E. coli* (EAEC) (9.3 versus 4%), enterohaemorrhagic *E. coli* (EHEC) (5.1 versus 3%) and *Salmonella* spp. (3.3 versus 1%). The following enteropathogens were detected exclusively in the diarrhoeal stools: *Shigella* spp. (5.1%), *Yersinia enterocolitica* (0.9%), *Aeromonas hydrophila* (1.4%), *Entamoeba histolytica* (0.5%), *Giardia lamblia* (0.5%), *Trichomonas hominis* (0.5) and *Trichuris trichiura* (0.9%). The detection rates of rotavirus, EPEC and EAEC were much greater in the diarrhoeal than in the control patients. No *Vibrio cholerae*, enteroinvasive *E. coli* (EIEC), *Plesiomonas* spp. or *Cryptosporidium* spp. were detected in this study. Our data suggest that both the traditional and newly recognised diarrhoeal agents are important causes of diarrhoea in the children under 5 years old in Lagos, Nigeria.

Introduction

Diarrhoea poses a very serious problem in developing countries where it is the leading cause of morbidity and mortality amongst children.^{1–3} It ranks second as a major cause of morbidity among the notifiable diseases in Nigeria² where, according to the Federal Statistic Bulletin,⁴ c. 300 children die every day from dehydration and malnutrition caused by diarrhoea. This could be an underestimate, as very few patients have access to the very limited number of hospitals and health centres that are available.

Aetiological agents of diarrhoea are many and varied. In Nigeria, data from various studies have implicated various enteropathogens.^{5–10} However, the relative occurrence of these pathogens in the defined age group most vulnerable to enteropathogenic infections has not been well documented. Therefore, it is difficult to generalise about the public health importance of these agents in children under 5 years old in Nigeria. Furthermore, the roles of relatively “new”

agents such as enterohaemorrhagic *Escherichia coli* (EHEC), enteroadherent *E. coli* (EAEC), *Plesiomonas* spp. and cryptosporidia in childhood diarrhoea in Nigeria still need to be investigated properly.

This study was undertaken to investigate the prevalence of enteropathogens and the role of newly recognised pathogens in diarrhoea amongst children under 5 years of age in Lagos.

Materials and methods

Patient population

Between December 1989 and May 1990, faecal specimens were obtained from children aged ≤ 5 years attending the Paediatric Emergency Room, Lagos University Teaching Hospital (LUTH), and the Children's Clinic at Gbaja Health Centre, Surulere, about 1 km from LUTH; 215 specimens were collected from children with acute diarrhoea. During the same period 100 faecal specimens, collected from age-matched children seen at the same hospitals for reasons other than gastrointestinal illness, were selected as controls. Neither patients nor controls had received

antibiotics in the preceeding 2 weeks. Detailed history to define age, sex, current diarrhoeal illness and frequency of bowel motions was documented carefully. Stool specimens were collected by one of us (T.I.O.) who noted the consistency and presence of frank blood or mucus or both. Specimens were transported in stool cartons to the laboratory where they were examined immediately by microscopy for the presence of red blood cells and leucocytes, and then examined for bacterial, viral and parasitic enteropathogens. All the specimens were processed within 2 h of collection.

Cell lines

Enterotoxin production and enteroadherent properties of *E. coli* were determined by assays with Vero and HEP-2 cell lines obtained from Yaba Vaccine Production Laboratories, Yaba, Lagos.

Animals

Suckling mice weighing *c.* 2.5 g and guinea-pigs weighing *c.* 500 g were obtained from the University of Lagos College of Medicine for the investigation of the toxigenic and invasive properties of the enteropathogens. They were housed in filter-topped cages and were fed with rat chow and water *ad libitum*.

Microbiological methods

Faecal samples were cultured for pathogenic *E. coli*, salmonellae, shigellae, aeromonads, *Plesiomonas* spp., vibrios and yersiniae on the following media (Unipath): MacConkey Agar, Sorbitol MacConkey Agar for *E. coli* O157:H7, Desoxycholate Citrate Agar (DCA), Xylose Desoxycholate Citrate Agar (XDCA), Salmonella-Shigella Agar (SS) and Thio-sulphate Citrate Bile Salt (TCBS) Agar. Specimens were also inoculated into phosphate-buffered saline (PBS), alkaline peptone water (pH 8.6) and selenite-F broth. All inoculated media were incubated at 37°C for 18–24 h. Cold enrichment for *Yersinia* spp. in Dulbecco's PBS (Unipath) at 4°C was performed on all specimens, which were thereafter subcultured on to MacConkey agar and DCA after 7, 14 and 21 days and incubated at room temperature. The alkaline peptone water was subcultured on to TCBS agar and XDCA, and selenite-F broth cultures were subcultured on to DCA and SS agar. These subcultures were also incubated at 37°C for 18–24 h. Representative colonies were selected and isolates were identified by standard methods.^{11,12} For *E. coli*, five colonies were selected randomly from each plate for testing.

All *Salmonella* spp., *Shigella* spp. and *E. coli* isolates were serotyped with Wellcome Diagnostic Antisera. *E. coli* isolates, whether sorbitol negative or not, were kept on nutrient agar slopes at room temperature for a period of up to 6 months. These storage conditions appeared to be suitable for the marker plasmids of

E. coli K12. They were then screened for toxin production by a modified Vero cell line assay.¹³

Heat-stable enterotoxigenic strains of *E. coli* (ETEC) were confirmed by the suckling mouse test described by Giannella.¹⁴ A mean ratio of the intestinal weight to the weight of the remaining carcass of ≥ 0.083 was taken to indicate the presence of heat-stable enterotoxin. The heat labile ETEC strains were identified by the modification of the Elek test described by Honda *et al.*,¹⁵ while the enteroinvasive strains of the *E. coli* (EIEC) isolates were identified by the Sereny test for invasiveness in the guinea-pig eye model.¹⁶

The enteroadherent *E. coli* (EAEC) strains were identified by testing for adherence to HEP-2 cell lines.¹⁷ For each strain, the pattern of adherence was noted, i.e., diffuse, localised or aggregative.

Wet mounts of each fresh faecal sample were made in saline and iodine solution and examined for trophozoites and cysts of *Entamoeba histolytica* and *Giardia lamblia*. Formalin-ether concentrates (FEC) and smears stained with iron haematoxylin were also prepared for each specimen and examined for other intestinal parasites. Another smear prepared from the FEC was stained by modified Kinyoun acid-fast stain and examined for cryptosporidial oocysts.

Rotavirus was detected by preparing a 10% suspension of each faecal sample in PBS (pH 7.4) and centrifuging at 2000 *g* for 15 min. The faecal supernate collected was tested for the presence of rotavirus antigen with an ELISA technique.¹⁸

Results

The comparative characteristic features of both the patients and controls are shown in table I: 166 (77%) of the 215 children with diarrhoea were aged ≤ 1 year; 98.4% of them were aged ≤ 3 years. None was above 4 years old. The sex distribution showed a male:female ratio of 2:1. Over 60% of diarrhoeal patients sought medical help within the first 7 days of the onset of symptoms; 46% were febrile, 4% and 14% had

Table I. Comparative characteristics among the diarrhoeal patients and control subjects

Presenting features	Patient group (215)	Control group (100)
Age in months		
Range	2–48	6–48
Mean	11.4	15.0
Male:female ratio	2:1	1.9:1
Time of seeking medical assistance		
1 week	131 (60.9%)	...
> 1 week	84	...
Fever	90 (46%)	...
Presence of blood		
Frank	9 (4.2%)	0
Microscopic	30 (14%)	3 (3%)
Presence of leucocytes on microscopy	103 (47.9%)	9 (8%)

Table II. Detection of enteropathogens in diarrhoeal patients and controls

Enteropathogen	Number (%) of positive results from	
	diarrhoeal group (215)	control group (100)
EPEC	23 (10.7)	5 (5)
EPEC*	31 (14.4)	6 (6)
EHEC	11 (5.1)	3 (3)
EAEC	20 (9.3)	4 (4)
<i>Salmonella</i> spp.	7 (3.3)	1 (1)
<i>Shigella</i> spp.	11 (5.1)	0 (0)
<i>Y. enterocolitica</i>	2 (0.9)	0 (0)
<i>A. hydrophila</i>	3 (1.4)	0 (0)
<i>Ent. histolytica</i>	1 (0.5)	0 (0)
<i>G. lamblia</i>	1 (0.5)	0 (0)
<i>T. hominis</i>	1 (0.5)	0 (0)
<i>Trichuris trichiura</i>	2 (0.9)	0 (0)
Rotavirus	48 (22.3)	9 (9)

*Enterotoxin type in patients: LT 6 (2.8%); ST 23 (10.7%); LT/ST 2 (0.9%). The six strains from the control group were ST-positive.

frankly bloody stools or red cells on microscopy, respectively. The control and the diarrhoeal cases were fairly well matched. For the control group, the mean age was 1.3 years, the male:female ratio 1.9:1 and its members belonged to a similar economic background and residential area.

Aetiological agents

One hundred and sixty-one (74.9%) of the 215 diarrhoeal stools and 28 (28%) of control specimens yielded pathogens. All the positive diarrhoeal stools and 25 of the 28 control stools contained single pathogens. The individual enteropathogens isolated in the patient and control groups are shown in table II.

EPEC strains were the most commonly isolated bacterial pathogen, particularly in children with diarrhoea (14.4% yielded EPEC): LT-producing EPEC strains were isolated from six stools, ST-producing strains from 29 and LT- and ST-producing strains from two. The mean age of diarrhoeal patients infected with EPEC strains was 9 months. By contrast, the mean age of the control children from whom EPEC strains were isolated was 30.1 months. EPEC was the second most common bacterial agent isolated, being found in 23 diarrhoeal and five control patients. EPEC serotypes O26 and O86 were detected in both patients and controls; serotypes O55, O111, O125, O128 and O18c were detected in the patients only. The most frequently isolated serotype was O55 (10 of 28:35.7%) followed by O125 (5 of 28:17.8%). None of the EPEC strains exhibited adherence to the HEP-2 cells. EAEC strains were detected in 20 patients and four controls: 14 exhibited aggregative type adherence on HEP-2 cells, six exhibited localised adherence and the remaining four showed the diffuse type of adherence. None of the EAEC strains belonged to any EPEC serogroups frequently associated with this phenotype. EHEC strains were isolated from 11

patients and three controls; the former were < 1 year old whereas the latter were > 2 years old. No EIEC strains were isolated during this survey.

The distribution of the other bacterial pathogens isolated from patients and controls showed that the majority of them were exclusively isolated from the patients. Only the salmonellae were isolated from both patients and controls; seven and one isolates, respectively. The majority (4 of 7) were detected in patients < 1 year old. The eight *Salmonella* isolates were identified as serotypes *typhimurium* (4), *paratyphi* A (2) and *typhi* (2). The mean age of patients infected with salmonellae was 12.0 months. The *Shigella* spp. were isolated exclusively from children with diarrhoea (11 isolates). The age range of the patients was 3–48 months with a mean of 21.0 months. Of the 11 isolates five (45.4%) were *Sh. flexneri*, three (27.3%) *Sh. boydii*, two (18.2%) *Sh. dysenteriae* and one *Sh. sonnei*. *Y. enterocolitica* and *Aeromonas hydrophila* were isolated from two and three patients respectively.

Overall, the commonest enteropathogen detected was rotavirus—detected in 48 (22.3%) patients and nine (9%) controls. Forty-five (93.8%) of the 48 rotavirus isolates were detected in patients under 2 years old with a mean age of 12.2 months. The four types of intestinal parasites isolated were mainly from the patient group; *E. histolytica* (1) *G. lamblia* (1), *Trichomonas hominis* (1) and *Trichuris trichiura* (2). *Vibrio cholerae* was not isolated from either patients or controls in this study.

Discussion

Previous studies in Lagos have documented the prevalence of some traditionally recognised agents of diarrhoea^{5,9,10} but none has reported the prevalence of the newly recognised agents of diarrhoea, such as enteroadherent *E. coli* (EAEC) or enterohaemorrhagic *E. coli* (EHEC). Furthermore, our study has highlighted pertinent presenting features, such as fever, duration of illness before seeking hospital attention and the presence of red and white blood cells in the faeces, and shown that the majority of urban patients under 5 years of age with diarrhoea sought medical attention within the first week of their illness.

Both the heat-labile toxin-producing ETEC (LT⁺) and heat-stable toxin-producing ETEC (ST⁺) are recognised causal agents of diarrhoea worldwide.^{18–22} ST⁺ ETEC strains were isolated with equal frequency from the diarrhoeal and the control groups whereas LT⁺ ETEC strains, though detected in fewer patients, were confined to the diarrhoeal group only. This suggests that in the urban population of patients in Lagos the LT⁺-producing ETEC strains are probably more associated with diarrhoea than the ST⁺ ETEC strains. However, a much larger study conducted over a longer period may be needed to confirm this tentative conclusion, especially as studies from some other developing countries have indicated that the carriage

of LT⁺ strains of ETEC is not statistically associated with diarrhoea.²³⁻²⁵ Although ST⁺ strains of ETEC were strongly associated with diarrhoea in studies conducted in a similar setting in China²⁶ and Australia,²⁷ our survey showed a relatively high carriage rate of ST⁺ strains in the control group, which makes interpretation somewhat difficult. A study from Djibouti²⁵ appears to be in agreement with our experience. The observation in this survey of a decreasing isolation rate of ETEC strains with age is also of interest; the majority of the diarrhoeal patients with ETEC were < 1 year old whereas the average age among the controls carrying ETEC strains was 30.1 months, thus confirming the experience in Mogadishu.²⁸

So far as we know, this report represents the first description of EHEC (O157:H7) and EAEC in patients with diarrhoea in Nigeria. Finding these two newly described diarrhoeal pathogens in our patients in a relatively higher proportion than that which has been reported in many developed countries^{29,30} is of great clinical importance. EHEC strains have been strongly associated with watery diarrhoea, haemorrhagic colitis and the haemolytic uraemic syndrome.^{31,32} However, more reports on studies conducted in other developing countries are necessary to determine whether or not there is significant geographic difference in the prevalence of EHEC and EAEC infections. Similar high isolation rates of EHEC to that in our study (5.1%), have been reported from Asian countries, i.e., 7% of patients in Thailand³³ and 6.8% of patients in Beijing.²⁶ Surprisingly, a lack of association between a history of faecal blood and the presence of microscopic faecal leucocytes was found with the isolation of our EHEC strains. None of the EAEC strains isolated from both patients and controls belonged to the recognised EPEC serogroups, just as none of the EPEC isolates were enteroadherent, which confirms the finding of Mikhail *et al.*²⁵

Many of the patients from whom EAEC strains were isolated in this study, as in two other reports,^{24,26} presented with fever, or faecal leucocytes and erythrocytes. These features are more frequently associated with invasive diarrhoeal pathogens and support the *in-vitro* observations of Donnenburg *et al.*³⁴ who showed that EAEC strains were not only entero-adhesive but also invasive towards epithelial cells. EAEC strains exhibiting localised adherence to HeLa or HEp-2 cell lines *in vitro* have been associated consistently with diarrhoea all over the world.^{26,35,36} This survey confirms that EAEC is positively associated with diarrhoea in children between 1 and 2 years old, an age higher than that usually associated with ETEC infection.

Our data show that EPEC is the second most common bacterial diarrhoeal pathogen isolated from both patients and controls after ETEC, although more frequently isolated from patients, which is similar to a report from South Africa.³⁷ The isolation rate of EPEC strains in diarrhoeal patients in Lagos remains consistently and relatively high. Previous studies^{5,38} support this assertion. Over 78% of the patients infected with EPEC strains were aged ≤ 2 years with an average age greater than that found with ETEC or EAEC infections. The preponderance of EPEC in the older age group in our survey is in contrast to observations in the developed countries, where neonates are the most vulnerable age group.³⁹ This may be related in part to the delayed weaning normally practised in developing countries.⁴⁰

Other bacterial pathogens, (*Shigella* spp., *Salmonella* spp., *A. hydrophila* and *Y. enterocolitica*) were confined mainly to the diarrhoeal group. We may assume that these bacteria are indeed pathogenic whenever they are found in the stool specimens of children with diarrhoea in Lagos. All patients with shigella infection had fever and faecal erythrocytes on microscopy. The mean age of these patients was 18 months. Our finding with shigellae is similar to the reports from Djibouti,²⁵ Beijing²⁶ and Somalia²⁸ with regards to species prevalence and positive association with faecal leucocytes and erythrocytes. The commonest species in our survey, as in these other reports, was *Sh. flexneri*.

The results of this study are in agreement with many other reports from elsewhere,^{9,25,41,42} in that rotavirus is the most frequent cause of diarrhoea in the defined age group that we surveyed. The overall attack rate of c. 22% in the diarrhoeal group compares with the reported rates of 19–27% in other countries. Children afflicted with diarrhoea caused by rotavirus were < 2 years old and leucocytes and erythrocytes were not present in their faeces.

The yield from examination of stools for cysts, ova and parasites was very low. No cryptosporidial oocysts were found. Limited information from Nigeria suggests that *Cryptosporidium* spp., *G. lamblia* and other parasitic infections are rare causes of diarrhoea.^{43,44} This low yield may also be explained by the limitations of detection from a single faecal specimen, the small quantity of samples obtained from some patients and the timing of the study. The survey was conducted during the dry season of the year and occurrence of *G. lamblia*, *Ent. histolytica* and *Cryptosporidium* spp. may be seasonal, as is generally the case in temperate countries, appearing more in the rainy season.

We thank Mrs Macaulay for her technical assistance and Ms Sheila Baker for her excellent secretarial assistance.

References

- Walsh JA, Warren KS. Selective primary health care. An interim strategy for disease control in developing countries. *N Engl J Med* 1979; **301**: 967–974.
- Barua D. The WHO Global Diarrhoeal Disease Control programme. In: Emejuaiwe SO, Ogunbi O, Sanni SO (eds) *Global impacts of applied microbiology* (Sixth international conference). London, Academic Press. 1981: 405–413.

3. Synder JD, Merson MH. The magnitude of the global problem of acute diarrhoeal disease: a review of active surveillance data. *Bull World Health Organ* 1982; **60**: 605-613.
4. Federal Ministry of Health Bulletin. Lagos Statistics. Lagos, Government Press. 1987: 20, 5.
5. Ogunbi O, Hunponu-Wusu OO, Coker AO *et al.* Bacterial and viral agents associated with diarrhoeal diseases in Lagos—Nigeria. In: Emejuiwe SO, Ogunbi O, Sanni SO (eds) Global impacts of applied microbiology (Sixth international conference). London, Academic Press. 1980: 427-434.
6. Agbonlahor DE, Odugbemi TO, Lasi Q. Isolation of *Yersinia enterocolitica* from stools of acute gastroenteritis cases in Lagos, Nigeria. *East Afr Med J* 1981; **58**: 520-524.
7. Olusanya O, Adebayo JO, Williams B. *Campylobacter jejuni* as a bacterial cause of diarrhoea in Ile-Ife, Nigeria. *J Hyg* 1983; **91**: 77-80.
8. Coker AO, Dosunmu-Ogunbi O. Isolation of *Campylobacter jejuni* in Lagos, Nigeria—a new bacterial agent of diarrhoea. *East Afr Med J* 1984; **61**: 52-55.
9. Coker AO, Dosunmu-Ogunbi O, Odugbemi T *et al.* A study on the prevalence of rotavirus diarrhoea in Ohoazara Local Government area, Imo State, Nigeria and Lagos University Teaching Hospital, Nigeria. *East Afr Med J* 1987; **64**: 587-589.
10. Oyerinde JPO, Dosunmu-Ogunbi O, Alonge AA. Age and sex distribution of infections with *Entamoeba histolytica* and *Giardia intestinalis* in Lagos population. *Int J Epidemiol* 1977; **6**: 231-234.
11. Kelly MT, Brenner DJ, Farmer JJ. Enterobacteriaceae. In: Lennette EH, Balows A, Hansler WJ, Truant JP (eds) Manual of clinical microbiology. Washington, DC, American Society for Microbiology. 1985: 263.
12. Cowan ST, Steel KJ. Identification of medical bacteria, 2nd edn. London, Cambridge University Press. 1974.
13. Pai CH, Gordon R, Sims HV, Bryan LE. Sporadic cases of haemorrhagic colitis associated with *Escherichia coli* O157: H7—Clinical, epidemiologic and bacteriologic features. *Ann Intern Med* 1984; **101**: 738-742.
14. Giannella RA. Suckling mouse model for detection of heat-stable *Escherichia coli* enterotoxin: characteristics of the model. *Infect Immun* 1976; **14**: 95-99.
15. Honda T, Taga S, Takeda Y, Miwatani T. Modified Elek test for detection of heat-labile enterotoxin of enterotoxigenic *Escherichia coli*. *J Clin Microbiol* 1981; **13**: 1-5.
16. Sereny B. Experimental shigella keratoconjunctivitis—a preliminary report. *Acta Microbiol Acad Sci* 1955; **2**: 293-296.
17. Mathewson JJ, Johnson PC, Dupont HL *et al.* A newly recognized cause of travelers' diarrhea: enteroadherent *Escherichia coli*. *J Infect Dis* 1985; **151**: 471-475.
18. Yolken RH, Wyatt RG, Kapikian AZ. ELISA for rotavirus. *Lancet* 1977; **2**: 819.
19. Echeverria P, Taylor DN, Suthienkul O. Recent advances in bacterial diarrhoea. *Southeast Asian J Trop Med Public Health* 1986; **17**: 627-634.
20. Shukry S, Zaki AM, DuPont HL, Shoukry I, El Tagi M, Hamed Z. Detection of enteropathogens in fatal and potentially fatal diarrhoea in Cairo, Egypt. *J Clin Microbiol* 1986; **24**: 959-962.
21. Levine MM. *Escherichia coli* that causes diarrhoea: enterotoxigenic, enteropathogenic, enteroinvasive, enterohemorrhagic and enteroadherent. *J Infect Dis* 1987; **155**: 377-389.
22. Ako-Nai AK, Laminkana A, Ola O, Fadero FF. A study of the incidence of enterotoxigenic *Escherichia coli* (ETEC) secreting heat-labile toxin in two communities in south-western Nigeria. *J Trop Med Hyg* 1990; **93**: 116-118.
23. Escheverria P, Taylor DN, Leksomboon U *et al.* Case-control study of endemic diarrhoeal disease in Thai children. *J Infect Dis* 1989; **159**: 543-548.
24. Kim K-H, Suh I-S, Kim JM, Kim CW, Cho Y-J. Etiology of childhood diarrhoea in Korea. *J Clin Microbiol* 1989; **27**: 1192-1196.
25. Mikhail IS, Fox E, Haberberger RL, Ahmed MH, Abbatte EA. Epidemiology of bacterial pathogens associated with infectious diarrhea in Djibouti. *J Clin Microbiol* 1990; **28**: 956-961.
26. Kain KC, Barteluk RL, Kelly MT *et al.* Etiology of childhood diarrhea in Beijing, China. *J Clin Microbiol* 1991; **29**: 90-95.
27. Valladares KD. Enteric pathogens in aboriginal and non-aboriginal children admitted to Port Augusta Hospital. *Aust J Med Lab Sci* 1990; **11**: 93-95.
28. Casalino M, Yusuf MW, Nicoletti M *et al.* A two-year study of enteric infections associated with diarrhoeal diseases in children in urban Somalia. *Trans R Soc Trop Med Hyg* 1988; **82**: 637-641.
29. Pai CH, Ahmed N, Lior H, Johnson WM, Sims HV, Woods DE. Epidemiology of sporadic diarrhea due to verocytotoxin-producing *Escherichia coli*: a two-year prospective study. *J Infect Dis* 1988; **157**: 1054-1057.
30. Harris AA, Kaplan RL, Goodman LJ *et al.* Results of a screening method used in a 12-month stool survey for *Escherichia coli* O157:H7. *J Infect Dis* 1985; **152**: 775-777.
31. Spika JS, Parsons JE, Nordenberg D, Wells JG, Gunn RA, Blake PA. Haemolytic uremic syndrome and diarrhea associated with *Escherichia coli* O157:H7 in a day care centre. *J Pediatr* 1986; **109**: 287-291.
32. Remis RS, MacDonald KL, Riley LW *et al.* Sporadic cases of haemorrhagic colitis associated with *Escherichia coli* O157:H7. *Ann Intern Med* 1984; **101**: 624-626.
33. Brown JE, Echeverria P, Taylor DN *et al.* Determination by DNA hybridization of Shiga-like-toxin-producing *Escherichia coli* in children with diarrhea in Thailand. *J Clin Microbiol* 1989; **27**: 291-294.
34. Donnenberg MS, Donohue-Rolfe A, Keusch GT. Epithelial cell invasion: an overlooked property of enteropathogenic *Escherichia coli* (EPEC) associated with the EPEC adherence factor. *J Infect Dis* 1989; **160**: 452-459.
35. Chatkaemorakot A, Echeverria P, Taylor DN *et al.* HeLa cell-adherent *Escherichia coli* in children with diarrhea in Thailand. *J Infect Dis* 1987; **156**: 669-672.
36. Mathewson JJ, Oberhelman RA, Dupont HL, de la Cabada FJ, Garibay EV. Enteroadherent *Escherichia coli* as a cause of diarrhea among children in Mexico. *J Clin Microbiol* 1987; **25**: 1917-1919.
37. Koornhof HJ, Robins-Browne RM, Richardson NJ, Canel R. Etiology of infantile enteritis in South Africa. *Isr J Med Sci* 1979; **15**: 341-347.
38. Dosunmu-Ogunbi O. Intestinal microbial and helminthics infections, Lagos University Teaching Hospital, 1965-1968. *J Niger Med Assoc* 1971; **1**: 53-59.
39. World Health Organization Scientific Working Group. *Escherichia coli* diarrhoea. *Bull World Health Organ* 1980; **58**: 23-36.
40. Rowe B. Enteropathogenic *Escherichia coli* (EPEC)—importance and pathophysiology. In: Holme T, Holmgren J, Merson MH, Möllby R (eds) Acute enteric infections in children: new prospects for treatment and prevention. Amsterdam, Elsevier. 1981: 101-107.
41. Escheverria P, Blacklow NR, Vollet JL *et al.* Rotavirus-like agent and enterotoxigenic *Escherichia coli* infections in paediatric diarrhea in the Philippines. *J Infect Dis* 1978; **138**: 326-332.
42. Stintzing G, Bäck E, Tufvesson B, Johnsson T, Wadström X, Habte D. Seasonal fluctuations in the occurrence of enterotoxigenic bacteria and rotavirus in paediatric diarrhoea in Addis Ababa. *Bull World Health Organ* 1981; **59**: 67-73.
43. Oyerinde JP, Odugbemi T, Benson RI *et al.* Investigation of *Cryptosporidium* in relation to other intestinal parasites at the Lagos University Teaching Hospital. *W Afri J Med* 1989; **8**: 264-269.
44. Eko FO, Rotimi VO. *Cryptosporidium* and *Cryptosporidiosis*: an epidemiologic and clinical review. *Niger Med Practit* 1992; **23**: 17-21.