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NEW AND OLD AGENTS IN DIARRHEA: A PROSPECTIVE STUDY OF AN INDIGENOUS ADULT AFRICAN POPULATION*

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Abstract. We conducted a prospective study of 77 indigenous African adults with acute diarrhea seeking care at the major hospital in Nairobi, Kenya, to determine the major pathogens responsible for this syndrome in adults. Fecal and blood specimens were collected and examined for enteric bacterial pathogens, viruses, and parasites. In 13 (26%) inpatients and 11 (49%) outpatients *Shigella* was found, and heat-labile and heat-stable forms of enterotoxigenic *Escherichia coli* were found in 9 (18%) inpatients and 1 (4%) outpatient. Human reovirus-like agent titers rose significantly in another 3 (6%). Amebic dysentery was not seen although hemagglutination-inhibition tests for invasive *Entamoeba histolytica* were positive in 4 inpatients. An etiologic agent was found in 65% of patients.

Diarrhea is one of the most common causes of admission to the hospital in Nairobi for both adults and children, and it is a common cause of death in the latter. There is a paucity of information on etiologic agents for adult diarrhea, particularly in developing countries. In this paper we have attempted to determine the major pathogens at the Kenyatta National Hospital, specifically seeking protozoans, *Shigella* and *Salmonella*, enterotoxigenic *Escherichia coli* (ETEC), and human reovirus-like agent (HRVLA).[1]

MATERIALS AND METHODS

Fifty-four inpatients admitted to the Adult Observation Ward of the Kenyatta National Hospital because of diarrheal disease, and 23 outpatients who were self-referred because of diarrhea but were not ill enough for admission, comprised the patients for this study. Ages ranged from 16 to 60 yr with most in their 20s (mean = 32 yr). All were seen between August 1975 and February 1976. The criteria for admission to the study required diarrhea of less than 3 wk's duration, age over 14, and at least four diarrheal stools daily. The major criteria for hospitalization included toxicity and fever (17%), diarrhea and pain (57%), and dehydration (26%). Three patients were excluded because antibiotics had been taken prior to admission, leaving 51 patients. The inpatients received a history and physical examination with particular emphasis on the clinical features of the diarrheal illness and evidence of dehydration. They were weighed, and a stool specimen was obtained. The warm stool was examined within 15 min, and a determination of gross appearance, gross or occult blood, motile trophozoites, red and white cell counts per high power field, and eggs and cysts was made. Fecal specimens were streaked on MacConkey agar. Portions of each specimen were placed in freshly mixed merthiolate-iodine-formalin (MIF) preservative, polyvinyl alcohol (PVA), and formol saline (FS) for parasitologic examination. Blood cultures (2), malaria smears, complete blood count, and an acute serum were obtained.

Inpatients were rehydrated, usually orally despite vomiting, using a formula recommended by the World Health Organization consisting of glucose 20 g, NaCl 3.5 g, $NaHCO_3$ 2.5 g, and KCl 1.5 g dissolved in each liter of water.[2] Thereafter, frequent temperature checks and daily examinations were done, and body weight, stool counts, and stool weights were recorded. Patients were treated supportively and with antibiotics as seemed indicated, but antispasmodics were not given.

Inpatients were followed 2 wk post-discharge with a history and examination as well as a convalescent serum. Repeat stool examination and

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parasitologic examinations were done at this time. Follow-up was obtained in 70%. The 23 outpatients were studied bacteriologically and parasitologically, but no serology could be performed as no follow-up was possible.

Bacteriology. *Salmonella* and *Shigella* were identified from the MacConkey plate using standard methods[3] and serotyped with Wellcome antisera. In addition, five individual lactose positive colonies typical of *E. coli* and a pool of ten other colonies typical of *E. coli* were inoculated onto nutrient agar slants which were then transported to Baltimore for subsequent testing for enterotoxin. Finally, a second stool culture was obtained on the outpatients and processed separately for *Salmonella* and *Shigella* species.

Enterotoxin assays. All isolates and pools of *E. coli* were tested for heat labile toxin (LT) production in the Y1 adrenal cell assay[4] in duplicate, and for heat stable toxin (ST) production using the infant mouse assay.[5] For the ST assay, supernates of cultures grown in brain-heart infusion broth with agitation (200 shakes/minute), and to which gentamycin (40 μg/ml) had been added, were injected percutaneously intragastrically into at least two 2- to 4-day-old mice. Positive strains produced fluid secretion as determined by a mean intestine weight to remaining body weight ratio of $\geqslant$0.083 at 4 h.

Parasitology. Stool samples were preserved in MIF and PVA solutions and examined up to 6 mo after collection by one of us (R.G.K.). Both acute and, when available, convalescent samples were examined for intestinal protozoa. Two methods were chosen: a direct MIF smear after bulk fixation,[6] and an iron-hematoxylin permanent stained smear[7] after PVA fixation. Both types of smears were examined under oil immersion and the protozoa encountered were closely characterized particularly in regard to nuclear morphology, size and cytoplasmic inclusions.[7,8] Because of a large amount of precipitate found covering many of the iron-hematoxylin smears as well as poor fixation, the inpatient specimens were studied only in a MIF direct smear after bulk fixation. Outpatient samples had been fixed in PVA only and, therefore, were not available for MIF.

Serology. Anti-LT antibody titers were determined using a microtiter adrenal cell LT neutralization assay.[9] Units of antitoxin were determined for each serum by determining the geometric mean titer of two assays done in triplicate and comparing the unknown serum to the standard serum (Swiss Serum Institute anti-cholera serum) which has been assigned 1,000 units against *E. coli* LT. HRVLA (rotavirus) antibody titers were determined using a complement fixation assay with Nebraska calf diarrhea virus and "O" antigen as substitute antigens[10] on the paired acute and convalescent sera. Antibodies to amebae were determined by Dr. George Healey of the Center for Disease Control, Atlanta, Georgia.

RESULTS

Bacteriology. The results of the bacteriologic studies are listed in Table 1. *Shigella*, ETEC, *Salmonella*, and HRVLA were detected in that order, and dual infections occurred. Among the ETEC cultures, of the five isolates tested, a mean of 3.1 colonies were positive (range 1–5). Blood cultures were negative in all except one patient with typhoid.

Duplicate stool cultures taken from the 23 outpatients were performed to assess the need for repeated cultures. Of the 11 *Shigella* isolates, three were only present in one of the cultures, suggesting a second sample might add another 15% or more of cases associated with *Shigella*.

Parasitology. Of a total of 40 inpatient and 23 outpatient samples examined, *Entamoeba histolytica* trophozoites of the non-invasive type or minuta form were found in 5. Other trophozoites seen included *Entamoeba coli* in 7 patients, *Endolimax nana* in 10, *Iodamoeba butschlii* in 2, *Chilomastix mesnili* in 4, and *Trichomonas hominis* in 3. Cysts of *Giardia lamblia* were found on one occasion. In addition, hookworm eggs were seen in 5 and *Schistosoma mansoni* in 4 patients.

A positive (titer 1:128) amebic indirect hemagglutination test was present in 4 of 43 inpatients indicating previous exposure to invasion with this organism. None of these four had confirmed invasive, hematophagous *E. histolytica* trophozoites although one with motile forms on initial warm smear received treatment before the stained smears were obtained. That PVA smear demonstrated only macrophages resembling trophozoites.

Serology. Anti-LT antibody titers were determined on the acute sera of 39 inpatients and on convalescent sera in 33 of these, representing 31

TABLE 1

Pathogens detected among 74 adult Kenyans (51 inpatients and 23 outpatients) with diarrhea

Etiologic agents*	Inpatients (51) No.	Inpatients (51) %	Outpatients (23) No.	Outpatients (23) %
Shigella species	13	25.5	11	47.8
S. flexneri—1, 2, 2a, 3, 4, 6	8		6	
S. dysenteriae—2, 3	4		2	
S. sonnei	1		2	
S. boydii	0		1	
Salmonella typhi	2	3.9	0	
Other *Salmonella*	3			
S. typhimurium	2			
S. cerro	1			
Enterotoxigenic *E. coli*	9	17.6	1	4.3
LT producing	4		1	
ST producing	2		0	
LT-ST producing	1		0	
By LT serology only	2		0	
HRVLA	3	5.9	0	
Mixed	3	5.8	0	
ETEC (LT)—*Shigella sonnei*	1			
ETEC (ST)—*Shigella flexneri*	1			
HRVLA—*Shigella flexneri*	1			
No etiologic agent	18	35.2	11	47.8
TOTAL	51		23	

* LT, heat labile toxin; ST, heat stable toxin; HRVLA, rotavirus; ETEC, enterotoxigenic *E. coli*.

paired acute and convalescent sera. A greater than fourfold change in titer was present in four patients, two of whom had positive cultures for ETEC. Of the other LT producing ETEC culture positive patients, one had a two- to threefold rise in titer, one had only a minimal rise, and two lacked paired sera. The geometric mean titer of the acute anti-LT sera was 8.2 (2 S.E. = 6.0–11, range 1.6–104). Of the 31 paired sera tested for antibodies to HRVLA, one was anticomplementemic, and four demonstrated a fourfold or greater rise in titer to HRVLA. One of these grew *Shigella flexneri-IV* on culture, and the major pathogen is unclear.

Other. One patient had a heavy parasitemia with *Plasmodium falciparum* and presented with fever, vomiting, headache, and diarrhea after a visit to an endemic area. Another had acutely increasing edema with vomiting and diarrhea for 2 days and had constrictive pericarditis confirmed on cardiac catheterization. A woman with shigellosis was found to have coexistent advanced, severe tuberculosis.

Clinical Features

Clinical features of the 34 inpatients in whom an etiologic agent was found are listed in Table 2. The remaining 18 patients without an agent found in association with their illness probably represent a collection of disorders: three of these had had some previous medication (non-antibiotic), and in eight paired sera were lacking. Seven of 18 had no fecal leukocytes whereas all but two of those with an infectious etiology had fecal leukocytes. Two had "classic" shigella stools (see below) but were culture negative. Finally, three had elevated HAI titers for amebiasis without trophozoites in the stool.

Abdominal pain was a frequent feature, present in 92%. Two of the remainder had no fecal leukocytes, one having typhoid septicemia and one having acute hypertrophic pulmonary osteoarthropathy as well. The abdominal pain was located variably, and no pattern correlating the pain with particular pathogens was found. Dehydration, best detected by a postural drop in

TABLE 2
*Clinical features of inpatients with diarrhea in Kenya**

Clinical features	*Shigella* (13)†	*S. typhi* (2)	Other *Salmonella* (3)	ETEC‡ (9)	HRVLA§ (3)
Symptoms					
Mean duration prior to admission (days)	2.4	5.4	2.5	1.5	1.3
Abdominal pain	12/13	1/2	3/3	8/9	2/3
Feverish	8/13	2/2	1/3	2/9	1/3
Headache	9/11	2/2	2/3	6/9	2/3
Vomiting	5/13	2/2	2/3	9/9	2/3
Signs					
Average peak fever	38.1	40.5	38.5	37.7	38.1
Volume depletion	5/13	1/2	3/3	3/9	1/3
Microscopic stool examination					
>10 leukocytes/hpf	9/12	0/2	2/3	5/8	2/3
>10 erythrocytes/hpf	10/13	0/2	2/3	2/8	1/3
Few microorganisms	4/6	–	0/2	–	–

* Excludes patients with mixed etiologies and no agent found.
† Number of patients.
‡ ETEC, enterotoxigenic *E. coli*.
§ HRVLA, rotavirus.

blood pressure and rise in pulse, correlated with vomiting rather than with stool frequency. Skin turgor was less reliable than the postural pulse and blood pressure changes, and stool volume measurements in these non-cholera patients were difficult to interpret and follow.

All but one stool examination in shigella patients had fecal leukocytes and 7/11 had over 50 white cells per high power field, and all but two patients had blood in their stool. Most of these had watery, alkaline stools, which were odorless with few organisms microscopically, the red watery stool with clumps of pus on the bottom of the container being characteristic. The non-typhoid salmonella cases (3) were similar to shigella, although the fever was somewhat higher. The nine patients with ETEC (on culture or by titer change) had the shorter duration of symptoms mentioned, all had vomiting except one with nausea only. Symptoms of fever and headache were mild and occurred in about one-half, a picture of volume depletion being present in only three. Stools were bloody in two but were usually watery, yellow, or brown in color. All had some stool leukocytes, occasionally large numbers.

The three patients with a HRVLA titer rise had a short lasting diarrhea. Fever was present in all, one reaching 39.7°C. Two had signs of volume depletion. All three had some fecal leukocytes, and two had stool blood present. In all three, the illness was severe enough to require hospitalization but was of short duration, permitting discharge in 1.5 to 2.5 days.

DISCUSSION

With the advent of assays for enterotoxin production, ETEC has been found to be a major pathogen of travelers in developing countries.[11, 12] However, relatively little previous work has been done in placing these agents in the perspective of diarrhea among adults indigenous to a developing country. Although American Peace Corps volunteers in Kenya have had ETEC implicated in 63% of their travelers' diarrhea[12] and HRVLA has been found in up to 42% of infants and young children in the United States,[1] neither has previously been studied in adult inpatients in East Africa.

Amebic dysentery is considered to be the major cause of acute diarrheal illness by many physicians in tropical countries and patients are often treated presumptively for this. Although certain difficulties, particularly proper identification of the parasite and clinical recognition of the disease, can make diagnosis difficult, particular efforts were made in this study to detect this pathogen, and no invasive forms of *E. histolytica* were found. The four patients with elevated IHA

titers indicate past or present invasive amebiasis, but in only one was the acute clinical picture suggestive. In reviewing all of the 24,086 stool reports for 1976 at the Kenyatta National Hospital, only three cases were found to have acute amebic dysentery, with invasive type trophozoites numerous on fresh direct smear. By the formol-saline concentration method 1,409 amebic cyst passers were noted, but *Entamoeba hartmanni* was not recognized separately.

The extraordinary geographical variation in virulence of *E. histolytica* is well-recognized. Elsdon-Dew has noted four foci of high virulence: Mexico, Durban in South Africa, West Africa, and Southeast Asia,[8] liver abscess being used as an index of invasiveness. A virus or other factor has been speculated to be responsible for transforming the harmless commensal ameba into a totally different invasive form.[13] Although we do not have definite data on liver abscess for Kenya, it does occur in Nairobi, and is more common than the acute amebic colitis in our experience.

Although iron hematoxylin stained smears are probably the most reliable and superior method for proper protozoal identification when properly done, the method is expensive and elaborate and not well suited for a routine laboratory in developing countries. We obtained good results using the MIF preserved samples, adding oil-immersion for examining the wet preparation. A single MIF smear in a busy lab will provide accuracy with simplicity, once the technicians are trained.[6]

The high percentage of cases with shigellosis is in contrast to the experience of Peace Corps volunteers in the same country, in whom no cases were found, but in whom ETEC was the predominant pathogen.[12] We felt this might reflect the inpatient population we initially studied, but the outpatient population also had a high rate of infection with *Shigella* as well. Presumably, this reflects the less than optimal home environments, toilets, and washing facilities available to the indigenous population. The percentage with shigellosis may be even higher in view of the well-recognized difficulty in culturing these organisms.

Travelers' diarrhea has recently been attributed most commonly to ETEC, which has accounted for from 45% to 75% of episodes in Mexico[11, 14] and in Kenya.[12] Little, however, is known about its prevalence among indigenous populations. We have demonstrated it does occur in Kenyans and that it can be associated with severe diarrheal illness requiring hospitalization here. A picture of severe, cholera-like diarrhea[15, 16] has been seen with ETEC in India, Bangladesh, and even North America.[17] Of interest is the relative infrequency of ETEC (18%) of our population of indigenous adults, when compared to the frequent occurrence of ETEC among the travelers and, conversely, the infrequency of shigellosis among the Peace Corps volunteers (particularly considering their propensity to get ETEC diarrhea), when compared to the frequent occurrence of shigellosis among the Kenyan population. This presumably reflects a difference in transmissability of the two organisms; also, the Kenyan population probably is exposed to ETEC at a young age and indeed has a higher mean anti-LT antibody titer which may reflect some protection.

The patients with antibody rises to HRVLA were important in that this agent, although causing mild illness in adults,[18] has only infrequently been associated with severe hospitalizing illness in adults. It is a particularly common cause of infantile winter diarrhea in temperate climates and may infect the parents of infected children, causing a mild illness. Here it was associated with four of the diarrheal illnesses among these inpatient adults.

Several clinical features became apparent during this study. The ease with which dehydration could be corrected by the oral glucose and electrolyte solution, despite the presence of vomiting, has been confirmed by our experience in these non-cholera patients. The typical stool of shigella (red, watery, mucoid, with clumps of pus on the bottom of the container) was quite distinctive of this disorder. However, some of the inpatients and most of the outpatients with *Shigella* lacked this characteristic stool. The shorter duration ETEC and HRVLA diarrheas were associated with severe vomiting and diarrhea, but there was less fever and less pus and blood in the stools.

Particularly unusual cases included one with hypertrophic pulmonary osteoarthropathy with bilateral tibial periostitis and no pathogen found, and one with constrictive pericarditis and diarrhea. The two typhoid cases represented two ends of that spectrum: one with fulminant septicemia

and shock which may be accompanied by diarrhea and the other more chronic case with diarrhea appearing late after initial constipation. In the latter patient the entire family had been ill with typhoid (but without diarrhea), and one of the children had just died.

Known diarrheal pathogens were encountered in 33 of our 51 (64.7%) patients. In the remainder complete serology was not available; some had had previous medication obtained from dispensaries and friends, and multiple stool cultures could not be obtained. These factors probably account for the bulk of patients in whom no etiologic agent could be found, and it is likely the remainder, particularly those without fecal leukocytes, represent infections with a lower concentration of organisms or non-infectious etiologies.

To extrapolate these figures to the general population of developing countries is, of course, unjustified. However, they do suggest that two "new" agents, ETEC and HRVLA, do cause severe disease among adults in Kenya and probably elsewhere, and that further studies elucidating their prevalence among the general population in all age groups are indicated. The frightening mortality caused by diarrheal illnesses in the pediatric populations of developing countries demands additional investigations into their causes as well as the dissemination of information regarding their treatment with oral glucose-electrolyte replacement solutions.

In summary, we have demonstrated that recognizable etiologic agents are associated with the majority of diarrheal illnesses in a developing country, and that these illnesses are not commonly due to amebiasis. Rather, shigellae, ETEC, salmonella including typhoid, and HRVLA in that order constitute the major pathogens among our inpatients and outpatients.

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