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PREVALENCE OF PATHOGENIC *ESCHERICHIA COLI* AND PARASITES IN INFANTS WITH DIARRHOEA IN KUMASI, GHANA

P. A. K. ADDY, G. ANTEPIM and E. H. FRIMPONG

ABSTRACT

Objectives: **To determine the relative frequencies and prevalence rates of pathogenic *Escherichia coli* and intestinal parasites in hospitalised infants (0-5 years) in Kumasi.**
Design: **A prospective descriptive study of screening 162 (83 males and 79 females) infants with diarrhoea and 122(64 males and 58 females) non-diarrhoeal infants controls for pathogens (*E. coli* and intestinal parasites) by standard microbiological methods.**
Setting: **Komfo Anokye Teaching Hospital and Maternal and Child Hospital, Kumasi, Ghana.**
Results: **From the 162 in the diarrhoeal group 96(59.6%) pathogens, and from the 122 in the control group, eight (6.6%) pathogens were isolated. Enteropathogenic *E. coli* (EPEC) was the most frequently detected pathogen, accounting for 24(14.8%) of the findings in the diarrhoeal group, five (4.1%) in the non-diarrhoeal control group. Of the 26 EPEC isolates, there were nine serotypes with the three dominant ones being 0125 (6), 0119 (5), and 026 (15). Other agents isolated included *Ascaris lumbricoides* 18(11.1%) and two (1.6%), *Cryptosporidium* 13(8.0%) and one (0.8%) for diarrhoeal and non-diarrhoeal infants respectively. The following were detected only in diarrhoeal stools. *Giardia lamblia*, six (3.7%); *Trichomonas hominis*, three (1.9%); *Trichuris trichiura* one (0.6%) and hookworm, one (0.6%).**
Conclusion: **From this study, EPEC and *Cryptosporidium* may be considered as important diarrhoeagenic pathogens and it is hoped that provision of potable water and good sanitation may decrease diarrhoeas in infants caused by these agents.**

INTRODUCTION

The World Health Organization defined diarrhoea as the voiding of more than two unformed watery stools in any 24 hour period, or any voiding of watery stools if accompanied by fever, abdominal pain and/or vomiting(1,2).

Diarrhoea accounts for more deaths in childhood than any other disease in the developing world. It has been pointed out that in the developing countries, a child of less than seven years of age still has a 50% chance of dying from diarrhoeal diseases(1-3).

Infantile diarrhoea remains one of the leading causes of childhood morbidity and mortality in developing countries. Children in the developing world have an average of 5-6 episodes a year(l). WHO has accordingly underlined the need for epidemiological surveys of infantile diarrhoea in all geographical areas(2,4).

Diarrhoea occurs when the ability of a normal or a damaged colon to re-absorb all excess fluid in the intestines fluid is impaired, hence resulting in the loss of fluids and electrolytes. The end result is dehydration which is the main cause of death(5).

There is a high mortality rate in urban slums and in rural farming communities(6,8), close association of diarrhoea to socio-economic conditions and poverty. Poverty creates overcrowding, illiteracy, poor sanitation and malnutrition; all related to diarrhoeas(6-8).

In malnourished infants, the attack rate and the severity of disease are increased, resulting in high mortality(7). Children under three years are the most affected.

Mackenjee *et al.*(9) reported that in developed countries, diarrhoea is seen in the winter with viruses being the major agents, whereas in the developing countries, diarrhoea remains a wet season disease with bacteria playing a greater role, though Anders *et al.* found rotavirus as the dominant agent in Ethiopia(4). The agents responsible for infantile diarrhoea may be bacteria, viruses, parasites, malnutrition and drugs. These organisms cause diarrhoea alone, or with others(1-5).

Parasites known to cause diarrhoea e.g. *Amoeba* and *Giardia lamblia*, may also cause infantile diarrhoea, but these tend to be commoner in older children in whom they cause either dysentery, or chronic diarrhoea and malabsorption(10).

However in this study, emphasis is on infantile diarrhoea caused by *Escherichia coli (E.coli). E. coli* is a gram-negative bacillus that may be found in the normal intestinal flora of humans and animals but can also be an important cause of enteric illness(5,11). Many serotypes of *E. coli* have been incriminated in infantile diarrhoea in many places.

E. coli serotypes are typed according to the somatic, flagella and capsular antigen groups 0, H and K respectively, e.g. *E. coli,* 0111, K58, H12. *E. coli* produces diarrhoea using four main mechanisms: (i) through production of toxin by enteropathogenic *E. coli* (EPEC); (ii) through invasion by enteroinvasive *E. coli* (EIEC); (iii) through adhering and effacing of the membrane of the intestinal microvilli by enteropathogenic *E. coli* (EPEC); and (iv) through the production of verotoxin by verotoxin *E. coli* (VTEC)(11,12).

E. coli was first considered enteropathogenic in 1945, when Bray, demonstrated that an antigenically distinct strain of *E. coli* was responsible for an outbreak of infantile diarrhoea(13). These strains were later named enteropathogenic *E. coli* (EPEC) by Neter *et al.* in 1955(14). The criteria for accepting such enteropathogenicity include the consistent isolation of a particular strain in an epidemic as the predominant coliform in the stools of affected infants and its relative rarity in the general community(14).

The *E. coli* serotypes known as enteropathogenic *Escherichia coli* (EPEC) are those pathogenic *E. coli* strains which do not produce heat-labile (LT) and/or heat stable (ST) toxins and are not invasive(5,12). Studies have reported as many as 170 serogroups as the causal agents of infantile diarrhoea in many parts of the world(4-12).

In Ghana, a number of papers have been published on the subject(15-18). In the Ashanti region, however, the role of EPEC and parasitic agents in diarrhoea in especially the 0-5 year group has not been adequately explored. Furthermore, the current status of the relatively new diarrhoeal agent *Cryptosporidium,* earlier reported in children(16) still needs to be evaluated. The objective of this study was to determine the relative frequency and serotypes of diarrhoeagenic *E. coli* and intestinal parasites in diarrhoeal infants (0-5 years).

MATERIALS AND METHODS

The study was undertaken in 1995. The study group included all diarrhoeal infants aged between 0 and 5 years who were on admission at the Mother-Child Clinic and the Komfo Anokye Teaching Hospital (KA.T.H.), both in Kumasi from March to June 1995. There were 162 children. Controls were selected from among healthy infants attending the out-patient department (OPD) of the two selected health facilities for minor skin ailments or upper respiratory tract infections and who were not obviously with diarrhoea. They were matched for age and gender. The selection was at random, using one-in eight systematic sampling, the first 122 who qualified and agreed to participate were included in the study. Data were compiled from interviews of mothers, using questionnaire, clinical notes, and laboratory results on faecal specimens from study subjects and controls.

Faecal specimen collections from study cases were done on the morning of the second day after admission. Control specimens were collected in the mornings at the O.P.D. Specimens were placed in a cold box and taken within two hours to the laboratory of the Department of Clinical Microbiology, KNUST, Kumasi.

Stool samples were collected into clean monowax containers and transferred on Cary-Blair medium and transported in an icebox to the laboratory. All samples were plated directly on MacConkey Agar, the primary plate, and incubated at 37°C overnight.

Stool specimens were processed to detect *E. coli* using standard microbiological methods(19). The *E. coli* isolated were typed by serology using a slide agglutination method to identify EPEC strains. Positive agglutinations were repeated. The study used EPEC antisera (Denka Seiken Co. Ltd, Tokyo, Japan) which was supplied by Noguchi Memorial Institute of Medical Research, Accra.

For parasitological diagnosis, stool specimens were examined visually for consistency, colour, mucus, blood, and parasites. Formed faeces from controls were first suspended in a tube containing lml sterile saline. All specimens were examined microscopically for parasites, leukocytes and blood cells, mucus and fats cell.

RESULTS

The study involved 162 infants aged 0-5 years with 122 controls making 284 in total (147 females and 137 males) (Table 1). The youngest was three months and the eldest was five years. The mean age of the diarrhoeal cases was 17.1 months with a standard deviation (S.D.) of 13.9. No difference was noticed between the genders.

The results of microscopic examination of stools collected from 162 diarrhoeal infants are set out in Table 2. No enteric pathogen was seen in 96(59.2%) and 114(93.4%) of the diarrhoeal and control stools, respectively. From the study group, 66(40.7%) pathogens were isolated as against eight (6.6%) from the control.

Table 1

Age and gender distribution in relation to diarrhoel and non-diarrhoel cases

Age group (Months)	No. of Females	Diarrhoel cases (n=612) Males	Females	Non-Diarrhoel cases (n=122) Males	Females	Males
0-11	60	68	34	39	26	29
12-23	42	36	21	29	21	7
24-35	24	23	13	8	11	15
36-47	10	3	8	0	2	3
48-60	11	7	7	3	4	4
Total	147	137	83	79	64	58

Mean age for diarrhoel cases= 17.1 months F:M 1.1:1.0, F:M 1.1:1. SD ±13.9

Table 2

Frequency of Enteropathogenic E. Coli and other parasites seen in 162 diarrhoeal and 122 non-diarrhoel stools

Pathogens in faecal specimens	Patients (n=162) No.	%	Controls (n=122) No.	%	(X^2)	(P)	Blood	Pus	Mucus	Mucus + Pus
Enteropathogenic *E. Coli*	24	14.8	5	4.1	8.72	<0.001	0	1	3	0
Ascaris lumbricoides	18	11.1	2	1.6	2.89	>0.95	0	0	0	0
Cryptosporidium	13	8.0	1	0.8	6.61	>0.10	0	0	3	0
Giardia lamblia	6	3.7	0	0	0.006	>0.95	0	0	0	0
Trichomonas hominis	3	1.9	0	0	2.28	>0.10	0	0	0	0
Trichuris trichiura	1	0.6	0	0	0.76	>0.10	0	0	0	0
Hookworm	1	0.6	0	0	0.76	>0.10	0	0	0	0
No enteropathogen observed	96	59.3	114	93.4	42.21	>0.10	3	16	0	1
Total	162	100	122	100	64.24		3	17	6	1

Table 3

EPEC serotypes isolated from a total of 224 diarrhoeal and non-diarrhoeal children in Kumasi, Ghana

EPEC O Serotype	Patients No. isolated	(%)	Controls No. isolated	(%)
0125	6	25		
0119	5	21		
0111	3	13		
026	3	13	2	40
0168	2	8		
086a	2	8	3	60
0169	1	4		
055	1	4		
015	1	4		
Total	9	24	5	100

Table 4

Prevalence of Enteropathogenic E. Coli in diarrhoeal and non-diarrhoeal children by age in Kumasi (Females in parenthesis)

Age (months)	Children with diarrhoea Total No.	No. with EPEC	%	Children without diarrhoea Total No.	No. with EPEC	%
0-6	30 (10)	10 (4)	41.7	32	0	0
7-12	45 (18)	6 (3)	25.0	25	3	60
13-24	34 (21)	4 (3)	16.6	36	1	20
25-36	21 (15)	2 (0)	8.3	16	0	0
37-48	15 (8)	1 (1)	4.2	6	1	20
49-60	17 (11)	1 (0)	4.2	5	0	0
Total	162	24	100	120	5	100

Mean age for diarrhoel infants 13.29 months, Standard deviation ± 5.28. Two of controls not sure of age, ommited from analysis

EPEC was the most frequent enteric pathogen, accounting for 24 (14.8%) of the pathogens isolated from diarrhoeal stools, whilst five (4.1%) isolates were from the control stools. (Table 4). In all, nine different EPEC serotypes were isolated from the study and control groups (Table 3). The most isolated serotype was *E. coli* 0119 (5/24-21.0%). EPEC serotypes *E. coli* 086a and 026 were detected in both diarrhoeal and non-diarrhoeal stools.

At 18 (11.1%), *Ascaris lumbricoides* was the second most frequent isolated non-bacterial enteric pathogen. *Cryptosporidium* was found in 13(8.0%) diarrhoeal stools while one (0.8%) was found amongst the control stools. *Giardia lamblia* was found in six (3.7%) of diarrhoeal stools, and none amongst controls. Three or 1.9% diarrhoeas were due to *Trichomonas hominis* while one diarrhoeal case was due to two aetiologic agents; *Trichuris trichiura* and hookworm (Table 2).

DISCUSSION

In this study, we investigated the relative frequency and prevalence of EPEC and intestinal parasites in hospitalised diarrhoeal children between the ages of 0-5 years. Our findings (Table l) show that 144 (88.9%) of the 162 in the diarrhoeal cases, involved the 0-3 year age group, as has been reported earlier(20,21).

The overall isolation rate of bacteria and parasites in this study is 40.1% which is higher than that reported from two regions in Ghana (15-17), but still lower than that reported from Nigeria (22). The reason for the higher isolation may be due to the study centre being a referral centre, which may selectively have received more ill patients.

From the results, in 59.3% of the diarrhoeal cases, no agent could be isolated; this may be due to the fact that the study did not investigate all possible agents. In our study, nine EPEC 0 serogroups were detected in 14.8% of cases (Table 3), as against 2.2% of Anteson *et al.* in Accra, Ghana, 10.7% of Rotimi *et al*(22), 59% of 83 *E. coli* of Akinyemi *et al*(23) both of Lagos, Nigeria and 13% of Joyce *et al.* in Kenya(24). The nine serogroups isolated were those commonly detected elsewhere(4,16,17,21-24). This finding supports the strong association between infantile diarrhoea and EPEC(4).

The second most significant finding was *Ascaris,* also reported elsewhere (16). *Cryptosporidium* was the third commonest agent. A prevalence rate of 80%(13) was seen in the study group, and one case in the non-diarrhoeal group. This is comparable to earlier study from Ghana(18). The high rate further supports Cry*ptosporidium* as an important diarrhoeagenic agent.

Some of the parasites found in this study namely: *Giardia lamblia, Trichuris trichiura, Ascaris lumbricoides* and hookworm are all known, to cause diarrhoea(16). The high incidence rate of faecally transmitted parasitic and bacterial agents, in this study suggests that more attention must be given to sanitation and personal hygiene to help improve the health of children. Again, it is known that diarrhoea is associated with consumption of contaminated food and non-potable water(1,2), hence much education must be given to mothers at the ante and post natal clinics on food preparation.

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