

## ORIGINAL ARTICLES:

## OBSERVATIONS ON PARASITES AND BACTERIA IN STOOLS OF CHILDREN WITH DIARRHOEAL DISEASE

By

\*Reginald K. Anteson, Korebumi Minakami, Patience P. A. Mensah

*Department of Microbiology, University of Ghana Medical School, Korle-Bu, Accra*

And

Maxwell A. Appawu

*Noguchi Memorial Institute for Medical Research, University of Ghana, Legon.***Summary**

*Studies involving the isolation of parasitic and bacterial agents in faecal samples of 363 children (age 3 months-15 years) with diarrhoeal disease in Accra revealed a 39.4% incidence rate of both parasites and bacteria. Helminthic and protozoan parasites accounted for 33.1% of the isolations.*

*All the bacteria isolated, are known causative diarrhoeal agents in children in Accra. Two enteral protozoa and 3 helminths known to cause diarrhoea in humans were found in this study and these were encountered alone or in various combinations in about 82.5% of the positive stools.*

*Concurrent isolation of parasitic and bacterial diarrhoeal agents was made in 5.6% of the positive stools.*

*It is suggested that helminths and or protozoa may play a major role in the causation of diarrhoea in children in Accra.*

Keys Words: Diarrhoea, Polyparasitic infections, *Ascaris lumbricoides*, *Trichuris trichiura*, *Strongyloides stercoralis*

**Introduction**

A number of research papers on the aetiology of diarrhoea in children in Ghana has been published in recent years<sup>1-7</sup>. In almost all the papers, the emphasis was on the isolation of bacteria and/or viruses as the causative agents of the disease. The relative role played by parasitic agents in diarrhoeal disease in the country has not been adequately explored, even though both protozoan and helminthic related diarrhoeas are known to be a serious disease condition especially in children<sup>8</sup>. The unfortunate outcome of the virtual neglect of studies on diarrhoeas of parasitic origin in the country is that, it has become almost impossible (in the absence of

the relevant data) to evaluate the problem of diarrhoea in terms of the known pathogenic bacteria, viruses and parasites in the production of the disease state in cases where these agents are recovered from the same faecal specimen.

In the present investigation bacteriological and parasitological studies were carried out on young children with diarrhoea. Additional independent virus studies were performed on duplicate specimens taken from the same children. These studies will be published separately. The present communication presents cases of single and multiple infections with known pathogenic enteral parasites and bacteria in the children investigated.

**Materials and Methods**

A total of 363 stool samples from children with acute diarrhoeal disease were collected during January-October, 1981. The study group included 193 in-patients from the Princess Marie Louise (P.M.L.) Hospital, and 170 out-patient cases from the Ussher Clinic, both in Accra. In all, 168 females and 195 males with age range from 3 months to 15 years were investigated.

The diarrhoeal stools were soft or watery and contained blood and/or mucus.

**Parasitological methods**

All stool specimens obtained were examined fresh for ova and protozoa by the direct smear method and the formol-ether concentration method according to Ridley and Hawgood<sup>9</sup>. For the specific identification of vegetative protozoan forms, we used fresh preparations and smears stained by the direct MIF method of Saper and Lawless<sup>10</sup>.

**Bacteriological methods**

Fresh stool samples were inoculated onto differential selective media (MacConkey agar and Sal-

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\*To whom request for reprints should be sent.

monella/Shigella agar) and into enrichment medium (Salenite F broth) and incubated for 48 hours at 37°C. Isolation and primary identification were carried out according to Edward and Ewing<sup>11</sup>. Confirmatory tests were performed serologically with type specific antisera.

Colonies on the enrichment broth were subcultured onto Salmonella/Shigella agar and treated as above after overnight incubation.

Typical lactose fermenting colonies were examined using the relevant biochemical reactions and specific antisera for enteropathogenic *Escherichia coli*.

Enterotoxin production was not studied.

### Results

Parasitic agents of possible aetiological significance were found in the faecal samples of 33.1% (120) of the 363 children in this study. These findings are shown in Table 1. *Ascaris lumbricoides* alone was recovered from 19.5% (71) of the stools. *Trichuris trichiura* was found in 3.3% (12) and *Strongyloides stercoralis* accounted for 2.3% (8) of all the cases studied. Hookworm accounted for 1.1% (4) of the parasites in these stools. Among the known protozoan disease agents *Entamoeba histolytica* and *Giardia intestinalis* appeared in low percentages, 0.3% (1) and 0.9% (3) respectively.

Polyparasitic infections encountered in this study from both Ussher Clinic and the Princess Marie Louise Hospital, Accra are shown in Tables 2 and 3 respectively. Again, it can be seen that *Ascaris lumbricoides* appears more often in the various combinations. There was a case of triple infection with hookworm, *Ascaris* and *Strongyloides* from a girl of 13 years and another case of a quadruple infection, hookworm, *Ascaris*, *Strongyloides* and *Trichomonas* in a five year old girl.

As shown in Table 4, bacteria generally accepted as enteric pathogens were isolated

TABLE 1: Parasitic agents recovered from diarrhoea patients investigated.

Parasitic agent	Ussher Clinic		P.M.L.	
	Positives No	%	Positives No	%
<i>Ascaris lumbricoides</i>	43	11.8	28	7.7
Hookworm	3	0.8	1	0.3
<i>Strongyloides stercoralis</i>	2	0.6	6	1.7
<i>Trichuris trichiura</i>	8	2.2	4	1.1
<i>Hymenolepis nana</i>			2	0.6
<i>Entamoeba histolytica</i>	1	0.3		
<i>Giardia intestinalis</i>	2	0.6	1	0.3
<i>Entamoeba coli</i>			2	0.6
<i>Iodamoeba butschilii</i>			1	0.3
<i>Trichomonas hominis</i>	11	3.0	5	1.4
Total	70	19.3	50	13.8

TABLE 2: Multiple parasitic infections in individual children with diarrhoea showing their ages and sex. P.M.L. cases.

Patients		Parasite species
Age (Yrs)	Sex	
2.6	M	<i>Trichomonas hominis</i> , <i>Ascaris lumbricoides</i>
2.5	M	<i>Ascaris</i> , <i>Strongyloides stercoralis</i>
14	M	<i>Ascaris</i> , <i>Trichuris trichiura</i> , <i>Entamoeba coli</i>
5	F	<i>Ascaris</i> , <i>Trichuris</i>
1.25	F	<i>Trichomonas</i> , <i>Ascaris</i>
1	M	<i>Ascaris</i> , <i>Trichuris</i>

TABLE 3: Multiple parasitic infections in individual children with diarrhoea showing their ages and sex. Ussher Clinic cases.

Patients		Parasite species
Age (Yrs.)	Sex	
3.5	F	<i>Ascaris lumbricoides</i> , <i>Trichuris trichiura</i>
11	M	<i>Ascaris lumbricoides</i> , <i>Trichuris trichiura</i>
13	F	Hookworm, <i>Ascaris</i> , <i>Strongyloides stercoralis</i>
8	F	<i>Trichomonas hominis</i> , <i>Trichuris trichiura</i>
1.3	M	<i>Trichomonas hominis</i> , <i>Ascaris lumbricoides</i> , <i>Trichomonas</i>
5	F	Hookworm, <i>Ascaris</i> , <i>Strongyloides stercoralis</i> , <i>Trichomonas</i>
3	F	<i>Trichuris</i> , <i>Trichomonas</i>
9	M	<i>Ascaris</i> , <i>Trichomonas</i>
13	M	<i>Ascaris</i> , <i>Trichuris</i>
7	F	<i>Ascaris</i> , <i>Entamoeba histolytica</i> (trophozoites)

TABLE 4: Bacterial agents isolated from Diarrhoea patients investigated.

Bacterial agent	Ussher Clinic		P.M.L.	
	Positives No.	%	Positives No.	%
<i>Salmonella typhi</i>	3	0.8	1	0.3
<i>Salmonella</i> Gp A	1	0.3		
Gp B	2	0.6		
Gp C			1	0.3
Gp E4	1	0.3		
Enteropathogenic				
<i>Escherichia coli</i>				
0125 K70	2	0.6		
0111 K58	1	0.3	1	0.3
055 K59			1	0.3
026 K60	1	0.3		
086a K61	1	0.3		
028 K73	1	0.3		
<i>Shigella flexneri</i>	3	0.8	3	0.8
Total	16	4.4	7	1.9

TABLE 5: Concurrence of parasitic and bacterial agents in diarrhoeal patients.

Bacteria	Patients		Parasites
	Age	Sex	
<i>Salmonella typhi</i>	8	F	<i>Ascaris</i>
Gp A	11	M	<i>Ascaris</i>
Gp B	2	F	<i>Ascaris</i>
Gp E4	1	F	<i>Ascaris</i>
<i>Shigella flexneri</i>	15	F	<i>G. lamblia</i>
<i>E. coli</i>			
0125 K70	1.5	F	<i>Ascaris</i>
0111 K58	1.5	M	<i>Ascaris</i>
026 K60	3.5	F	<i>Ascaris</i>

from 23 or 6.3% of the diarrhoeal cases. *Salmonella* was isolated from 2.5% (9), and enteropathogenic *Esch. coli* was found in 2.2% (8) of the stool samples. *Shigella flexneri* also accounted for 1.7% (6) of the positive faecal specimens. Among the *Salmonella* isolations, *S. typhi* accounted for 1.1% (4) and a variety of serotypes was found in 1.4% (5). Six serotypes of enteropathogenic *Esch. coli* were also recovered but no single type in great preponderance. There were no dual bacterial infections.

The concurrence of bacterial and parasitic agents in the same diarrhoeal stool is shown in Table 5. All the three enteropathogenic bacteria isolated in this study were represented. However, only *Ascaris* and *Giardia*, among the parasites encountered in this study, were found in association with the bacteria. It is worth noting that, once again, *Ascaris* was the most common parasite in these combinations.

### Discussion

The present investigation is probably the first attempt at correlating the roles played by enteropathogenic bacteria and parasitic agents in acute diarrhoeal disease in children in Accra. The data here presented show that bacteria such as *Salmonella*, *Shigella* and *Esch. coli*, whose casual role in infantile enteritis has already been established in the country<sup>2-4, 7, 12</sup> were isolated from the stools examined.

The isolation rate of these recognised pathogens in the present work was however lower than in previous studies in Accra. The reason for this difference may be that fewer subjects were investigated in the present study.

Some of the parasites found in this study: *Entamoeba histolytica*, *Giardia*, *Trichuris*, *Ascaris* and hookworm are known on their

own to cause diarrhoea<sup>8</sup>. These intestinal parasites were found either alone or in various combinations in about 82.5% of the positive stools. One may be tempted to speculate, therefore, that these parasites played an important, if not a major role in the causation of diarrhoea in the patients from whom they were recovered. Furthermore, a comparison of the incidence rates of the known diarrhoea-causing bacteria and parasites isolated in the study population may lead to the conclusion that parasites may be more important than bacteria in the aetiology of infantile diarrhoea when both forms occur in the same population. Until subsequent studies show otherwise such a possibility should be borne in mind. Again, the high incidence rate of the faecally transmitted parasitic and bacterial agents in this study suggest unsatisfactory environmental conditions in the study area. This is further confirmed by our data which show that polyparasitism accounted for about 13% of the positive cases of parasitic infections in the study.

Helminthic or protozoan infections were observed in concurrence with bacteria in 5.6% of the total positive faecal samples investigated. It has been observed earlier in this paper that parasitic infections may be more important in the production of diarrhoea in children in Accra. Guardiola-Rotger *et al*<sup>13</sup> have also suggested that some type of synergism could exist between special groups of parasites and bacteria which are isolated in the same diarrhoeal patient. Indeed, it has been observed<sup>8</sup> that when amoebic infection and shigellosis co-exist, either infection may aggravate the other to produce a severe condition with copious diarrhoea. Such a combination was, however not encountered in this study and the clinical observation recorded on the patients investigated did not suggest an unusually severe diarrhoeal condition in any of the subjects with mixed parasitic and bacterial infections.

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