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Enteropathogenic *Escherichia coli* Among Children*

by

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INTRODUCTION

Extensive investigations into the incidence of salmonellosis in Ceylon have been carried out by Schmid and Velaudapillai (1951) and de Silva, Schmid and Soysa (1953). Falisevac, Padley and Gulsekharam (1959) have reviewed all the work done in this field in Ceylon up to 1959. Gulasekharam and Velaudapillai (1961) carried out a survey in a rural area and reported the incidence of salmonellosis and shigellosis in children who did not have symptoms of these diseases.

Although reference to the isolation of enteropathogenic *Escherichia coli* is made in the Administration Report of the Director of Health Services for 1952 (Report, 1953), no intensive work was done to determine the role of this group of organisms in causing diarrhoea among children. This paper describes the incidence of enteropathogenic *E.coli* among children under two years with and without diarrhoea and is based on investigations carried out from June, 1964 to January, 1965.

MATERIALS AND METHODS

Rectal swabs were taken from children under two years who attended the Out Patient Department of the Lady Ridgeway Hospital for Children, Colombo. Most of the children came from the Colombo Municipality and the rest were from the suburbs. The parents of these children generally belonged to the poorer class, but some came from the middle class.

The swabs were plated, within two hours of collection, on MacConkey, S. S. and Wilson and Blair's agar media. The swabs were subsequently dipped into tetrathionate and selenite broths. Examination for the evidence of salmonellas and shigellas were done by means of standard methods (Velaudapillai, Nithiananda and Meedeniya, 1963). For

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detecting *E.coli*, direct slide agglutination was done with pooled antisera against the known 14 pathogenic strains from colonies appearing on MacConkey agar. Positive colonies were tested against individual sera. Colonies giving positive agglutination were picked into Kligler's medium. Tube agglutination tests were done on the following day to confirm the slide agglutination results.

The flagellar antigens of *E.coli* strains were determined by serially passing them five times through Craigie's tubes, and thereafter they were tested against H antisera.

RESULTS

Table 1 reveals the incidence of salmonellas, shigellas and pathogenic *E.coli* according to age groups.

TABLE 1

Distribution of Cases with Organisms Isolated According to Age Groups

Age in months	0—3	4—7	8—11	12—15	16—24	TOTAL
			Diarrhoea Cases			
Total No. of Cases	23	64	65	71	92	315
Salmonella & %	1(4.3)	6(9.4)	2(3.1)	5(7.0)	2(3.2)	16(5.1)
Shigella & %	—	3(4.7)	1(1.5)	5(7.0)	5(5.4)	14(4.4)
Pathogenic *E.coli* & %	3(13.0)	3(4.7)	5(7.7)	5(7.0)	2(2.2)	18(5.7)
Total Positives & %	4(17.3)	12(18.8)	8(12.3)	15(21.0)	9(10.8)	48(15.2)
			Non Diarrhoea Cases			
Total No. of cases	25	59	87	97	133	401
Salmonella & %	—	—	4(8.7)	2(2.1)	2(1.5)	8(2.0)
Shigella & %	1(4.0)	—	1(1.1)	2(2.1)	5(3.8)	9(2.2)
Pathogenic *E.coli* & %	2(8.0)	5(8.5)	2(2.3)	4(3.1)	5(3.8)	18(4.5)
Total Positives & %	3(12.0)	5(8.5)	7(12.1)	8(7.3)	12(9.1)	35(8.7)

Percentages are given in brackets.

Salmonellas and shigellas were found among children from 4—7 months and onwards. *E.coli* were found among the 0—3 months age group also. Enteropathogenic *E.coli* were present to a lower extent among 16—24 months age group. In the diarrhoea group 5.7% had pathogenic *E.coli* while the corresponding percentage for non-diarrhoea group was 4.5.

Table 2 lists all the individual organisms that were isolated.

TABLE 2

Individual Organisms Isolated

Name of Organism	Diarrhoea Group Frequency of Isolation	Non Diarrhoea Group Frequency of Isolation	TOTAL
Salm. stanley	12	6	18
Salm. saint-paul	—	1	1
Salm. bareilly	4	1	5
Shig. flexneri 2	9	2	11
Shig. flexneri 3	1	2	3
Shig. sonnei	4	5	9
E.coli. 026:K60	1	1	2
E.coli. 055:K59	3	1	4
E.coli. 086:K61	—	1	1
E.coli. 0111:K58	2	1	3
E.coli. 0112:K66	1	—	1
E.coli. 0114:K90	2	2	4
E.coli. 0119:K69	5	1	6
E.coli. 0125:K70	2	3	5
E.coli. 0126:K71	1	2	3
E.coli. 0127:K63	1	—	1
E.coli. 0128:K67	3	3	6
E.coli. 0142:K86	2	4	6

Three different salmonellas were isolated 16 times from diarrhoea cases and 8 times from non-diarrhoea cases. Three different shigellas were isolated 14 times from diarrhoea and 9 times from non-diarrhoea cases. Of the known 14 serotypes of pathogenic *E.coli*, 12 types were detected in this study. An almost equal number of isolations were made from diarrhoea and non-diarrhoea patients. *E.coli O 119:K69* was encountered more often in diarrhoea cases than the other strains of *E.coli*.

The flagellar antigens of 12 strains of *E.coli* from diarrhoea and 8 from non-diarrhoea cases are tabulated in Table 3.

TABLE 3

Pathogenic *E.coli* with H Antigens

Serial No.	Register No.	Antigenic Formula	H Antigens Associated with Diarrhoea (Ewing, 1963)
		Isolated from Diarrhoea Cases	
1	3643	055:K59:H2	Non motile (β), 6 or 7
2	3804	,,	,,
3	3881	,,	,,
4	3846	0111:K58:H18	Non motile (α), 2, 4, 12 or 21
5	3879	,,	,,
6	2420	0114:K90:H2	

7	2289	0119:K69:H6	Non motile or 6
8	2514	,,	,,
9	2037	0128:K67:H12	Non motile, 2, 7, 8, 9 or 12
10	2291	,,	,,
11	6791	0128:K67:H18	,,
12	3454	0142:K86:H—	6

Isolated from Non Diarrhoea Cases

1	3246	055:K59:H7	Non motile (β), 6 or 7
2	3302	0111:K58:H2	Non motile (α), 2, 4, 12 or 21
3	2757	0119:K69:H6	6
4	2452	0125:K70:H21	15, 19 or 21
5	1845	0128:H67:H12	Non motile, 2, 7, 8, 9 or 12
6	3041	0142:K86:H38	6
7	3294	0142:K86:H?	6
8	3257	0142:K86:H?	6

The first 6 and last 2 strains from the diarrhoea group possess antigens which have not been reported from America (Ewing, 1963). The flagellar antigens of the other 4 strains have been found in diarrhoea cases. Of the 8 strains picked up from non-diarrhoea cases, only the first 5 possess H antigens associated with diarrhoea.

With a view to determining how long the pathogenic strains of *E.coli* were excreted from the time of first isolation, reswabbings were done in some of the diarrhoea cases and the results are shown in Table 4.

TABLE 4

Time taken to Clear *E.coli* Infection

Serial No.	Register No.	Organism	Date when swab was first positive	Date when swab became first negative	Time taken to become negative
1	774	0112:K66	3.3.65	29.3.65	26 days
2	822	0128:K67	5.3.65	1.4.65	27 days
3	1047	0142:K86	18.3.65	—	Positive on 7.4.65 (20 days)
4	1086	0111:K58	19·3.65	31.3.65	12 days
5	2037	0128:K67	27·4.65	—	*E.coli 0125*:K70 on 25.5.65 (28 days)
6	2072	055:K59	28.4.65	26.5.65	28 days
7	2215	0126:K71	8.5.65	25.5.65	17 days
8	2216	0125:K70	8.5.65	25.5.65	17 days

The 'carrier state' investigation was done after the main survey was completed.

One case became negative within two weeks from the time the organisms were first isolated, two within three weeks and three in four weeks.

DISCUSSION

Although the title of the paper is enteropathogenic *E.coli*, salmonellas and shigellas are also listed in the different tables. They were present in the rectal swabs and were isolated by the routine methods used. Lubin, Girola and Grinstein (1963) found in their study in Argentina that the maximum rate of infection with these organisms occurred between the ages of 12—15 months. The same conclusion is arrived at in our study. However, the younger (4—7 months and 8—11 months) age groups too appear to have nearly this rate of infection. Ceylon is a tropical country and the children are commonly put to sleep on mats on the ground. People walk in and out of the houses with bare feet and thereby pass on the infection to the infants even before the latter begin to walk. As regards *E.coli*, it was found even among the 0—3 months age group. One child two-weeks old who had diarrhoea had *E.coli O 119:K69* as the causative organism. Enteropathogenic *E.coli* is not so prevalent among the 16—24 months group with diarrhoea.

The number of cases studied and the number of isolations of *E.coli* are not sufficient to draw any justifiable conclusions. We are not in a position to explain why in the non-diarrhoeal cases, the age groups 0—3 months and 4—7 months have about 8% positive rate for *E.coli* and thereafter the rate is about 3%. They might be symptomless carriers or they may be in the incubation period. Velaudapillai and Sabanathan (1966) found that in the case with infection with *E.coli O 119:K69*, the organism appeared in the faeces first and diarrhoea developed later. Diarrhoea due to enteropathogenic *E.coli* is not always severe and mothers might not take any notice if the children have sight diarrhoea.

Ramos-Alvaresz and Olarte (1964) reported from Mexico that pathogenic *E.coli* were found in 6% of the diarrhoea cases and in 7% of non-diarrhoeal cases. In our series enteropathogenic *E.coli* were isolated from 5.7% of the diarrhoea cases and from 4.5% of non-diarrhoea cases. *E.coli O 119:K69* was most frequently isolated from diarrhoea children. During the latter part of 1963, this organism caused an outbreak of diarrhoea at the Premature Baby Unit, Castle Street Maternity Hospital, Colombo, (Velaudapillai and Sabanathan, 1966).

An investigation was carried out in 1964 at the shanty part of Peliyagoda. Rectal swabs from 78 children under two years and without diarrhoea were examined. None of them was positive for pathogenic *E.coli*. Faecal droppings from 196 animals reared by the people living there were also examined. *E.coli O 112:K66* was isolated from the droppings of a cockbird and *E.coli O 128:K67* from a cockbird and a goat. Although these organisms were present in the environment, none of the children harboured these organisms. From the negative findings of the study at Peliyagoda and the higher rates of positives among non-diarrhoeal cases there is a strong suggestion that most of the strains of pathogenic *E.coli* must have caused diarrhoea earlier or might have done so in future in those children who had no diarrhoea at the time of isolation.

Ewing (1963) found that certain H antigens of *E.coli* were associated with diarrhoea. It was thought that identifying the flagellar antigens might be of value in distinguishing the strains that caused diarrhoea from those that did not. Of the 8 strains that were isolated from non-diarrhoeal cases, 3 had flagellar antigens not met with in such cases, and the H antigens from 8 out of 12 strains from children with diarrhoea had different antigens from those reported from America. Identification of H antigens is a time consuming task as the organisms must be first made actively motile, which might take about two weeks. By that time the children might be discharged from the hospitals. The identification of the flagellar antigens appears to be of not much practical value. Therefore, determining the O and K antigens of enteropathogenic *E.coli* will be sufficient from the bacteriological point of view.

Reswabbings were done from 8 cases of diarrhoea, 6 of them became bacteriologically negative. The period ranged from 12—28 days. One was positive on the 28th day with an organism different from the strain that was isolated first. At this time the child had diarrhoea, probably it must have got another infection. The second was positive on the 20th day. Further follow up studies could not be made as they were not brought to the clinic subsequently. However, broadly speaking the organisms in most diarrhoea cases disappeared within a period of one month from the time of first isolation.

SUMMARY

From the children attending the Out Patient Department of Lady Ridgeway Hospital, Colombo, twelve different serotypes of enteropathogenic *E.coli* were isolated. *E.coli O 119:K69* is the most prevalent organism causing diarrhoea in children.

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