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Comparison of the Organisms Causing Diarrhoea in a Children's Hospital and in Estates*

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INTRODUCTION

Schmid and Velaudapillai (1951), Schmid (1955) and Velaudapillai, Mendis and Niles (1966) have reported the incidence of salmonellas, shigellas and enteropathogenic *Escherichia coli* in Ceylon. The cases that were studied were from patients who underwent treatment at the Government hospitals. Before they sought treatment at the hospitals, they usually obtained treatment from private practitioners. It was not always possible to isolate the organisms that caused diarrhoea as a certain proportion of the infecting organisms would have been killed by the antibiotics or sulpha drugs that were administered before admission to the hospitals.

In order to get an accurate picture of the incidence of salmonellas, shigellas and pathogenic *E.coli* that were responsible for causing diarrhoeal diseases, a population which does not usually seek treatment at Government hospitals had to be selected. The employees on estates belong to this category. Therefore, this investigation of the diarrhoeal diseases was done on the estate population from 10th September, 1965 to 31st August, 1966.

The labourers in each estate live as a separate unit. Matters concerning health are looked after by estate medical staff. When labourers fall ill, they seek treatment at the estate medical institutions.

Through the efforts of the Medical Officer, Planters' Association Health Scheme, 32 large estates were selected for this study. The average extent of each estate is about 1000 acres. With the exception of one which is in the Southern Province, these estates are situated in the Central, Sabaragamuwa and Uva provinces. The principal crop is tea. In 7 estates small extents are planted with rubber. The altitude ranged from 2000 feet to 5000 feet. In the tea estates, more than 90 % of the labour force consisted of Indians and the rest Sinhalese. They were provided with residential facilities inside the estates. In the rubber plantation sections more Sinhalese than Indians were employed. Rubber is grown in the estate in the Southern province. Sinhalese made up to 3/4 of the labour force. The average altitude of this estate is about 500 feet above sea level.

The investigation on the children was made from one ward of Lady Ridgeway Hospital, Colombo from 26th August, 1965 to 23rd April, 1966.

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MATERIALS AND METHODS

In all cases faecal specimens prior to the administration of drugs were collected in Stuart's transport medium. Instructions regarding collection of specimens were given to the hospital and estate medical staff. Specimens from hospital were delivered by hand and those from the estates were sent by post. They were cultured by methods described by Velaudapillai *et al.* (1966). The specimens were not examined for evidence of amoebae, cysts and ova. Microbic sensitivity tests were done with discs obtained from Messrs Difco Laboratories, U.S.A. The strength of the antibiotics was 30 micrograms (mcg) for streptomycin, chloramphenicol, tetracycline, oxytetracycline, neomycin, paramomycin, nalidixic acid. Sulphadiazine was used at a strength of 300 mcg. It was incorporated in a medium with 5 % laked blood.

Nutrient agar plates were flooded with 18—24 hr. lemco broth culture of test organism, excess fluid was pipetted off. When the plates were dry, discs were overlaid and incubated overnight. Organisms were arbitarily considered sensitive to an antibacterial drug if there was an inhibition zone of 3 mm or more.

RESULTS AND DISCUSSION

Table 1 gives the incidence of salmonellas, shigellas and enteropathogenic *E.coli* among the children at the hospital and among the estate populations. The figures for the estates are split into two columns, one for the children up to 9 years and the other for the adults. Wherever possible, in the subsequent tables, presentation will be made in this pattern.

TABLE 1

Incidence of Salmonellas, Shigellas and Enteropathogenic *E.coli*.

	Hospital		*Estates*					
			Children		Adults		Total for estates	
	+	Total	+	Total	+	Total	+	Total
Salmonellas & %	209 (18.0)	1126	17 (4.9)	347	19 (4.1)	456	36 (4.4)	803
Shigellas & %	51 (4.5)	1126	49 (14.1)	347	83 (18.2)	456	132 (16.4)	803
Enteropathogenic *E.coli* & %	46 (5.4)	846	4 (1.6)	236			4 (1.6)	236

Percentages are given in brackets.

The total number of specimens from the hospitals was 1126, of which 846 were from children below the age of two. From the estates a total of 803 specimens was examined, which comprised 236 from children under the age of two, 111 from the 3—9 age group and 456 from adults.

At the children's hospital, the ratio of salmonella to shigella was 4:1, whereas for the estate children it was 1:3 and for adults 1:4. Broadly speaking the ratio is almost the reverse of the hospital. Schmid (1955) reported nearly the same ratio for salmonella and shigella for hospital children. The frequency of isolation of enteropathogenic *E.coli* in children at the hospital was more than on the estates. The infection rate for salmonellas among hospital children was 18.0%, the rate for estate children was 4.9%. Similar percentages for the shigellas were 4.5 for hospital and 14.1 for the estates. Among the adults at the estates, shigella rate was 18.2%. Shigella organisms appeared to play a major role in causing diarrhoea in the estates.

Table 2 analyses the infection rate according to age groups. Salmonellas, shigellas and *E. coli* are grouped together.

TABLE 2

Infection Rate According to Age Groups

Age Group	*Hospital*		*Estates*	
	+ & %	Total	+ & %	Total
0— 2	231 (27.3)	846	48 (20.3)	236
3— 9	72 (26.6)	271	22 (19.8)	111
10—19	3 (33.3)	9	29 (28.7)	101
20—29			39 (28.4)	137
30—39			17 (16.1)	105
40—49			8 (13.5)	59
50—59			4 (15.3)	26
60—69			4 (16.6)	24
70—79			—	2
80—89			1 (50)	2

Percentages are given in brackets.

The children at the hospital and on the estates had almost the same rate of infection irrespective of the organisms. Children of the 0—2 age group in addition to picking up salmonella and shigella infection, are also likely to be infected with enteropathogenic *E.coli* but the overall infection rate for 0—2 and 3—9 age groups did not show any significant difference. Among the estate population, the rate of infection rose up to 20—29 age group. For instance, it was 28.7 % for the 10—19 age group and 28.4 % for 20—29 group, thereafter the rate gradually dropped. The labourers of these age groups, being young would have mixed more freely with the others in the estate and with those in the neighbouring estates. Therefore, the risks of infection would have been greater for these age groups.

The salmonellas, shigellas and enteropathogenic *E. coli* that were isolated from the estates are classified according to age groups and sex in Table 3.

TABLE 3

Salmonellas, Shigellas and Enteropathogenic *E.coli* According to Age and Sex.

Age Groups	Males			Females		
	Salmonellas	*Shigellas*	*E.coli*	*Salmonellas*	*Shigellas*	*E.coli*
0— 2	6	18	3	5	15	1
3— 9	3	9	—	3	7	—
10—19	1	13	—	6	9	—
20—29	—	17	—	5	17	—
30—39	1	8	—	1	7	—
40—49	2	5	—	—	1	—
50—59	1	3	—	—	—	—
60—69	1	2	—	1	—	—
70—79	—	—	—	—	—	—
80—89	—	—	—	—	1	—
Total	15	75	3	21	57	1

Salmonellas affected males and females equally up to the age of 9. Salmonella infection was still detected in women among the 10—19 and 20—29 age groups, whereas they were hardly seen in males. The women, as mothers, might have been infected from their children. It is also possible that girls of the 10—19 age group, prior to getting married would have become infected by looking after sick children in their families. The shigella group of organisms had affected those of the 10—19 and 20—29 age groups to a greater extent than adults of other age groups. Men were affected with shigellas to a greater extent than women. The infection in men was seen up to 60—69 age groups, whereas it was rare in women after 30—39. A similar analysis of the children at the hospital was not done as the range of age groups was narrow.

Statistical analysis was not done separately for individual estates as the number of specimens received from each was not sufficiently large.

The individual pathogens isolated from the hospital and from the estates are listed in Table 4.

TABLE 4

Individual Pathogens

	Hospital	Estates		
		Children	*Adults*	*Total for estates*
Salm.paratyphi B ..	9	—	2	2
Salm.stanley ..	159	1	—	1
Salm.sandiego ..	1	1	—	1
Salm.saint paul ..	1	1	1	2
Salm.typhimurium ..	8	3	6	9
Salm.virchow ..	2	1	—	1

Salm.richmond	..	2	—	—	—
Salm.bareilly	..	16	1	—	1
Salm.newport	..	—	1	1	2
Salm.typhi	..	4	—	2	2
Salm.enteritidis	..	3	—	—	—
Salm.javiana	..	—	1	—	1
Salm.weltevreden	..	1	—	—	—
Salm.newbrunswick	..	1	—	—	—
Salm.hvittingfoss	..	—	—	1	1
Salm.saphra	..	—	—	1	1
Salm.urbana	..	1	—	—	—
Salm.waycross	..	1	7	5	12
Total (salmonellas)	..	209	17	19	36
Shig.dysenteriae 2	..	1	1	4	5
Shig.dysenteriae 10	..	1	—	—	—
Shig.flexneri 1	..	2	10	12	22
Shig.flexneri 2	..	26	30	48	78
Shig.flexneri 3	..	2	4	9	13
Shig.flexneri 4	..	4	—	2	2
Shig.flexneri 6	..	—	—	1	1
Shig.boydii 7	..	1	—	—	—
Shig.boydii 8	..	2	1	1	2
Shig.boydii 11	..	2	—	—	—
Shig.boydii 14	..	1	—	—	—
Shig.boydii 15	..	1	—	—	—
Shig.sonnei	..	7	3	6	9
Total (shigellas)	..	51	49	83	132
E.coli 055:*K*59	..	4	—	—	—
E.coli 086:*K*61	..	3	—	—	—
E.coli 0112:*K*66	..	3	—	—	—
E.coli 0114:*K*90	..	4	2	—	2
E.coli 0119:*K*69	..	2	—	—	—
E.coli 0125:*K*70	..	5	1	—	1
E.coli 0126:*K*71	..	4	—	—	—
E.coli 0142:*K*86	..	21	1	—	1
Total (*E.coli*)	..	46	4	—	4
Total Pathogens	..	306	70	102	172

Among the hospital children, 14 different serotypes of salmonellas were isolated. From the estates 9 serotypes were identified from the children and 8 from adults. *Salmonella stanley* was isolated 159 times, *Salm.bareilly* 16 times, *Salm.paratyphi B* 9 times and *Salm. typhimurium* 8 times from the hospital. In the estates salmonellas appeared sporadically. From the children's hospital 12 different shigellas were isolated but only 6 from children in the estates. The most predominating organism from both groups was *Shigella flexneri* 2. Forty six strains of enteropathogenic *E.coli* belonging to 8 different serotypes were obtained from children at the hospital whereas only 4 strains belonging to 3 different serotypes were isolated from the estate children.

One of the estates at which the survey was carried out is in the Southern province. Unlike the other estates the principal crop here is rubber. The total number of salmonellas isolated was 10 and shigellas 13. As in the other estates, the predominating organisms were shigellas, but the salmonellas were much more prevalent than on the tea estates. This estate

is close to the villages. The Sinhalese labourers usually mix freely with the villagers. Gulasekharam and Velaudapillai (1961) found the rural children to harbour more salmonellas than shigellas. Therefore, there is every possibility of the Sinhalese labourers transferring salmonella infection to the estate from the villages.

When the results of the examinations done on the estates were scrutinized it was found that on many occasions shigellas and salmonellas belonging to the same serotypes were isolated from two or more specimens examined at the same time from the same estates. There were 17 such incidents due to *Shig.flexneri* 2. three to *Shig.flexneri* 1, one of each caused by *Shig.dysenteriae* 2, *Shig.flexneri* 3 and *Shig.sonnei*. There were 4 outbreaks caused by salmonellas, two were due to *Salm.typhimurium* and two to *Salm.waycross*. *Salm.waycross* affected children. There were a total of 27 outbreaks. The incidents occurred right round the year. In March, there were 4 incidents and in June, 6. The sources of infection might have been common for each incident as the labourers live as a closed community. Epidemiologically it might be interesting to trace the sources of some of these outbreaks.

Out of 1126 cases studied at the children's hospital, 25 had mixed infections. All were below 2 years of age. There were 11 cases infected with two different salmonellas, 3 with salmonellas and shigellas and 9 with salmonellas and enteropathogenic *E.coli*. Two cases under one year had triple infections. From one, two salmonellas and *E.coli* were isolated and from the other a salmonella, shigella and *E.coli*. From the estates three cases with mixed infections were detected, but they were not from children. The infections were due to salmonellas and shigellas. This is probably due to the fact that the tea estate population being a closed community is less likely to receive infection from outside.

The microbic sensitivity of the organisms isolated at the children's hospital is shown in Tables 5, 6 and 7.

TABLE 5

Microbic Sensitivity (Salmonellas)

	Strepto-mycin		*Chloram-phenicol*		*Tetracy-cline*		*Oxytetra-cycline*		*Neomycin*		*Paramomycin*		*Sulphadia-zine*		*Nalidixic acid*	
	S	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*
Salm.stanley ..	7	43	8	42	2	14	4	42	34	16	30	12	—	50	7	—
Salm.barcilly ..	4	4	5	3	—	1	4	4	6	2	5	3	—	8	—	—
Salm.saint paul ..	—	1	1	—	1	—	1	—	1	—	1	—	—	1	—	—
Salm.virchow ..	—	1	1	—	—	1	—	—	—	1	—	—	—	1	—	—
Salm.typhimurium ..	3	1	4	—	—	—	4	—	4	—	—	—	—	4	—	—
Salm.paratyphi B ..	8	—	8	—	2	—	6	—	6	2	7	—	—	8	1	—
Salm.enteritidis ..	3	—	3	—	3	—	—	—	3	—	—	—	—	3	3	—
Salm.newbrunswick ..	1	—	1	—	1	—	—	—	1	—	—	—	—	1	—	—
Salm.urbana ..	1	—	1	—	1	—	—	—	1	—	—	—	—	1	—	—
Salm.richmond ..	1	—	1	—	1	—	—	—	1	—	—	—	—	1	—	—
Salm.waycross ..	1	—	1	—	1	—	—	—	1	—	—	—	—	1	—	—

R=resistant S=sensitive

All the strains were not tested against tetracycline, oxytetracycline, paramomycin and nalidixic acid; hence the total for these tests will be different from the rest.

Salm.stanley was almost resistant to streptomycin, chloramphenicol tetracycline and oxytetracycline. The majority of them were fairly resistant to neomycin and paramomycin. Only 7 strains were tested on nalidixic acid and all were sensitive. All the strains were resistant to sulphadiazine. Of the strains of *Salm.bareilly* tested nearly half were resistant. *Salm.stanley* and *Salm.bareilly* were frequently isolated not only from the children's hospital but also from other hospitals. They had varying patterns of microbic sensitivity. They might be called "hospital strains". *Salm. typhimurium*, *Salm.paratyphi B*, *Salm.saint paul* and *Salm. virchow* also showed resistance but to a lesser extent than the "hospital strains". Strains like *Salm.enteritidis*, *Salm.newbrunswick*, *Salm.urbana* and *Salm.waycross* were less frequently isolated. They were sensitive to all the antibiotics but resistant to sulphadiazine. They might be called "wild strains".

Some of the strains of *Salm.stanley* have now become completely resistant to all the antibiotics and sulphadiazine. Table 6 shows when it began to acquire resistance to the antibiotics.

TABLE 6

Antibiotic Sensitivity of *Salm.stanley*.

Date	*Streptomycin*	*Chloramphenicol*	*Neomycin*	*Chlortetracycline*
April, 1954 ..	S	S	S	S
June, 1963 ..	R	S	S	R
October, 1963 ..	R	R	S	R
November, 1965 ..	R	R	R	R

R=resistant S=sensitive

The sensitivity tests were done on strains isolated from the hospital. *Salm.stanley*, according to Nityananda and Schmid (1954) was sensitive to streptomycin, chloramphenicol, chlortetracycline and neomycin; in June, 1963 we observed that it was resistant to streptomycin and neomycin; in October, 1963 its resistance to chloramphenicol was also noted. During the present investigation in November, 1965 it has turned out to be resistant to neomycin as well. It might be mentioned that strains sensitive to all the antibiotics and strains sensitive to some of these are also being isolated in addition to completely resistant ones.

The microbic sensitivity of enteropathogenic *E.coli* and shigellas isolated from the children's hospital are presented in Table 7.

TABLE 7

Microbic Sensitivity (*E.coli* and shigellas)

	Strepto-mycin		*Chloram-phenicol*		*Tetra-cycline*		*Oxytetra-cycline*		*Neomycin*		*Paramomycin*		*Sulphadia-zine*		*Nalidixic acid*	
	S	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*
E.coli 055:*K*59 ..	—	2	—	2	—	—	—	2	2	—	—	2	—	2	—	—
E.coli 0125:*K*70 ..	—	4	—	4	—	—	—	4	3	1	4	—	—	4	—	—
E.coli 0142:*K*86 ..	3	6	3	6	1	—	2	7	7	2	7	2	—	9	—	—
E.coli 0126:*K*71 ..	4	1	4	1	—	—	2	3	5	—	5	—	—	5	—	—
E.coli 086:*K*61 ..	2	—	1	1	—	—	1	1	2	—	2	—	—	2	—	—
E.coli 0112:*K*66 ..	2	—	—	2	—	2	—	—	2	—	2	—	—	2	—	—
E.coli 0114:*K*90 ..	2	—	2	—	1	—	1	—	2	—	2	—	—	2	—	—
Shig.dysenteriae 2 ..	1	—	1	—	1	—	1	—	1	—	1	—	—	1	1	—
Shig.flexneri 2 ..	6	4	6	4	4	3	2	1	8	2	8	2	—	10	2	1
Shig.flexneri 3 ..	2	1	3	—	2	—	1	—	3	—	1	—	—	3	2	—
Shig.flexneri 4 ..	3	—	3	—	3	—	3	—	3	—	3	—	—	3	3	—
Shig.boydii (4 different serotypes)	4	—	4	—	4	—	4	—	4	—	4	—	—	4	2	—
Shig.sonnei ..	7	—	7	—	7	—	7	—	7	—	2	—	—	7	5	—
Alkalescens-Dispar 01	1	—	1	—	1	—	1	—	1	—	1	—	—	1	1	—

R=resistant *S*=sensitive

All the strains were not tested against tetracycline, oxytetracycline, paramomycin and nalidixic acid; hence the total for these tests will be different from the rest.

Strains of *E.coli* gave reactions almost similar to those of the salmonellas. Of the 26 strains tested 23 were sensitive to neomycin. *E.coli* 0142:*K*86 was not reported in Ceylon up to 1956 (Velaudapillai, 1966). Attempts to isolate this organism were made from 1964 and its isolation in the same year was reported by Velaudapillai (1966). From the pattern of antibiotic resistance it appears that this pathogen would have caused diarrhoea prior to 1964. If efforts were made to isolate this organism, it might have been done much earlier.

Among the shigellas, only *Shig.flexneri* 2 and *Shig.flexneri* 3 acquired resistance. Strains of *Shig.flexneri* 2 resistant to all the drugs and strains sensitive to all except sulphadiazine are being isolated from children's hospital. *Shig.sonnei* did not acquire resistance to the antibiotics.

All the strains of enteropathogenic *E.coli*, shigellas and salmonellas except one strain of *Salm.virchow* isolated from the estates were sensitive to all the antibiotics but resistant to sulphadiazine. *Salm.virchow* was resistant to chloramphenicol. Nityananda *et al.* (1954) found this strain to be resistant to chloramphenicol. It might be possible that this strain during these years might have found its way into the estate where it was reported.

As a result of this investigation a question might be asked 'What are the most suitable chemotherapeutic agents for the treatment of diarrhoeal diseases due to salmonellas, shigellas and enteropathogenic *E.coli* ?' The antibiotics and sulphadiazine used for this

study are recommended for clinical use. Those like streptomycin and chloramphenicol which have been used for considerable time have produced resistant strains. Oxytetracycline is useful for bacillary dysentery. With it the larger the oral dose given, the smaller is the proportion absorbed, and so the concentration in the faeces rises sharply as the dose is increased (Herrell, Heilman, Wellman and Bartholomew, 1950). Owing to this advantage clinicians prefer to use it even if the strain is not fully sensitive. Then there are newer agents like paramomycin, nalidixic acid which have not produced much resistant strains. Every strain isolated from the hospital and from the estates was resistant to sulphadiazine. Sulphadiazine or other sulpha compounds are in use for the last 30 years and as a result all the strains have become resistant. Sulphas are preferred because they are cheap. When chemotherapeutic agents to which organisms are not quite sensitive are used in treatment, the defensive mechanisms of the body play a major role in clearing the infection. The "wild strains" from the hospital and the estates were found sensitive to all the antibiotics. Here the clinicians will have their discretion in the choice of antibiotics. But for the "hospital strains" like *Salm.stanley*, *E.coli* 055:*K*59 and *Shig.flexneri* 2, a controlled clinical study will be useful to evaluate the efficacy of these chemotherapeutic agents.

SUMMARY

1. The ratio of the infections due to the salmonellas and shigellas among the hospital children was 4:1 whereas for the estate children it was 1:3.

2. The most frequently isolated organisms from the hospital children were *Salm. stanley*, *Shig.flexneri* 2 and *E.coli* 0142:*K*86. Some of the strains were completely resistant to all the chemotherapeutic agents.

3. *Shig.flexneri* 2 was most commonly isolated from the estates. This organism along with others that were isolated from the estates were sensitive to all the antibiotics and nalidixic acid.

4. The estate labourers of age groups 10—19 and 20—29 were mostly affected by shigellas.

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