

DISTRIBUTION OF ENTEROPATHOGENS ASSOCIATED WITH
DIARRHOEA AMONG INFANTS AND CHILDREN OF HIGH
AND LOW SOCIO-ECONOMIC CLASSES IN TEHERAN*

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ABSTRACT

Bacteriologic, parasitic, fungal and viral investigation of stools of 268 sick and 105 healthy infants and children during a two-year period from March 1970, through March 1972, revealed that: Shigella and Salmonellae are still the major causes of infantile endemic diarrhoea in Teheran. Enteropathogenic bacteria were isolated from 26.86% of sick vs 7.61% healthy children.

Salmonella typhimurium was found to be the most common enterobacterial pathogen in young sick children of the low social class, whereas in the sick children of high class, Shigella was the most prevalent organism and *Shigella sonnei* the predominant serotype. Cases of diarrhoea due to EPEC and Salmonella other than *Sal. typhimurium* occurred mainly in the high socio-economic class in this age group.

Protozoa and helminthic ova were detected in 17.91% of sick vs 30.47% of healthy and positive fungal cultures were obtained in 5.97% of sick vs 0.95% healthy, while enteroviruses were recovered from 38.43% of sick vs 32.38% healthy children. In 32.08% of diarrhoea cases, no pathogenic bacte-

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Bacterial Methods :

Each fresh fecal sample was divided into three portions; one portion was cultured immediately for enteric bacterial and fungal organisms. The other two portions were sent for parasitic and viral investigation to the laboratories concerned.

for the detection of enteric pathogens, the method described by Edwards and Ewing(1) with modification by Mohadjer and Badalian(2) was used. Identification of Salmonella types and pathogenic *E. coli* was done by the techniques described by Le Minor.(3)

Parasitological Methods:

Protozoa and the ova of helminths were identified by the following methods :

1. Direct examination with Ringer Solution;
2. Direct examination by M.I.F. (Merthiolate and Lugol's Iodine Formaldehyde) Sapero, J.J. *et al.*;(4)
3. Flotation method of Willis;(5)
4. Trichrom staining method of Weatly (6)

Fungi Isolation Methods :

For the isolation of fungi from focal samples, the Vanbreuseghen method(7) was used. *Candidia albicans* and other candidia species were identified by the production of Calmidospores on PCB medium(8) and assimilation and fermentation of various sugars.(9)

Isolation and Identification of Viruses :

Stool specimens suspended in Hanks' solution, containing penicillin, streptomycin and amphotericin B, were centrifuged at 4000 rpm for 30 minutes and 0.2 ml of the supernatant fluid was inoculated into tissue culture tubes. Viruses were isolated and typed on monkey kidney cell culture and on a continuous cell line of Amnios (Am57).(10) for identification of enteroviruses (Polio, ECHO, Coxsackie B), the neutralisation test was carried out according to the methods described by Lennette.(11)

RESULTS

Organisms Recovered :

Organisms of possible significance recovered from the 268 sick and 105 healthy children are presented in Table 3.

Enteropathogenic Bacteria (Salmonella, Shigella and path. *E. coli*)

were isolated from 7.6% of the 105 healthy and 26.8% of the 268 sick individuals, showing a significant difference.*

The infrequent recovery of *Proteus*, *Klebsiella*, *Arizona* and *Providencia* in the present study show that these species are probably not important as causative agents in diarrhoeal diseases in this region and in this age group.

As reported in our previous papers,(12, 13) again the enteric pathogens isolated showed a high degree of resistance to tetracycline, streptomycin, ampicillin, chloramphenicol and triple-sulfa, and *Sal. typhimurium* and path. *E. coli* (O111B4) were the most resistant strains.

PARASITES: The difference in the recovery rates of protozoa and helminthic ova (although high rate) was found to be not statistically significant.*

Giardia lamblia was the most common protozoan isolated in both classes. *Entamoeba histolytica* was rarely encountered in this age group.

Helminthic Ova: The Ova of 4 species of helminths were found in the stools of 373 infants and children examined. *Hymenolepis nana* was the most predominant, *Ascaris lumbricoides* the second, followed by *Trichiuris trichiura* and *Enterobius vermicularis*.

Fungi: Sixteen patients with diarrhoea had high colonial counts of fungi (5.9% in the sick vs 0.9% in the healthy) in their stool cultures. Eight of these (50%) were *Candida albicans*, 6 were various candida species and 2 were yeasts.

Two of the *Candida albicans* were associated with *Salmonella* and one with virus. One of the yeasts was in association with *Salmonella*.

Enteroviruses: The isolation rate of enteroviruses was 38.4% in the sick and 32.4% in the healthy and did not show a statistically significant difference* between the controls and the sick cases. From the three types of Viruses isolated, ECHO had the highest frequency and Polio was the second by Coxsackie B, while 35 of the isolated enteroviruses were unclassified.

ANALYSIS BY SOCIAL CLASS

Bacteria: From Table 4, it can be concluded that in the age group considered, bacterial infection (excluding *Sal. typhimurium*) is more prevalent in the sick of high class (28.2%) than in the sick of low class infants (13.7%).

Salmonella typhimurium was found to be the most prevalent bacterial pathogen in the sick of the lower class (10.8%) and shows a significant difference* compared with 3.8% in the sick of the higher class. In the case of *Shigella*, the situation is reversed and this organism was recovered from 13.9% of the sick of high class and 5.8% of the sick of the low class. The same applies to the prevalence of enteropathogenic *E. Coli* recovered from the sick of high class (7.7%), which is more than twice the rate in the sick of the low class

* Statistically significant difference proven by group a Chi square test at a 95% confidence level.

DISCUSSION

In endemic diarrhoea among the pre-school population in two different socio-economic communities, as observed in the present study, a noteworthy percentage is associated with bacterial infection: 31% of high income sick and 23% of low income sick vs 8.8% high income healthy and 5.4% low income healthy infants and children, which confirms the high incidence of bacterial pathogens in both communities as compared with other enteropathogens.

The 8 cases among healthy individuals may be attributed to asymptomatic infection, as it is rare to come across carriers in this age group. Our findings in this age group are very similar to the results obtained by others.(14, 15, 16, 17, 18)

Salmonella typhimurium is the prevailing bacterial pathogen in the low socio-economic community: 10.8% in low sick vs 3.8% in high sick. Similar results have been obtained from infants and children of low socio-economic class hospitalized in Teheran for diarrhoeal diseases. In 1969, of 88 enteropathogens, 17 (19.3%), (19) and in 1971 of 123, 17 (13.8%), were *Sal. typhimurium* (unpublished data).

However, salmonellae other than *Sal. typhimurium* are more prevalent in the sick of the high socio-economic group and the infection rates in high and low classes were 5.4% and 2.9%, respectively. This phenomenon can be explained in the following way: one of the major causes of human Salmonellosis is the consumption of contaminated foods. These foods are available at an earlier age to infants and children of the higher social class, who usually start eating eggs, canned baby food, powdered milk, etc., after they are 3 months old. On the other hand, as reported by others,(20) Salmonellosis is more likely to be symptomatic when children are young. The results of this study show that the infection rate is higher in less than 2-year old children of the high social class. In the low social class most of the children are breast-fed until their second year, and the possibility of coming in contact with *Salmonella* (other than *Sal. typhimurium*) is very limited for them.

Shigella was recovered from only 5.8% of the low social class, but increased to 13.9% in the high social class. This difference in the rate of infection may be related to two factors:

1. Foods, mainly fruits and vegetables, are available to this age group in the higher class, but are not consumed by the lower class, which indicates the possibility of food-borne shigellosis.(21)
2. According to Kourany,(22) in pre-industrial countries shigellosis is rare in the first months of life, which means that breast-fed children have a special intestinal flora that protects them from this type of infection. Differences in the intestinal microflora of breast-fed and artificially-fed infants or other factors such as antibodies or anti-

bacterial substances in the mothers' milk, may be responsible for the varying behaviour of shigella infection.(23) (As we have already stated, children in our low class group are breast-fed until 2 years of age.)

Contrary to Salmonella infections, which show a high incidence in summer, the shigella infections observed occurred mostly during spring, as has been reported by others as well.(24) although some authors have reported a higher incidence in seasons other than spring.

Shigella sonnei is the predominant shigella in both social classes. This prevailing infectious agent is replacing *Sh. flexneri* II, which was the predominant bacterial pathogen in our previous study.(2)

Enteropathogenic *E. coli* was recovered from 5.6% of sick and 1.9% of healthy children; thus the rates of infection in our high and low social class groups were 7.7% and 3.6%, respectively. In this study, these sporadic cases of diarrhoea due to EPEC occurred mostly in the under 2 years age group and were recovered from 10.8% of the high and 2.3% of the low social class. This shows another bacterial pathogen with high incidence in industrialized countries.(25) The serotype most often isolated was O111B4, as reported by others as well.(17, 26)

Infection with bacteria alone or concurrently with other enteropathogens was invariably associated with diarrhoea (Table 10). The rate of bacterial isolation alone or in association with other enteropathogens was 28% and 42% for the diarrhoea and diarrhoea with fever groups. This shows a significant difference* in the rate of bacterial isolation by severity of disease. The association of bacteria with other organisms does not seem to affect the severity of the disease. In both groups of diarrhoea alone and diarrhoea with fever, 50% of the isolations were of single bacteria or bacteria in association with other enteropathogens, as has also been shown by others. (27).

Protozoa :

Giardia is well represented in both communities and appeared with approximately equal frequency in diarrhoea and control cases in the present investigation. Contrary to the bacterial findings, *Giardia* detection was higher in the less severe cases: 21.4% in the diarrhoea group and 11.6% in the diarrhoea with fever group, even though it has been reported that *Giardia* may cause acute attacks of gastroenteritis.(28)

Candida albicans: *Candida albicans* can be considered the causative agent of a small fraction of diarrhoea cases in the study, due to the fact that the number of less than one-year old infants, the most vulnerable age group to this agent, was relatively low.(28, 29)

Negative Cases :

The results of the study agree with the conclusion drawn from the

studies of Pierce *et al.*, Joe *et al.* and Mata *et al.* (31, 32, 33) that only a part of the diarrhoeal disorders of children are caused by direct infectious agents, since 32.1% of our diarrhoeal cases were not associated with pathogenic bacteria, parasites, viruses or fungi. Other potential causes such as intolerance to some foods and drugs, milk allergy, metabolic diseases, feeding mismanagements, certain surgical conditions, malnutrition, etc., were not considered here.

No difference was observed in the two socio-economic groups in this respect; 32.3% of low sick and 34.8% of high sick of our diarrhoeal cases were not positive for any of the infectious agents.

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TABLE 1
DISTRIBUTION OF INFANTS AND CHILDREN BY CLASS

HEALTH CLASS	SICK	HEALTHY	TOTAL
LOW	139	37	176
HIGH	129	68	197
TOTAL	268	105	373

TABLE 2
AGE DISTRIBUTION OF SICK AND HEALTHY INFANTS
AND CHILDREN BY CLASS

HEALTH AGE	LOW CLASS		HIGH CLASS	
	SICK	HEALTHY	SICK	HEALTHY
0- $\frac{1}{2}$ YEARS	85	13	74	21
2- $\frac{1}{2}$ YEARS	26	6	28	18
4-7 YEARS	28	18	25	29
UNKNOWN	-	-	2	-
TOTAL	139	37	129	68

TABLE No. 3

ORGANISMS RECOVERED IN HEALTHY AND SICK INFANTS AND CHILDREN

ORGANISMS RECOVERED	NO. TESTED	SALMONELLA TYPHIMURIUM	SALMONELLAE	SHIGELLA	PATH. E. COLI	PROTOZOA	HELMINTHS' OVA	FUNGI	ENTERO VIRUSES	MIXED INFECTIONS	NONE
HEALTHY	105	1 (0.9)	3 (2.8)	2 (1.9)	2 (1.9)	20 (19)	12 (11.4)	1 (0.9)	34 (32.4)	8 (7.6)	46 (43.8)
SICK	268	20 (7.4)	11 (4.1)	26 (9.7)	15 (5.6)	37 (13.8)	11 (4.10)	16 (5.9)	103 (38.4)	46 (17.1)	86 (32)

TABLE No. 4

ORGANISMS RECOVERED IN HEALTHY AND SICK INFANTS AND CHILDREN BY CLASS

ORGANISMS RECOVERED	NO. TESTED	SALMONELLA TYPHIMURIUM	SALMONELLAE	SHIGELLA	PATH. E. COLI	PROTOZOA	HELMINTHS' OVA	FUNGI	ENTERO VIRUSES	MIXED INFECTIONS	NONE
HEALTHY (LOW)	37	-	1 (2.70)	1 (2.70)	-	12 (32.4)	7 (18.9)	1 (2.70)	12 (32.4)	7 (18.9)	15 (40.5)
SICK (LOW)	139	15 (10.8)	4 (2.9)	8 (5.8)	5 (3.6)	21 (15.1)	7 (5)	11 (7.9)	53 (38)	25 (17.9)	45 (32.4)
HEALTHY (HIGH)	68	1 (1.5)	2 (2.9)	1 (1.5)	2 (2.9)	8 (11.7)	5 (7.3)	0 -	22 (32.3)	1 (1.5)	31 (45.5)
SICK (HIGH)	129	5 (3.8)	7 (5.4)	18 (13.9)	10 (7.7)	16 (12.4)	4 (3.1)	5 (3.8)	50 (38.7)	21 (16.7)	41 (34.8)

METHOD OF STUDY

Eight villages were randomly selected from the entire province. In each village, all men and women aged 40-60 who were present in the village were studied. Each person was first interviewed, and a questionnaire on socio-economic status, occupation, education, number of children, cigarette smoking, etc., was filled out; then height, weight and blood pressure were measured. Afterward, a 12 lead ECG was taken using a Cardiopan apparatus with a speed of 25 mm per second. ECG tracings were coded according to the modified Minnesota code described by Rose and Blackburn in 1968(10). Because of difficulties in obtaining the correct ages of the subjects, we could not limit ourselves to 40-59 years.(1)

RESULTS

Out of a population of 3451 in the villages studied, 599 persons were listed as eligible for study (men and women aged 40-60), including 276 women and 324 men. We examined 198 women (71.7%) and 178 men (54.6%) who were present in the villages at the time of the study. The rest were absent, most of them on trips to urban areas. There were only a few who refused and did not want to cooperate.

Table I shows the percentage of various types of ECG abnormalities. It is seen that only 45% of the tracings are completely normal. Important ECG abnormalities are shown in Table II. We may discuss them as follows:

Q/QS items (1 :-2-3): These abnormalities were seen in 7 men (3.9%) and 11 women (5.5%). Out of these 18 persons, 5 of them showed these abnormalities only in V1 - V3 leads and 4 others only in V1 - V2 leads.

Left axis deviation (2:1): This was recorded in 6 men (3.3%) and 9 women (4.5%).

Increased R amplitude (3r - 3): This was seen in 10 men (5.6%) and 4 women (2%).

ST Segment depression (4r - 3): This was seen in 7 men (3.9%) and 23 women (14.1%). T wave inversion (5r - 2) was recorded in 4 men (2.2%) and 23 women (11.6%). Flat T wave (5₃) was seen in 5 men (2.8%) and 19 women (9.65%).

Table III and Figure 1 show ECG abnormalities by blood pressure categories. For a definition of these, we have used the values and cutpoints recommended by the Expert Committee of the World Health Organization in 1959 (13) to differentiate three blood pressure groups. It can be seen that 93.7% of normotensive men and 74.4% of normotensive women have completely normal ECG tracings while about 40% of hypertensive individuals (men and women) have at least one important abnormality in their ECG.

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ria, virus, parasite or fungi were found in the stool specimens. The rate of mixed infections was considerably greater in diarrhoeal cases than in the control group, 17.1% vs 7.6%.

The enteric pathogens isolated in this study showed a high degree of resistance to tetracycline, chloramphenicol, streptomycin, ampicillin and triple-sulfa.

INTRODUCTION

Diarrhoea is a clinical syndrome induced by various organisms as well as non-infectious factors. In this study, data is presented concerning the bacterial, protozoal, helminthic, fungal and viral findings and their association with diarrhoea in children of low and high socio-economic classes in Teheran.

The study was conducted on 268 infants and children sick with diarrhoea (more than 4 bowel movements per day with and without fever, vomiting, loose stools with and without mucus and blood) and 105 healthy individuals who were brought to pediatric clinics for treatment, check-up and vaccination during a period of two years (1970-1972).

MATERIALS AND METHODS

Patients:

Fresh fecal specimens were obtained from both healthy and sick (acute or chronic diarrhoea) infants and children. The condition of the disease was diagnosed by one of two pediatricians, who referred the cases to the laboratory.

In the study, the healthy and sick children were classified according to age and socio-economic group by the income of the family: 10,000 rials (about \$135) or less per month was classified as "low" and more than 10,000 rials as "high" socio-economic groups. The occupational status of the family head was also taken into consideration and the low social class consisted mainly of the children of non-commissioned officers of the army and laborers.

The distribution of cases according to social class is shown in Table 1.

The age of infants and children ranged from 15 days to 7 years, with the distribution shown in Table 2.

A stool specimen was considered positive when any of the organisms was found on appropriate single examination. In cases where a first specimen was found to be negative for any of the organisms, a second and third specimen were obtained and the case was labeled negative after three consecutive examinations only. Two different enteric pathogens from the same patient within a one-month interval were considered as two positive cases.

(3.6%). Table 5 shows bacterial findings in sick and healthy children by social class.

Parasites: No significant difference* could be observed between the two classes regarding protozoal and helminthic infestation. Table 6 shows the prevalence of protozoa and helminthic ova in the fecal material of these two groups.

Giardia lamblia was the most common parasite found alone or in combination with other enteropathogenic agents: 13.6% in the low income sick, 29.7% in low healthy, 11.6% in high sick and 10.2% in high healthy, no significant difference* being detected between the high and low classes in this respect.

Fungi: It appears that the recovery of fungi in sick children is more frequent in the low (7.9%) than in the high class (3.8%). None of our control group had *Candida albicans*.

Viruses: No difference was observed in the isolation rates of enteric viruses in the high and low classes.

Seasonal Distribution

Table 7 shows the seasonal distribution of the enteropathogens. Salm onella show a higher prevalence in summer; Shigella increase considerably in the spring and decline in autumn. From 17 EPEC, 8 were isolated in summer. No difference of any significance was observed in protozoal and helminthic infestation by season of the year, and the number of yeast-like organisms isolated was too small to allow any comparisons in this respect.

Age :

From Table 8, it is evident that in the less than 2 age group of both social classes, *Salmonellae* and path. *E. cloi* are the most prevealene organisms, while in the over two years age group the predominant bacterial pathogens are the Shigellae.

The incidence of protozoal infestation increases with age in both social classes. As expected, helminthic infestation in this age group was very low; no infestation in the less than 2 age group was encountered but there was a considerable increase in childcen older than 4 years in the low social class, from 7.7%to 17.8%.

The fungi were mainly limited to the less than 2 age group, 12 out of 16 (75%) of the positive cases being in this age group.

Mixed Infections :

In children with diarrhoea, there was a high incidence of association of pathogenic bacteria with viruses: 19/268 (7.08%) in sick vs 1/105 (0.95%) in healthy (Table9).