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Salmonellae and shigellae in a group of rural South African Bantu school children

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INTRODUCTION

From a survey of the recoveries of salmonellae from specimens submitted to the South African Institute for Medical Research for bacteriological investigation, it is apparent that infections with these organisms in man are common in South Africa (Bokkenheuser & Greenberg, 1959). Thus, as indicated by notifications, the annual morbidity of typhoid fever for all races is about 40 times higher than in England and Wales (Bokkenheuser, 1959). Furthermore, salmonellae other than *Salmonella typhi* have been recovered with increasing frequency from excreta submitted to this Institute, e.g. in 1957, 4·0% of such specimens yielded a growth of salmonellae other than *Salm. typhi* (Bokkenheuser & Greenberg, 1959).

Outbreaks of shigella infections were reported by Finlayson (1943) and Finlayson & Siemelink (1947). Kahn (1957) and Kahn, Wayburne, Shnier, Stein, Cronje, Freiman, Levin, Orska, Reef & Theunissen (1958) stressed the role of the shigellae as causative agents of infantile gastrointestinal disorders and drew attention to the serious prognosis of such cases.

In search of possible sources of these infections, Bokkenheuser & Richardson (1959) studied the apparently healthy Bantu food-handlers, and found that 4·3% of them were infected with salmonellae and 0·6% with shigellae. On the evidence available it was impossible to decide if the infected persons should be considered chronic carriers or merely short-time infected individuals.

When Dr A. R. P. Walker, in May 1958, suggested a systematic bacteriological investigation of the faeces in a rural group of Bantu school children, we felt that it afforded an opportunity to study further the problems related to pathogenic Enterobacteriaceae in a section of the African population.

MATERIAL AND METHODS

Bantu children, aged between 6 and 16 years, were chosen for this study; they were pupils of Tlaseng Bantu School in a Native Reserve area, situated about 100 miles west of Johannesburg and 13 miles north-east of Rustenburg (Western Transvaal). The school accommodated about 300 children who were comparatively well dressed and appeared to be in good physical condition. According to information obtained by Dr F. W. Fox of this Institute (personal communication), the parents were mostly farmers, keeping cattle, goats, pigs and fowls, and growing maize, kaffir corn, beans and a few vegetables. The main component in the diet

was porridge prepared from maize or kaffir corn, eaten sometimes with milk, but seldom with sugar. The role of meat in the diet was not assessed. Green leaves of various plants were cooked, squeezed into small cakes, dried in the sun and stored and, when required, were cooked again. Food could be scarce in late summer before the cereals were harvested. The drinking water was drawn principally from shallow surface wells in the veld, situated about half a mile from the nearest building. Some of the wells were fenced with thorn-bush, others were open, and allowed access to grazing cattle. A small quantity of the water was obtained from tanks collecting rain water from galvanized iron roofs.

During the year of observation no alterations were made in the tribe's mode of life. It was not known whether the children from time to time received herbal or dietetic treatment, administered according to tribal customs. No one received modern medical treatment, not even those from whom pathogenic organisms were recovered. It was decided to break this rule if malignant gastrointestinal disorders should occur, but fortunately this did not happen.

The children were examined seven times at fairly regular intervals over a period of a year. Faecal specimens for bacteriological investigation were planted on SS-agar and Selenite-F media within 10 min. of voiding. A note was made of the consistency of the specimen. The oral temperature was recorded and the general condition of the child noted. Plates and enrichment media were taken to the laboratory in Johannesburg and incubated. The following morning a loopful of the enriched culture was plated on fresh SS-agar and incubated. The primary plates were examined and, as a rule, not more than three of the non-lactose fermenting colonies were isolated from any one plate. After 24 hr. incubation the secondary plates were dealt with as above. Thus, the number of colonies isolated from each individual per collection seldom exceeded 6. The organisms were tested in composite media for biochemical reactions, and those conforming to the pattern of salmonella were submitted to detailed antigenic analysis. The shigellae were classified according to their principal group antigen.

Employing the disk method, the organisms were tested for their resistance/sensitivity to a range of antibiotics. Disks, 6 mm. in diameter and weighing 3·3 mg., were impregnated with antibiotics of which the following were used: sulphatriad (50 μg.), penicillin G (10 units), streptomycin (100 μg.), aureomycin (50 μg.), chloromycetin (50 μg.), terramycin (50 μg.), erythromycin (50 μg.), achromycin (50 μg.), neomycin (50 μg.) and novomycin (50 μg.). The biological activity of the impregnated disks was checked by parallel tests, using the Oxford staphylococcus as the control. The size of the zone of inhibition around the disks was expressed arbitrarily: 4 mm. or more = '3', 4 to 2 mm. = '2', and less than 2 mm. = '0'.

Several of the observations were examined by the χ^2 test. 'Significant differences' refer to P values of 0·05 or less.

RESULTS

The survey comprised 124 children, fairly evenly distributed between the ages of 6 and 16 years (Table 1). There were 67 girls and 57 boys. During the year of observation they provided 772 faecal specimens for bacteriological investigation

Table 1. *Salmonella and shigella infections by age and sex*

Age in years	No. of individuals	No. of specimens	Specimens infected with Salmonellae No.	%	Specimens infected with Shigellae No.	%	Individuals infected with Salmonellae No.	%	Individuals infected with Shigellae No.	%	All infected individuals No.	%
6	2	14	1		1		1		1		1	
7	7	46	1		4		1		3		3	
8	13	81	2	5·8	2	4·1	2	32·1	2	20·8	4	45·3
9	18	123	10		1		8		1		9	
10	13	80	6		6		5		4		7	
11	12	75	3		5		3		4		6	
12	7	49	5		2		4		2		5	
13	22	146	11		4		10		3		12	
14	7	45	3	7·0	4	5·4	2	38·0	4	28·2	6	57·7
15	9	55	1		3		1		3		4	
16	14	58	7		5		7		4		8	
Total	124	772	50	6·5	37	4·8	44	35·5	31	25·0	65	52·4
Females	67	434	28	6·5	18	4·2	23	34·3	16	23·9	35	52·3
Males	57	339	22	6·5	19	5·6	21	36·8	15	26·8	30	52·6

and from these salmonellae were recovered from 50 (6·5%) and shigellae from 37 (4·8%). Two of the specimens yielded a growth of two salmonella types and, from another two, both salmonella and shigella organisms were isolated. The latter are tabulated under both salmonella and shigella. During the year of investigation more than half of the individuals (52·4%) experienced either salmonellosis or shigellosis. Of these, 44 (35·5%) were infected with salmonellae and 31 (25·0%) with shigellae; the difference was not significant. The infections were unrelated to sex, and there was no statistically significant difference between the proportions of infected individuals in the different age groups.

Out of the 65 infected children, 28 had one, and 6 had two salmonella infections, while 19 experienced one and 2 two shigella infections. Both salmonellae and shigellae were isolated from 10 children. There was usually a time interval between these different infections, but in four cases more than one type was isolated from the same specimen. A simultaneous triple infection of *Salm. norwich*, *Salm. mobeni* and *Sh. sonnei* was found in one child, a girl of 13 years of age.

Because some children left school before the termination of the survey and others were absent on the days of examination, only 75 children were available for all seven investigations. Their data are presented in Table 2, which shows that the incidence varied considerably with the season.

There was an accumulation of infections in December (early summer), when salmonellae were recovered from 22·6% of the children. Six different types were involved in this outbreak, and *Salm. mobeni* was responsible for the majority of infections. A smaller outbreak, caused by *Salm. johannesburg*, occurred in April (autumn). The other types encountered in this survey were isolated sporadically. In no instance was the same salmonella type recovered twice from the same person.

Table 2. *Seasonal distribution and duration of salmonella and shigella infections in 75 children*

Month of examination	Recovered salmonellae: *Salm. orion*	*Salm. dublin*	*Salm. oranienburg*	*Salm. mobeni*	*Salm. typhi mur*	*Salm. irumu*	*Salm. urbana*	*Salm. norwich*	*Salm. cerro*	*Salm. birkenhead*	*Salm. johannesburg*	*Salm. adelaide*	% salmonella-infected individuals	Recovered shigellae: *Sh. schmitzii*	*Sh. flexneri*	*Sh. sonnei*	*Sh. boydii*	% shigella-infected individuals	Total infected individuals: No.	%
May	1	.	.	.	.	.	.	.	.	.	.	.	1·3	2	1*	.	.	4·0	4	5·3
Aug.	.	.	.	.	.	.	.	.	.	.	.	.	—	.	3	.	.	4·0	3	4·0
Oct.	.	1	1	.	.	.	.	.	.	.	.	.	2·7	.	.	5†	.	6·7	7	9·3
Dec.	.	.	1	11‡§	2‡	3	1	1§	.	.	.	.	22·6	.	4	2§†	.	8·0	22	29·3
Jan.	.	.	.	.	.	.	1	.	1	.	.	.	2·7	.	1*	.	.	1·3	3	4·0
Mar.	.	.	.	1	.	.	.	.	.	3	.	.	5·3	.	.	.	1	1·3	5	6·7
Apr.	.	.	.	.	.	.	.	.	.	.	6	1	9·3	.	3	.	.	4·0	10	13·3
Total	1	1	2	12	2	3	2	1	1	3	6	1	44·0	2	12	7	1	29·3	54	72·0

*, † Same organism from same individual. ‡ Double infection. § Triple infection.

In contrast to the seasonal fluctuation in incidence of salmonelloses, the shigella infections were distributed fairly evenly throughout the year. All shigella subgroups (Kauffmann, 1954) occurred in this survey. It will be noticed that a flexner organism was isolated twice from the same individual with an interval of 9 months, and that *Sh. sonnei* was recovered from the same person in October and again in December, but not later.

Of these 75 children, 72·0 % experienced infections with either salmonellae (44·0 %) or shigellae (29·3 %).

Table 3 gives the relationship between the oral temperature of the individual, the consistency of the faecal specimen and the result of bacteriological examination of the stools.

This table contains only 645 observations because: (*a*) the consistency of the faeces and the temperatures were not recorded on the first occasion of investigation, in May 1958; (*b*) specimens with concurrent salmonella and shigella infections have been excluded; and (*c*) a note of consistency and temperature is missing in a shigella-infected case occurring later in the investigation.

About 25 % of the salmonella/shigella organisms were recovered from 'hard' faeces; the presence of the organisms in the stools, however, had a significantly softening effect on their consistency, although manifest diarrhoea was comparatively rare.

The oral temperatures of the infected groups did not differ from those in the non-infected controls.

In all three cases of double infection, the consistency of the stools was soft and the temperatures were 98·2°, 98·2° and 99·2° F. In the only case of triple infection the stool was liquid, but the temperature was 98·4° F.

Table 3. *Clinical observations related to infection*

	No. of specimens	Consistency of faeces: Hard No.	Hard %	Soft No.	Soft %	Liquid No.	Liquid %
Pathogens not recovered	571	235	41·1	331	58·0	5	0·9
Salmonella infected	44	13	29·5	29	65·8	2	4·5
Shigella infected	30	7	23·2	22	73·3	1	3·3
Total infected	74	20	27·0	51	69·0	3	4·1

		Temperature (°F.): 98·4 No.	98·4 %	98·4–99·8 No.	98·4–99·8 %	100+ No.	100+ %
Pathogens not recovered	571	115	20·1	423	74·0	33	5·8
Salmonella infected	44	5	11·4	37	84·0	2	4·5
Shigella infected	30	9	30·0	20	66·7	1	3·3
Total infected	74	14	18·9	57	77·0	3	4·1

Altogether twenty different salmonella types were found; nineteen of these were fully identified and one, at present under investigation, is believed to be a hitherto undescribed type. Neither *Salm. typhi*, nor *Salm. paratyphi A*, *B* or *C* were observed. Seventy per cent of the strains belonged to the somatic groups B–E (Kauffmann, 1954).

The isolated strains of salmonellae and shigellae were tested for sensitivity/resistance to antibiotics. Where a specimen yielded several strains of the same type, all strains were tested (Table 4). For comparative purposes the table also records the sensitivity of sixty-four salmonella strains isolated from individual cases in the Johannesburg area towards the end of the observation period.

Table 4. In vitro *resistance to antibiotics of isolated strains*

Origin of strain	Genus	No. of strains	Percentage strains resistant to: Sulphatriad	Penicillin	Streptomycin	Aureomycin	Chloromycetin	Terramycin	Erythromycin	Achromycin	Neomycin	Novomycin
Tlaseng School	*Shigella*	44	70·5	100	0	2·2	0	0	0	0	0	100
	Salmonella	99	96·9	100	0	10·1	0	0	7·1	0	0	100
Johannesburg	*Salmonella*	64	89·0	100	1·6	70·3	6·3	1·6	9·4	3·1	1·6	100

Salmonella and shigella strains recovered from pupils of Tlaseng School differed little in their sensitivity to antibiotics, although there were slight indications that some of the salmonellae were more resistant to sulphatriad, aureomycin and erythromycin. Salmonella strains from the Johannesburg district, on the other hand, appeared to be more resistant, not only to the same antibiotics which caused inhibition of the Tlaseng strains, but also to a wider range of antibiotics. These observations have not been examined statistically, as it was felt that it would be desirable to investigate more strains before forming a definite opinion.

The drinking water was suspected of being involved in the transmission of the infections. The water drawn from the rain water tanks was clear and without smell. It contained from a few to 'innumerable' bacteria per ml.; a few presumptive *Escherichia coli* were occasionally found in 100 ml. samples, but faecal *Esch. coli* were absent. The shallow well water was odourless, turbid, brownish grey in colour and 'innumerable' bacteria were present in 1 ml. samples. All specimens yielded growth of presumptive *Esch. coli*, ranging from 50 to more than 1800 per 100 ml. and faecal *Esch. coli*, ranging from 2 to 900 per 100 ml. Salmonellae and shigellae were not isolated from the water samples.

DISCUSSION

The results of incidence surveys of infections depend on the sampling methods (Hardy, Mackel, Frazier & Hamerick, 1953), the technical procedures, the number of examinations made and the epidemiological environment at the time of investigation. In regard to technical methods, the results are influenced by the chemical composition of the differential plates (Mollov, Winter & Steinberg, 1943; McCullough & Byrne, 1952; Meyer, 1953; Shipolini, Konstantinow, Trifonowa & Atanassowa, 1959), by the number of colonies examined (Hormaeche, Surraco, Peluffo & Aleppo, 1943) and by the nature of the enrichment medium (McCullough & Byrne, 1952; Meyer, 1953; Rappaport, Hirschberg & Konforti, 1956). While enrichment procedures favour the growth of most salmonellae and permit the examination of a comparatively large volume of material (Greifinger & Silberstein, 1944; Felsenfeld, 1945; Galton & Hardy, 1948), they appear to be without beneficial effect on the growth of the fastidious shigellae, and are often even inhibitory to these organisms (Felsenfeld, 1945; Broh-Kahn, 1946). Most shigella strains are obtained from primary plates, inoculated with moderate or small amounts of material. Thus, the finding of a statistically significant difference between the recoveries of salmonella and shigella from a population does not necessarily reflect a corresponding difference in the incidence of these infections. Until further information is available on the efficiency of the media in detecting salmonellae and shigellae, it must suffice to state that both genera occurred frequently among the Tlaseng school children.

The incidence of salmonellosis was related to seasons (Table 2). The highest incidence occurred in early summer, a finding that agrees with results reported from South Africa (Bokkenheuser & Greenberg, 1959) and with observations from Uruguay (Hormaeche *et al.* 1943). In Northern Europe and North America, the peak incidence occurs from mid-summer to autumn (Harhoff, 1948; Report,

1950*a*, *b*; Feig, 1950; MacCready, Reardon & Saphra, 1957). If this difference in seasonal incidence is real, it suggests that the optimal conditions for acquiring salmonella infections are present after a 2 or 3 months' period of warm weather. The absence of seasonal variations in shigella infections is in agreement with findings of Watt & Hardy (1945) and Feig (1950), but is in contrast to information from England where the highest incidence is claimed to be in winter (Report, 1959).

An assessment of the annual infection rate would require many observations closely spaced throughout the year. From Table 1 it is seen that salmonellae or shigellae were recovered from 52·4 % of the individuals in the group. If the survey is reduced to comprise those who were examined on all seven occasions (Table 2), the incidence rose to 72·0 %. It seems likely, therefore, that during the year of observation nearly all individuals experienced at least one infection of pathogenic Enterobacteriaceae and that many were infected several times.

There is no evidence to show that the infected individuals were chronic carriers (Table 2). Elevated temperatures and marked diarrhoea were uncommon (Table 3) which tend to exclude severe gastrointestinal disorders. It thus appears that the infections were mild, mainly subclinical and of short duration. It is possible, however, that some of the infected children were convalescents, although the school principal pointed out that diarrhoea was rare among the pupils and that it had not been associated with deaths in recent years. It is conceivable therefore that the incidence of salmonelloses and shigelloses in the group studied reflects the distribution of these infections in the whole tribe. If so, it must be envisaged that the infections contribute significantly to infantile mortality (Hormaeche *et al.* 1943; Seligmann, Saphra & Wassermann, 1946; Feig, 1950; Stein, 1955; Kahn, 1957).

The multiplicity of types, their intermittent appearance and the lack of highly predominating strains, are striking features of this study. While similar observations have been made among healthy Bantu food-handlers (Bokkenheuser & Richardson, 1959) a few strains usually account for most cases of symptomatic acute gastrointestinal infections (Seligmann *et al.* 1946; Felsenfeld & Young, 1949; Taylor, 1951; MacCready *et al.* 1957). That differences in pathogenicity between the types may exist is suggested by the works of Seligmann *et al.* (1946) and Saphra & Winter (1957), who showed that the prognosis was related to the infecting type. It is possible that some salmonella types of low pathogenicity may cause widespread infections of a subclinical nature, like those encountered in the school children and that only a smaller, more pathogenic group is responsible for major clinical outbreaks.

The mode of transmission of infections was not established, although it is highly probable that water from the surface wells was implicated. According to the standards laid down by the South African Bureau of Standards (1951) it was unsuitable for human consumption. One can well envisage that this water supply, common to man, birds and reptiles and occasionally to animals, easily contaminated with their excreta, could provide the ideal environment for the perpetual circulation of endless numbers of salmonella and shigella types.

In agreement with the findings of Rozansky, Birnbaum & Benari (1958) and Olarte & de la Torre (1959) the salmonellae and shigellae from the Tlaseng area

were highly sensitive *in vitro* to a range of antibiotics, whereas the salmonellae recovered from the Johannesburg area (Table 4) showed increased resistance. Strains resistant to all antibiotics were not encountered. The differences in sensitivity to tetracyclines of the Johannesburg salmonellae were surprising, but further investigations are required to assess the significance of these observations. Meanwhile, it may be a warning of the dangers of the liberal use of antibiotics and a reminder of the incorrectness of assuming that all cases of salmonellosis and shigellosis respond to a given antibiotic (Nityananda & Schmid, 1954; Stein & Shaff, 1958).

SUMMARY

1. Faeces from rural, outwardly healthy Bantu school children from a Native Reserve region have been bacteriologically examined seven times at regular intervals over a period of one year.

2. Of 75 children, 44·0% experienced at least one salmonella infection and 29·3% one shigella infection; considering the infections together, salmonellae or shigellae were recovered from 72·0% of the individuals. The observations are discussed and the view is expressed that over a period of one year, practically all children experience one, and many of them several, attacks by these pathogens.

3. In the majority of cases, the infections appeared to be subclinical and of short duration. There was no evidence proving the existence of chronic carriers.

4. Salmonelloses showed a seasonal dependence with the highest incidence in early summer, i.e. December. Shigelloses was distributed evenly throughout the year.

5. The recovered salmonellae were of twenty types. *Salm. typhi*, *Salm. paratyphi A*, *B* and *C* were not isolated. Members of all shigella groups were encountered.

6. All the recovered strains from the Tlaseng area were sensitive to streptomycin, chloromycetin, terramycin, achromycin and neomycin, and most of them to aureomycin and erythromycin. In comparison, salmonella strains isolated from the Johannesburg urban area showed signs of increased resistance to the same antibiotics.

7. Water was probably implicated in the conveyance of the infections.

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