A bacteriological assessment of meat, offal and other possible sources of human enteric infections in a Bantu township

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Two previous surveys undertaken at the South African Institute for Medical Research showed that many Bantu school children in rural and peri-urban areas (Bokkenheuser & Richardson, 1960; Richardson & Bokkenheuser, 1963) were infected with salmonellas and shigellas. It was suggested that the poor quality of water might possibly be incriminated in the spread of these organisms. In a third study subsequently carried out in an urban area using a similar group of school children, the only water available came from the Rand Water Board and was of excellent quality (Richardson, Koornhof & Hayden-Smith, 1966). The earning capacities of the families living in this area, although inadequate, were an improvement on the others. These factors, however, together with better hospital and clinic facilities, improved housing and sanitary conditions associated with urbanization, did not result in a noticeable decrease in the isolation of salmonella and shigella organisms.

Since human salmonellosis as a public health problem is attributable mainly to foods of animal origin, egg products, human carriers, food handlers and contaminated water (Sickenga, 1964; Bowmer, 1964; American Public Health Association, 1963) a study on the main items of food of animal origin consumed by the urban population was planned to assess their role in the high incidence of salmonellosis and shigellosis in the group of children investigated.

MATERIAL AND METHODS

Since previous surveys showed that the highest number of isolations of salmonellas and shigellas occurred during the warmer months, collections were arranged fortnightly for 14 months to see whether the isolated organisms followed a similar pattern.

Specimens

From the Johannesburg Municipal Abattoir

Each fortnight specimens of liver, spleen, lymph node, surface meat cut, bile and faeces were taken from a sheep and a bovine.

From the Offal Pool

Offal, consisting mainly of cattle and sheep tripe and intestine, plays an important part in the staple diet of the Bantu, both from an economic and nutritional

point of view. As well as being cheaper, it is often eaten in preference to the conventional meat cuts. After slaughtering, the offal from the Municipal abattoir is rinsed and then transported to a building nearby known as the Offal Pool. From there, by various means of transport it is taken to the Soweto township near Johannesburg and sold to the inhabitants by licensed vendors.

From Soweto township

Soweto township is a housing scheme built by the government, the Johannesburg Municipality and other bodies, to accommodate a population of about 750,000 Bantu, many of whom are employed in various walks of life in Johannesburg. It is situated about 15–20 miles south-west of the centre of the city and essential services, including water-borne sewerage to all houses, are administered by the Johannesburg Municipality. The inhabitants are served by shops of all types to satisfy their daily needs.

Specimens of tripe, intestine and intestinal fat were collected fortnightly from two offal stalls and less frequently, specimens of liver, spleen and muscle. From the butcher shops an occasional specimen of polony, meat, mince and snoek (fish) was tested. In addition, specimens of sour milk, fowl and dog faeces were collected regularly for bacteriological assessment.

Bacteriological methods

About 2·0 g. of each animal tissue specimen was pulverized in Griffith's tubes using about 1·0 g. of sterile sea sand and then ground further after the addition of 1·0 ml. of sterile normal saline. All specimens including the sour milk, fowl and dog faeces were then tested for the following organisms.

Salmonellas and Shigellas

For the primary isolation, an S.S. agar and a Wilson and Blair plate were planted with a loopful from each suspension and incubated overnight. The ground-up tissue was introduced into selenite F medium and after incubation plated out on deoxycholate agar. From the S.S. and deoxycholate plate three non-lactose-fermenting colonies from each, if present, were tested for biochemical reactions. Likewise, characteristic salmonella colonies from the Wilson and Blair plates were tested after 24 and 48 hr. incubation. Those colonies conforming to salmonellas were typed serologically. Throughout the investigation no shigellas were encountered.

After the study had been in progress for 6 months it was noted that on no occasion were salmonellas found on the Wilson and Blair plate that were not present on either the primary S.S. or secondary deoxycholate plate. The use of the Wilson and Blair plate was then discontinued.

Coagulase-positive Staphylococcus aureus

From the original inoculum on Chapman's medium (1945) after 48 hr. incubation, typical colonies were picked off into plasma/mannite media. Positive cultures were then phage-typed.

Clostridium welchii

Willis & Hobbs medium (1959) was used for primary isolation. A secondary isolation using the same medium was made from Robertson's cooked meat medium the next day. All plates were incubated anaerobically. Colonies of *Cl. welchii* were confirmed by subculturing on a further Willis and Hobbs plate half of which contained *Cl. welchii* antitoxin; no opalescence caused by the lecithinase in this half indicated a positive result.

To test for heat-resistant *Cl. welchii* strains, the original ground suspension, at the end of all culturing procedures, was heated in a boiling water bath for 1 hr. Additional Willis and Hobbs plates were planted and examined the next day for the presence of *Cl. welchii* colonies. No typing of the strains was carried out.

Faecal Escherichia coli

All specimens submitted were tested for the presence of faecal *E. coli* by planting in MacConkey single-strength broth and, if positive with the production of acid and gas, were subcultured into brilliant green bile broth and peptone water. After incubation at 44° C. a positive indole reaction and gas production in the brilliant green bile broth was taken as evidence of faecal *E. coli*.

Bacillus cereus

Willis and Hobbs plates were inoculated from the original ground suspensions and incubated aerobically. The organisms were identified on colonial morphology with typical opalescence, caused by lecithinase production, and the characteristic microscopic appearance of the bacilli.

Antibiotic sensitivity

Using the disk method, all salmonella organisms isolated were tested for sensitivity to the following antibiotics: penicillin G (10 units), ampicillin (25 μg.), streptomycin (50 μg.), tetracycline hydrochloride (50 μg.), chloramphenicol (30 μg.), erythromycin (15 μg.) colistin (10 μg.), novobiocin (10 μg.), gentamycin (10 μg.). During the survey nitrofurantoin (100 μg.), kitasamycin (5 μg.), and naladixic acid (30 μg.) were added. A zone of inhibition of less than 2 mm. from the edge of the disk was taken to indicate a resistant organism. An Oxford strain of *Staphylococcus aureus* was used routinely as a control.

RESULTS

Of the specimens submitted from the thirty sheep (Table 1A) salmonellas were isolated from only two faeces. These were identified as *S. typhimurium* and *S. duval*. *Cl. welchii* isolations from tissue specimens ranged from nil in the lymph nodes to 5 (17 %) in the liver. In addition, this organism was recovered from 20 (67 %) faecal and 7 (23 %) bile specimens. *Bacillus cereus* was not evident in any of the tissue specimens and coagulase positive *Staph. aureus* very occasionally. Faecal *E. coli* were found extensively in most specimens.

The results of similar specimens from thirty cattle show that there were fewer

isolations of all organisms, salmonellas excepted, than from the sheep (Table 1 B). *S. carrau* was isolated from one lymph node and *S. london* from a specimen of faeces. The highest isolation from tissue specimens of faecal *E. coli* was 5 (17 %) from liver, and of *Cl. welchii* 3 (10 %), also from liver specimens. A heat-resistant strain of *Cl. welchii* was cultured from 1/12 (8 %) specimens of faeces.

Table 1. *Isolation of pathogens from sheep and cattle at abattoirs*

(Figures in parentheses indicate percentages.)

	Number of isolations of					
	No pathogens	*Salmon-ella* spp.	*Faecal E. coli*	*Cl. welchii**	*B. cereus*	Coagulase-positive Staph.
A. Specimens from thirty sheep						
Liver	6 (20)	0	24 (80)	5 (17)	0	2 (7)
Spleen	6 (20)	0	24 (80)	2 (7)	0	1 (3)
Lymph node	10 (33)	0	20 (67)	0	0	1 (3)
Surface meat cut	24 (80)	0	6 (20)	1 (3)	0	0
Bile	4 (13)	0	26 (87)	7 (23)	0	0
Faeces	0	2 (7)	29 (97)	20 (67)	6 (20)	0
B. Specimens from thirty cattle						
Liver	23 (77)	0	5 (17)	3 (10)	0	0
Spleen	28 (93)	0	1 (3)	1 (3)	0	0
Lymph node	24 (80)	1 (3)	2 (7)	2 (7)	0	1 (3)
Surface meat cut	27 (90)	0	3 (10)	0	0	0
Bile	5 (17)	0	24 (80)	8 (27)	0	0
Faeces	0	1 (3)	30 (100)	29 (97)	9 (30)	0

* No heat-resistant *Clostridium welchii* isolated from sheep specimens. One heat-resistant strain of *Cl. welchii* from twelve specimens of cattle faeces tested.

Isolations from specimens from the Johannesburg Offal Pool and Soweto Township are presented in Table 2. From the Offal Pool, salmonellas were found in 14 (50 %) samples of tripe investigated and 11 (39 %) of intestine. Faecal *E. coli* were abundant and *Cl. welchii* were isolated from 11 (39 %) of tripe specimens and 22 (79 %) of intestine. No heat-resistant *Cl. welchii* were found and *B. cereus* and coagulase-positive *Staph. aureus* were cultured on only three occasions.

From the Soweto Offal stalls, similar results were obtained. Of note are the salmonella isolations, of which 26 (47 %) came from tripe samples, 13 (24 %) from intestine, 9 (16 %) from intestinal fat and 4 (20 %) from miscellaneous specimens. On 2 occasions out of 24 (8 %), heat-resistant *Cl. welchii* were found in intestinal fat. One specimen from an illegal unlicensed offal dealer yielded *S. typhimurium*, *B. cereus* and faecal *E. coli*.

Of twenty samples of polony, mince, meat cuts and snoek (fish) taken from butcher shops, no salmonellas were found and there were fewer isolations of the other organisms.

Specimens of sour milk were collected at random from street pedlars. No salmonellas were isolated, but 24 out of 28 samples (86 %) yielded faecal *E. coli*.

In the Bantu population, poultry is traditionally sold by the shopkeeper in its live state. The fowls are displayed in cages outside the shops and are fed by the shopkeeper until sold. Samples of fresh faeces were thus collected from the bottom of the cages for culture. Of 28 specimens, 4 (14 %) gave salmonellas, 22 (79 %) *Cl. welchii*, of which 3 out of 12 (25 %) were heat-resistant, and 7 (25 %) *B. cereus*. No coagulase positive *Staph. aureus* was isolated.

Table 2. *Isolation of pathogens from Johannesburg offal pool and from Soweto township*

(Figures in parentheses on each line indicate the total number of specimens examined.)

	Percentage of specimens showing					
	No pathogens	*Salmonella* spp.	Faecal *E. coli*	*Cl. welchii**	*B. cereus*	Coagulase-positive *Staph.*
Johannesburg offal pool						
Tripe (28)	0	50	96	39	0	4
Intestine (28)	0	39	100	79	4	4
Soweto offal stalls†						
Tripe (55)	0	47	98	74	13	7
Intestine (55)	0	24	100	96	13	2
Intestinal fat (57)	17	16	81	35	7	3
Liver, spleen and muscle (20)	0	20	100	75	5	0
Soweto butchers' shops						
Polony, meat, mince and snoek (20)	35	0	60	20	15	5
Other sources						
Sour milk (28)	14	0	86	4	7	0
Fowl faeces (28)	4	14	96	79	25	0
Dog faeces (28)	0	21	96	50	21	4

* Heat-resistant *Cl. welchii* were isolated from 2/24 specimens of intestinal fat, 3/12 specimens of fowl faeces and 7/12 specimens of dog faeces.

† One specimen from an unlicensed illegal offal vendor yielded *Salmonella typhimurium*, *Bacillus cereus* and faecal *Escherichia coli*.

Since dogs are known to be salmonella carriers, specimens of fresh faeces from some of the many township dogs were included in the survey. The samples came from the pavements, usually in the vicinity of the offal stalls which supplied the tripe and intestine specimens. Salmonellas were isolated from 6 (21 %) of 28 specimens, one of which had a double infection of *S. dakar* and *S. tel-el-kabir*, *Cl. welchii* from 14 (50 %), *B. cereus* from 6 (21 %) and there was one isolation of coagulase positive *Staph. aureus*. Heat-resistant *Cl. welchii* were found in 7 (58 %) of 12 specimens.

From all the specimens in the survey, ninety-three salmonellas were isolated comprising 28 different serotypes. Reference to Table 3 shows the frequency of isolations from various sources.

All salmonella strains were sensitive to tetracycline HCl, chloramphenicol, colistin, and gentamycin (Table 4). One strain of *S. duval* was resistant to ampi-

cillin and streptomycin and another *S. duval* to ampicillin only. The conventional pattern was shown, all strains being resistant to penicillin G, novobiocin and kitas-amycin, and 95 % to erythromycin.

Table 3. *Number, type and source of* Salmonella *strains isolated*

	Abattoir sheep and cattle specimens	Offal Pool tripe and intestine	Offal traders, all specimens	Dog faeces	Fowl faeces	Specimen from illegal offal trader	Total
S. typhimurium	1	9	9	.	1	1	21
S. london	1	1	13	.	1	.	16
S. newport	.	2	3	1	.	.	6
S. duval	1	1	3	.	.	.	5
S. montevideo	.	3	1	.	1	.	5
S. eastbourne	.	1	3	.	.	.	4
S. mission	.	1	2	.	.	.	3
S. stanley	.	2	1	.	.	.	3
S. labadi	.	1	2	.	.	.	3
S. saintpaul	.	.	3	.	.	.	3
S. tel-el-kabir	.	.	1	2	.	.	3
S. cerro	.	.	3	.	.	.	3
S. hato	.	.	2	.	.	.	2
S. poona	.	.	1	1	.	.	2
S. adelaide	.	1	.	.	.	.	1
S. johannesburg	.	1	.	.	.	.	1
S. anatum	.	1	.	.	.	.	1
S. pomona	.	1	.	.	.	.	1
S. paratyphi B	.	.	1	.	.	.	1
S. thompson	.	.	1	.	.	.	1
S. dublin	.	.	1	.	.	.	1
S. bulawayo	.	.	1	.	.	.	1
S. reading	.	.	1	.	.	.	1
S. minnesota	.	.	.	.	1	.	1
S. infantis	.	.	.	1	.	.	1
S. rowbarton	.	.	.	1	.	.	1
S. dakar	.	.	.	1	.	.	1
S. carrau	1	.	.	.	.	.	1
Total	4	25	52	7	4	1	93

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

	No. of strains tested	Percentage resistant		No. of strains tested	Percentage resistant
Penicillin G	92	100	Colistin	92	0
Ampicillin	92	2	Novobiocin	92	100
Streptomycin	92	1	Gentamycin	92	0
Tetracycline HCl	92	0	Nitrofurantoin	14	0
Chloramphenicol	92	0	Naladixic acid	14	0
Erythromycin	92	95	Kitasamycin	67	100

DISCUSSION

Salmonellas

The prevalence of salmonellas in the various meat samples tested in this survey supplements the findings of various workers in other countries. (Report of a Working Party of the Public Health Laboratory Service, 1964; Kampelmacher, Guinée & Clarenburg, 1962; Galton, Steele & Newell, 1964.) Furthermore these workers have indicated the close correlation between animal and human infections.

Of the 240 specimens of cattle and sheep tissue from the abattoir examined over the period, only one specimen of bovine lymph node (0·4 %) yielded salmonellas (*S. carrau*). This compares favourably with the findings of the Working Party of the Public Health Laboratory Service in their investigation of meat samples from retail butcher shops.

The salmonella isolations from the tripe and intestines present an entirely different picture. From the Offal Pool half the specimens of tripe and 39 % of intestine yielded salmonellas. As one would expect, the number of salmonellas isolated from the distribution stalls in the township for those items was correspondingly high. Other meat samples sold from the stalls showed a 20 % salmonella incidence, probably due to contamination from the tripe and intestine through handling. No salmonellas were isolated from the twenty specimens from the butcher shops, which appeared to observe a higher hygienic standard.

Although the number of samples tested was small, the salmonella isolations are significant. The amount of tripe and intestine consumed annually by the population of Soweto is estimated at 12,000 tons, indicating the popularity of this food. Were this amount evenly distributed, consumption per head per diem would be 40 g., which would afford 8 g. animal protein (A. R. P. Walker, personal communication). In Britain, the corresponding figure for animal protein derived from *all* meat products is about 30 g. (Domestic Food Consumption and Expenditure, 1961). It will therefore be appreciated that the protein contributed to the Bantu by offal is highly significant. With a combined result of nearly 28 % salmonella isolations from all the specimens from the stall distributors and 44 % from the central pool, the risk to the population is obvious.

Previous investigations on the incidence of salmonellosis (Bokkenheuser & Greenberg, 1959) in various populations have shown that there are far more isolations of salmonellas in the hot summer months than in the winter. A similar trend was not observed in the recovery of salmonellas from these meat samples.

In keeping with reports of high isolations in other countries (Report of a Working Party of the Public Health Laboratory Service, 1964; Galton *et al.* 1964), *S. typhimurium* was the type most frequently found from the specimens of offal (18/77 = 23 %). This was followed by *S. london* (14/77 = 18 %) and *S. newport* (5/77 = 6 %) (Table 3).

In contrast, however, *S. dublin*, which is predominantly of bovine origin and relatively host specific, was only found in one instance. This organism has in the past been reported as being most common in cattle in South Africa (Henning, 1949),

but has shown a decreasing trend in recent years (H. J. W. Botes, personal communication). An interesting feature of this survey was the single isolation of *S. johannesburg* in one of the Offal Pool specimens, since, during the period of the investigation, there were outbreaks of gastroenteritis due to *S. johannesburg*, particularly in infants and toddlers, in hospitals serving this area. During this period, a survey was conducted by Dr I. Spencer of the Johannesburg City Health Department and the South African Institute for Medical Research into the incidence of salmonellosis and shigellosis in children up to 2 years of age from clinics in Soweto. *S. johannesburg* was isolated on one occasion only (I. H. F. Spencer, personal communication). Unlike *S. typhimurium*, which is readily transmitted from animal to man, *S. johannesburg* appears to be far more host-specific to man.

The recovery of salmonellas, including *S. typhimurium*, from fowl faeces in this investigation serves to confirm another potential source of salmonellosis to the community. The salmonellas in fowl faeces might have been higher if media without bile salts had been used to allow all strains of *S. gallinarum-pullorum* to grow. It is well known that poultry, eggs and their subsequent products provide a large reservoir of salmonella organisms and adequate control is essential.

It is not surprising that, with the high salmonella incidence in the offal samples studied, there should be a relatively high recovery (21%) from the dog faeces investigated. Butler & Herd (1965), investigating the incidence of human enteric pathogens in dogs in Alaska, found that approximately 16% of family pets harboured salmonellas. Mackel *et al.* (1952) found that 15% of 1626 household dogs were infected with salmonellas. Other workers (Watt & De Capito, 1950; Floyd, 1945) have found a much lower incidence.

Coagulase-positive Staphylococcus aureus

Phage typing carried out on thirteen of the sixteen isolations of coagulase-positive *Staph. aureus* isolated from all types of specimens studied showed that 6 (46%) belonged to phage group III. According to Wilson & Miles (1964) coagulase-positive *Staph. aureus* organisms causing food poisoning may belong to this group and a smaller number to group IV, of which none were isolated in this survey. The significance of the small number isolated as a possible source of food poisoning is unknown.

Faecal Escherichia coli

Using the isolation of faecal *E. coli* as an indicator of faecal contamination, 62% (74/120) of the liver, spleen, lymph node and meat-cut specimens from sheep at the abattoir were positive in contrast to the similar sampling from cattle which gave only 9% (11/120). Since abattoir procedures on all animals are standard, one possible explanation may be the increased risk of tissue contamination with intestinal contents when working on smaller animals.

Nearly all specimens from the Offal Pool and Soweto Offal dealers showed gross contamination with faecal *E. coli*, as did 86% of the specimens of sour milk tested.

Clostridium welchii

It is well known that *Cl. welchii* organisms cause food poisoning outbreaks. Vernon (1966) in reviewing food poisoning and salmonella infections in England estimated that half of the cases were from salmonella infections and a third were due to *Cl. welchii*. Hobbs & Wilson (1959), investigating the contamination of wholesale meat supplies, found heat-resistant *Cl. welchii* in 11 % boneless meat and 2 % carcass meat samples.

Theories as to the pathogenesis of the disease due to *Cl. welchii* are varied. Evidence exists for and against it being either intoxication or infection. Hobbs and her colleagues (1953), on examining many outbreaks of food poisoning, found that the organism responsible was a variant of Type A, being feebly toxigenic and heat-resistant. Nygren (1962) maintains that the food poisoning due to *Cl. welchii* is not caused by the organism or the exotoxin, but by phosphorylcholine produced by the enzymic action of lecithinase on lecithin in the food. If sufficient phosphorylcholine is present in the food, the intestine will be affected in 8–12 hr. by this substance, causing increased peristalsis and intestinal hurry. It is apparent, however, that more experimental studies are necessary to supplement Nygren's postulates (Nelson *et al.* 1966).

The extent of outbreaks in Soweto township of food poisoning due to *Cl. welchii* is unknown. Because of the high incidence of faecal *E. coli* in the samples from the Offal Pool and the offal vendors in the township, it is not surprising that the isolations of *Cl. welchii* are correspondingly high. Of the few samples in each group tested for heat-resistant *Cl. welchii*, only 2 of 24 (8 %) were isolated from intestinal fat. It is noteworthy that heat-resistant *Cl. welchii* were isolated from a quarter of the fowl faeces and over half of the dog faeces tested.

Bacillus cereus

Hauge (1950, 1955) and Christiansen (1951), as quoted by Wilson & Miles (1964), have produced evidence that foods containing large numbers of *B. cereus* may cause food poisoning. In all the specimens treated from all sources, fowl faeces yielded the highest number of isolations (25 %). However, in every specimen containing *B. cereus*, the number of organisms was so scanty that, in this survey, their significance is doubtful.

CONCLUSIONS

Although the number of specimens tested from the abattoir was small, the results obtained show that reasonable care is taken to ensure that the slaughtered animals are processed in a satisfactory manner. As far as the Offal Pool is concerned, it is clear that the tripe and intestines are not adequately treated after leaving the abattoir to render them comparatively free of faecal contamination.

The risk of infection, particularly salmonellosis, from this source is very real considering the vast quantities consumed and the lack of knowledge of modern hygienic habits. It is accepted that the transient human carriers among the Bantu population in Soweto township may constitute an important reservoir of salmonellas responsible for diarrhoea and can be as high as 14·5 % in school children in

summer. (Richardson *et al.* 1966). Although salmonellas attack all age groups, infants and elderly people, whose resistance is weakened by other conditions, are more susceptible. In the Soweto township, the infant mortality rate (number of deaths of infants under one year of age per 1000 live births) was reduced over the last 10 years from 125·7 to 64·5, with a corresponding drop due to gastroenteritis over the same period from 41·4 to 19·3. The infant mortality rate in white children in Johannesburg due to gastroenteritis was 2·59 in 1957 and 1·02 in 1967 (I. H. F. Spencer, personal communication). Although the gastro-enteritis infant mortality rate in the Bantu shows a marked improvement over the 10-year period it is high when compared with that of the white infants. The infected offal may well be directly responsible for this high incidence.

Health authorities should take cognizance of the findings of this study. It is realized that any scheme to improve the condition of the offal must be costly, but great care must be taken to ensure that the price to the consumer does not rise so high that they cannot afford this valuable source of protein.

SUMMARY

1. From the Municipal abattoir, specimens of liver, spleen, lymph node, surface meat, bile and faeces from a sheep and a bovine, were examined fortnightly for 14 months for the presence of possible pathogenic bacteria. The results suggest that slaughtering procedures are satisfactory.

2. Offal, consisting mainly of tripe and intestine, is eaten in large quantities by the Bantu population and is both nutritious and economical. The high incidence of salmonella isolations in the tripe (48 %) and intestines (29 %), and faecal *E. coli* and *Cl. welchii*, show that this commodity is distributed in an inadequately cleansed condition.

3. Faecal *E. coli* was isolated from 86 % of samples of sour milk collected from street pedlars.

4. Dog faeces collected from the township pavements yielded 21 % salmonellas, and faeces from fowls sold live by shopkeepers 14 %.

5. From the offal specimens, *S. typhimurium* (23 %) and *S. london* (18 %) were the salmonella types most frequently isolated. *S. dublin* was isolated on only one occasion.

6. Throughout the survey no shigellas were isolated.

7. The significance of the *Cl. welchii*, coagulase positive *Staphylococcus aureus* and *B. cereus* isolations from the various specimens tested is not known.

8. It is emphasized that although there is a definite need for improved treatment of the offal before distribution to the consumer, the resulting increase in cost must not be such as to deprive the population of this important source of protein.

to Dr A. Smith, Medical Officer of Health, and Dr I. H. F. Spencer of the Johannesburg City Health Department for help and advice; to Mr A. J. MacG. Bryce for collecting all the specimens from the Soweto township; to Mrs Leonie Jonk for typing the salmonella organisms and Mr R. G. Robinson for investigating the micro-organisms' sensitivity to antibiotics.

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