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The prevalence of human isolates of *Salmonella* subspecies II in southern Africa

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SUMMARY

The Salmonella Reference Centre in Johannesburg received 14059 strains of human origin between 1979 and 1984. A significant proportion (6·3%) proved to belong to subspecies II. The majority were cultured from faecal material, usually associated with symptoms related to the gastrointestinal tract. They comprised 884 isolates, represented by 203 serotypes, of which 45 were new serotypes.

The poor hygienic conditions found in many rural areas, together with possible contamination of food and water by wild animals, may contribute to the greater frequency of human S.II infections and the widespread occurrence of unusual serotypes in man in this geographic region.

INTRODUCTION

Salmonella cultures, usually from human sources, are submitted from many diagnostic laboratories in South Africa and Namibia (South West Africa). Depending on the resources available, individual laboratories vary in the range of serotypes they are able to identify. The decision to request further identification is at the discretion of the laboratory concerned, as the public health authorities do not require the notification of salmonella isolations other than *Salmonella typhi*. As a result, the 3000–4000 cultures received at the Centre each year tend to be the difficult or unusual strains, and the distribution of serotypes is probably not truly representative of the country as a whole. A similar bias may exist in the reports of all National Salmonella Centres.

As in other parts of the world, most Southern African strains belong to *Salmonella* subspecies I, but S.II isolates, including those of human origin, are much more frequently seen in this region. It should be borne in mind that subspecies I and II correspond to Kauffmann's subgenera I and II (Kauffmann, 1960).

MATERIALS AND METHODS

Salmonella strains were isolated from source material and identified by conventional methods in hospital and public health laboratories situated throughout Namibia and in many parts of South Africa. The great majority of the population served by these laboratories live in the summer rainfall regions of southern Africa, including distant rural communities, where water supplies and sanitation may be rudimentary.

On receipt at the Reference Centre, each culture was replated onto a MacConkey agar medium for a purity check, before studying a set of biochemical characters. Most strains showed the typical reactions of subspecies I. Malonate and gelatin positive, ONPG negative (after 2 h) strains were assigned to subspecies II.

The few examples of the other subspecies isolated were excluded from this report, along with rough, mucoid and inagglutinable strains.

The antigenic formula was established by slide agglutination with polyvalent and specific factor sera. Swarm agar plates incorporating appropriate antisera were used for the selection of alternative flagellar phases. Additional biochemical tests were done if indicated. Strains that showed hitherto unknown formulae, or that gave rise to diagnostic problems, were sent to the World Health Organisation Collaborating Centre for Reference and Research on Salmonella in Paris, France, for further investigation. Extensive biochemical studies were done there, including testing for the characters selected to differentiate the six subspecies of *Salmonella* (Le Minor, Véron & Popoff, 1982*a*, *b*). Serological reactions were studied using sera prepared according to the Guidelines for the preparation of *Salmonella* antisera (Le Minor & Rohde, 1986).

RESULTS

Out of a total of 15188 confirmed salmonella strains received at the Reference Centre in Johannesburg in the 5-year period July 1979 to June 1984, 14059 originated from humans. Most of these (more than 90%) were cultured from faecal material, which was usually examined in the first instance because of symptoms referable to the gastrointestinal tract. A small proportion was isolated in the course of screening apparently healthy food-handlers. The isolates comprised 224 serotypes belonging to subspecies I and 203 to subspecies II. Two new serotypes from subspecies I (3 isolates) and 45 from subspecies II (68 isolates) were isolated and subsequently confirmed or analysed in detail at the Reference Centre in Paris.

The human isolates included 884 S.II strains (6·3%), of which 528 belonged to 34 serotypes, represented by at least 6 strains each (Table 1). The remaining 356 isolates were distributed among 169 serotypes, 87 of which occurred only once each. Table 2 lists the 45 new S.II serotypes identified during this 5-year period. It was noted that a much higher proportion of S.II isolates originated in Namibia than in the Republic of South Africa (19·5% of 2202 and 3·8% of 11857 respectively). Namibian isolates accounted for 21 of the 45 new S.II serotypes.

Table 1. *The more common S.II serotypes isolated from human sources, 1979–84*

Serotype	No. of isolates
luanshya	51
6, 7: *g*, *m*, [*s*], *t*: *e*, *n*, *x*	46
mobeni	34
sofia	33
1, 9, 12: *g*, *m*, [*s*], *t*: [1, 5, 7], [z_{42}]	28
kuilsrivier	27
wynberg	26
caledon	23
nachshonim	23
bechuana	20
gilbert	17
louwbester	15
bloemfontein	14
nordenham	14
9, 12: *a*: z_{39}	14
grabouw	12
durbanville	9
llandudno	9
makumira	9
9, 12: *l*, *v*: *e*, *n*, *x*	9
goodwood	8
lindrick	8
neasden	8
1, 4, 12, 27: *z*: 1, 5	8
hueningen	7
tosamanga	7
1, 9, 12: *a*: *e*, *n*, *x*	7
khami	6
mondeor	6
1, 4, 12, 27: *l*, *v*: *e*, *n*, *x*	6
1, 4, 12, 27: *l*, *v*: z_{39}	6
6, 7:–: 1, 6	6
1, 9, 12: z_{42}: 1, [5], 7	6
16: *d*: 1, 5	6
Total	528

DISCUSSION

Numerous reports have described the occurrence of a variety of salmonella serotypes, many of them belonging to 'subgenera' II and III, in reptiles and other cold-blooded animals, e.g. Iveson, Mackay-Scollay & Bamford, 1969. S.II salmonellae have also been found in other animal species, including cattle in Botswana, a territory bordering on both Namibia and South Africa (Miller, 1971).

S.II serotypes are regarded as an uncommon cause of disease in man (Rohde, 1965; Farmer *et al.* 1984), a view that is supported by retrospective analysis of the serotypes reported in a number of surveys published over the last three decades. Only 3 isolates of a single S.II serotype were reported among 3216 salmonellae cultured from humans before 1960 in Central Africa (the former Belgian Congo and Ruanda-Urundi). All 40 new serotypes isolated from man in the same period belonged to subspecies I, while only a few S.II strains were found

among many hundred of salmonellae cultured from a wide range of domestic and wild animals (Van Oye, 1964). A few years later, 24 S.II strains (3 serotypes) out of 814 human isolates were recorded in the same region (Gatti *et al.* 1968). More recently, 10 strains of a single S.II serotype were identified among 10953 human isolates in Malaysia (Jegathesan, 1984) and one S.II strain was found in a young child with enteritis in Spain, out of 1027 salmonellae cultured from human faeces (Prats *et al.* 1985). Only 14 S.II strains were identified among 67767 salmonellae isolated in France from 1980–3 (Le Minor, Le Minor & Grimont, 1985).

In southern Africa, however, significant numbers of human isolates belong to subspecies II, with a relatively higher frequency in Namibia than in the neighbouring Republic of South Africa. Prior to 1958, 14 S.II serotypes (16 isolates) were identified out of a total of 962 salmonellae cultured from acutely ill patients, convalescents and apparently healthy excretors (Bokkenheuser & Greenberg, 1959). An additional 35 S.II serotypes occurred among some 200 serotypes found in man in South Africa up to 1964, and it was observed that about a third of reported serotypes in this region were new, most of which belonged to subspecies II (Brede, 1964). The majority of a list of 36 S.II serotypes which had been associated with disease in man originated from South Africa (Winkle, 1966).

We now report 884 S.II strains isolated from human sources, the majority being cultured from faecal material, usually from patients with symptoms of gastrointestinal infection. They belonged to a wide range of serotypes, most of which occurred sporadically and infrequently. Clustering in time and place was observed on a few occasions, the most notable involving 12 children in an institutional outbreak of gastroenteritis, from all of whom S.II *mobeni* was isolated.

The mean number of isolates per serotype was 4·35, compared with 58·8 for the S.I strains included in the survey. The marked difference between these figures could be explained by less efficient propagation of S.II salmonellae between humans, because of poor adaptation from the primary host (presumably tortoises and other cold-blooded animals in many cases), or by the sporadic contamination of food and water from these unusual sources. Such organisms would continually be introduced into the community, but with limited opportunity for human to human dissemination in sparsely populated areas such as Namibia.

Poor hygienic conditions in many rural areas of southern Africa, particularly regarding sewage disposal and sources of drinking water, are probably responsible for the widespread prevalence of salmonellae among the local population. Several surveys have shown that communal shallow wells and streams, used by man and his domesticated animals, are probable reservoirs of salmonella infection in these areas (Bokkenheuser & Richardson, 1960; Richardson & Bokkenheuser, 1963; Richardson & Koornhof, 1965). In a frequently drought-stricken subcontinent, with many arid areas and few flowing rivers, wells dug in dry river-beds are often the only source of water for small communities in the dry season. Stagnant conditions and relatively high ambient temperatures would encourage bacterial multiplication in such water supplies. Pollution by birds and wild animals is common and this is likely to be aggravated by the advent of the rains, with flooding of the wells by surface run-off (Brede, 1964). Drinking water is not necessarily the only mode of transmission, because improving the quality of water may have little

Table 2. *New S.II serotypes of human origin 1979–84*

Paris reference number	Formula	Source	Age	Diagnosis
3340/81	1, 4, 12, 27, *a*: z_{39}	Faeces	Adult	Routine: food handler
4259/83	4, 12: *z*: 1, 7	Faeces	Adult	Routine: food handler
3339/81	4, 12: *z*: z_{39}	Rectal swab	10 mo.	Diarrhoea
4262/83	6, 7: *d*: z_{42}	Faeces	Child	Typhoid
4260/83	6, 7: *l*, *w*: z_{42}	Faeces	Child	Diarrhoea
4622/84	6, 7: *l*, z_{28}: z_6	Faeces	4 mo.	Dysentery
4049/83	6, 8: *a*: z_{39}	Rectal swab	8 years	Diarrhoea
4060/83	6, 8: *b*: 1, 5	Faeces	2 mo.	Gastroenteritis
4062/83	6, 8: *l*, z_{28}: *e*, *n*, *x*	Rectal swab	9 mo.	Gastroenteritis
4048/83	6, 8: z_{29}: *e*, *n*, *x*	Faeces	3 mo.	Diarrhoea
4058/83	9, 12: *d*: z_{39}	Faeces	22 years	Diarrhoea
4258/83	9, 12: *l*, z_{28}: 1, 5: z_{42}	Faeces	Child	Typhoid
3025/80	3, 10: *a*: z_{39}	Rectal swab	2 wk	Diarrhoea
3338/81	3, 10: *m*, *t*: 1, 5	Rectal swab	2 mo.	Gastroenteritis
4256/83	1, 13, 23: *g*, *m*, *s*, *t*: z_{42}	Faeces	Child	Gastroenteritis
3626/82	1, 13, 23[37]: *z*: z_{42}	Faeces	Child	Diarrhoea
3341/81	16: z_{29}: *e*, *n*, *x*	Rectal swab	6 mo.	Diarrhoea
4047/83	17: *g*, *m*, *s*, *t*: –	Pus	75 years	Septic leg
4621/84	21: *b*: 1, 5	Blood	15 years	Pyrexia
3648/82	21: *g*, *m*, *s*, *t*: –	Rectal swab	Child	Typhoid
4052/83	21: *m*, *t*: –	Faeces	4 years	Gastroenteritis
4059/83	28: *a*: *e*, *n*, *x*	Faeces	Adult	Routine: food handler
4261/83	28: *b*: *e*, *n*, *x*	Faeces	54 years	Diarrhoea
3780/82	28: *z*: 1, 5	Pus	Adult	Wound infection
4050/83	28: z_{29}: *e*, *n*, *x*	Faeces	Adult	Diarrhoea
3337/81	28: *l*, z_{28}: 1, 5	Faeces	1 year	Diarrhoea
3027/80	30: z_6: 1, 6	Rectal swab	3 mo.	Diarrhoea
4616/80	39: *e*, *n*, *x*: 1, 7	Sputum	69 years	Pleural effusion
4053/83	39: *l*, *v*: 1, 5	Rectal swab	Adult	Routine: food handler
4061/83	39: *l*, z_{28}: z_{39}	Faeces	4 mo.	Gastroenteritis
3183/81	39: *m*, *t*: *e*, *n*, *x*	Faeces	2 years	Diarrhoea
4043/83	1, 40: *e*, *n*, *x*: 1, [5], 7	Faeces	30 years	Routine: food handler
3629/82	40: z_{39}: 1, 7	Rectal swab	Adult	Routine: food handler
3501/82	1, 40: z_{42}: 1, 6	Faeces	Child	Diarrhoea
4618/84	41: *g*, *m*, *s*, *t*: z_6	Rectal swab	16 years	Typhoid
3500/82	1, 42: *l*, *w*: *e*, *n*, *x*	Faeces	Child	Diarrhoea
3776/82	43: *a*: 1, 5	Faeces	Adult	Typhoid
4056/83	45: z_{29}: *e*, *n*, *x*	Faeces	2 years	Dysentery
3773/82	47: z_{29}: *e*, *n*, *x*, z_{15}	Faeces	7 wk	Gastroenteritis
4066/83	50: *k*: *e*, *n*, *x*, z_{42}	Faeces	1 wk	Diarrhoea
3622/82	51: *g*, *s*, *t*: *e*, *n*, *x*	Faeces	2 mo.	Gastroenteritis
3336/81	53: *c*: 1, 5	Faeces	11 mo.	Gastroenteritis
4252/83	57: *a*: z_{42}	Faeces	Adult	Typhoid
4045/83	1, 59: *z*: z_6	Rectal swab	1 mo.	Gastroenteritis
3779/82	60: *b*: –	Rectal swab	5 years	Diarrhoea

direct effect on the prevalence of salmonellae in the community (Richardson *et al.* 1968).

A very wide distribution of serotypes must exist in human and animal hosts, as many of our rarely isolated serotypes (S.I and S.II) were found both in South Africa and in Namibia, by laboratories situated up to 2000 km apart. In such a

large and varied reservoir, antigenic 'shift' by mutation, hybridization or other modes of gene transfer (Iino & Lederberg, 1964) probably contribute to the emergence of so many new serotypes. The fact that 45 out of the 47 new serotypes found during the survey period belonged to subspecies II tends to support the belief that humans in this geographic region are constantly exposed to non-human sources of infection. There is little doubt that these strains are pathogenic to man, since most of the patients presented a clinical pattern of gastroenteritis or dysentery, often with pyrexia and, in at least one case, with bacteraemia (Table 2). Spread beyond the index case appears to be negligible, as a total of only 68 strains of the new S.II serotypes were isolated.

There is clearly a need for further investigation of the part played by wild animals and birds in the distribution of salmonellae in southern Africa, which could provide an explanation for the greater frequency of S.II infections in man in this region.

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